BMJ Open

Does paired genetic testing improve targeted therapy choices and screening recommendations for patients with upper gastrointestinal cancers and their families? A prospective cohort of 42 patients

Kevin Tatunay,[1] Stacey Cohen ,[1,2] Lorraine V Naylor,[1] Cynthia L Handford,[1] Angela Jacobson,[2] Veena Shankaran,[1,2] Brant Oelschlager,[2] William M Grady,[1,2] Britta Sjoding,[1] Everett Lally,[1] Lauren Facchini,[1] Qin Sun,[1] Mercy Y Laurino,[1] Colin Pritchard,[2] Eric Q Konnick,[2] Marianne E Dubard-Gault [1,3]

To cite: Tatunay K, Cohen S, Naylor LV, *et al*. Does paired genetic testing improve targeted therapy choices and screening recommendations for patients with upper gastrointestinal cancers and their families? A prospective cohort of 42 patients. *BMJ Open* 2025;**15**:e091745. doi:10.1136/bmjopen-2024-091745

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2024-091745).

Received 15 February 2025
Accepted 02 May 2025

[1]Fred Hutchinson Cancer Center, Seattle, Washington, USA
[2]University of Washington, Seattle, Washington, USA
[3]Oncology, Providence Swedish Caner Institute, Seattle, Washington, USA

Correspondence to
Dr Marianne E Dubard-Gault; Marianne.Dubard-Gault@Swedish.org

ABSTRACT

Objectives Our study was designed to assess whether paired normal-tumour testing increased access to targeted therapy, clinical trials and influenced cancer screening recommendations given to patients and their families.

Design Prospective cohort study.

Setting Academic cancer centre in the Pacific Northwest region of the USA.

Participants Patients newly diagnosed between 01 January 2021 and 31 December 2022 with cancers of the oesophagus, gastro-oesophageal junction and stomach (CEGEJS) were included. All other cancer diagnoses such as head and neck, duodenal and lower gastrointestinal tract cancers were excluded.

Intervention Paired germline and tumour genetic test within 90 days of new patient visit.

Primary outcome measures Number of targeted therapies received (or not) when eligible, follow-up treatment data and number of inherited predispositions to cancers identified. No secondary outcome measures.

Results Of 42 patients, 32 (76.2%) were eligible for at least one targeted therapy. 19 patients received immunotherapy, when 16 had a biomarker predicting immunotherapy benefit, and benefit of immunotherapy was unclear for 3. Another 11 did not have this biomarker, and 6 of them received immunotherapy. Six pathogenic variants were identified in four high-risk genes. By 01 January 2024, 18 patients (42.9%) had died of complications of cancer.

Conclusion More than 75% of patients who received tumour testing were eligible for a targeted therapy regardless of their stage at diagnosis, emphasising the need to expand access to testing with staging workup to improve survival outcomes. Six families received personalised screening recommendations, thanks to this study.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a prospective cohort characterising 42 patients newly diagnosed with upper gastrointestinal cancers between 01 January 2021 and 31 December 2022.
⇒ Retrospective review of claims from major payors was performed to assess characteristics of prior patients with upper gastrointestinal cancers and frequency of genetics referral in our region.
⇒ We offered paired germline and tumour genetic testing and assessed its impact on choice of targeted therapy, access to clinical trials and cancer screening recommendations.
⇒ Our study is limited to one large academic cancer centre and to genetic testing that was clinically available in 2021.
⇒ Sample size was small, limiting our ability to perform comparative analyses between subgroups.

INTRODUCTION

Thousands of patients diagnosed with cancers of the oesophagus, gastro-oesophageal junction and stomach (CEGEJS) face a dire prognosis[1–3] every year, impelling us to develop better methods for early diagnosis and treatments.

A subset of CEGEJS exhibits mismatch repair (MMR) or homologous DNA damage repair deficiency (dHRD).[4] Treatments targeting deficient DNA-repair damage pathways such as immunotherapy and/or poly (ADP-ribose) polymerase (PARP) inhibitors are associated with better tolerance, fewer long-term side effects and better outcomes than conventional cytotoxic chemotherapy and radiation.[5 6–12] A recent study in advanced

gastric cancer where patients with dHRD were treated with neoadjuvant durvalumab (programmed death Ligand −1 inhibitor (PD-L1)), paclitaxel and olaparib (PARP inhibitor) demonstrated promising results.[13 14]

The aetiology of CEGEJS is heterogeneous and population-dependent.[15 16] Familial CEGEJS case studies suggest a hereditary component for up to 15% of patients.[17–20] Drawing from the overall survival benefit gained with PARP inhibitors in germline mutated breast and ovarian cancer, understanding inherited genetic factors in CEGEJS would augment our ability to identify the most appropriate targeted therapy and predict response.[21] There are rare genetic predispositions to CEGEJS, including hereditary diffuse gastric cancer syndrome (HDGC), tylosis with oesophageal cancer syndrome (TEC) or chromosome breakage disorders.[4 22–26] However, patients with more common hereditary cancer syndromes such as Lynch syndrome[27] and hereditary breast and ovarian cancer syndrome (HBOC) have an increased lifetime risk of upper gastrointestinal malignancies.[22 28–30] Uncovering HBOC would unlock access to targeted treatment with a PARP inhibitor.[13 14] Furthermore, a delay in identifying a hereditary cancer syndrome at the time of a patient's diagnosis closes a window of opportunity for early detection and prevention of hereditary cancers for at-risk relatives. National treatment guidelines, including the National Comprehensive Cancer Network (NCCN) guidelines,[31] did not specify guidance for appropriateness of genetics referral for all CEGEJS diagnoses in 2021, limiting access and insurance coverage of genetic services.

The goal of this project was to report on the clinical utility of paired normal-tumour profiling results in guiding choice of therapy, access to clinical trials and assess the prevalence of hereditary cancer syndromes in patients with CEGEJS. With this project, we reviewed retrospective registry and claims data for patients with CEGEJS diagnosed between 2015 and 2019, and we prospectively followed newly diagnosed patients with CEGEJS after they received paired clinical normal-tumour testing.

METHODS

This project included a retrospective review of registry and payor claims and a prospective cohort study of patients newly diagnosed with CEGEJS. For the retrospective review, we collected and analysed deidentified health metrics from the Surveillance, Epidemiology and End Results (SEER) data for the 13 counties of the Puget Sound region (see online supplemental figure S1) and claims data submitted to Centers for Medicare & Medicaid Services, Washington state Medicaid, Premera Blue Cross and Regence Blue Shield and shared with Hutchinson Institute for Cancer Outcomes Research (HICOR) between 2015 and 2019. Retrospective dataset contained demographic and ethnicity information, cancer diagnosis and treatment data, family history, payor, area of deprivation index[32 33] and reports of referral to genetics or reimbursement for genetic testing for patients diagnosed with CEGEJS. During the prospective cohort study, we estimated the number of patients newly diagnosed with a CEGEJS diagnosis at Fred Hutch by querying an institutionally generated deidentified dashboard of annual completed appointments (see online supplemental figures S4-S6). 2 weeks prior to the study start date, we met with the Fred Hutch gastrointestinal oncologists at each location to share the protocol, eligibility criteria and how to refer to the study. We sent a departmental update on this study after 1 year of enrolment. Between 01 January 2021 and 31 December 2022, gastrointestinal oncologists referred new patients with CEGEJS for a cancer genetics evaluation and study participation. The visit with genetics included collection of demographic information and ancestry, confirmation of histology, construction of a 3-generation family tree, pretest counseling, review of the purpose of the study, documentation of interest for genetic testing and research participation. Following the genetic visit, patients were contacted by a research coordinator who obtained informed consent to participate. Paired somatic and germline genetic testing was ordered by the genetics team and performed using the clinical genetic tests called OncoPlex and BROCA[34 35] developed by the Laboratory Medicine at the University of Washington in Seattle, Washington, USA (see online supplemental figure S7). Post-test genetic counseling visit included result disclosure and recommendations for familial cascade testing if indicated. Patients and family members confirmed to have a hereditary cancer syndrome were offered a referral to a gastrointestinal cancer high-risk programme and enrolment in a long-term surveillance programme. The study team performed periodic chart review and recorded participant demographics, personal risk factors, cancer diagnosis based on histology report, treatment sequence, genetic test results and vital status at follow-up. All histologies were included. We also assessed whether each patient met criteria for genetic testing per the NCCN guidelines available in January 2021.[36 37] Testing for microsatellite instability (MSI) was performed with next generation sequencing,[38] testing for mismatch MMR repair deficiency with immunohistochemistry (IHC) and testing for Human Epidermal Growth Factor 2 (HER2) overexpression with IHC and Fluorescence In Situ Hybridisation (FISH).[39] Testing for PD-L1 in a tumour sample was performed by measuring the ratio of tumour cells expressing PD-L1 over the total number of viable tumour cells and reported under a Combined Positive Score (CPS).[40 41]

The study was approved by the Institutional Review Board (IRB) of the University of Washington with IRB number: 11 490. Data were stored in a password-protected REDCap database only accessible to the study team. Our study team performed descriptive data analysis using Excel V.2307 and no complex statistical tests were performed. Authors of this manuscript have no competing interests.

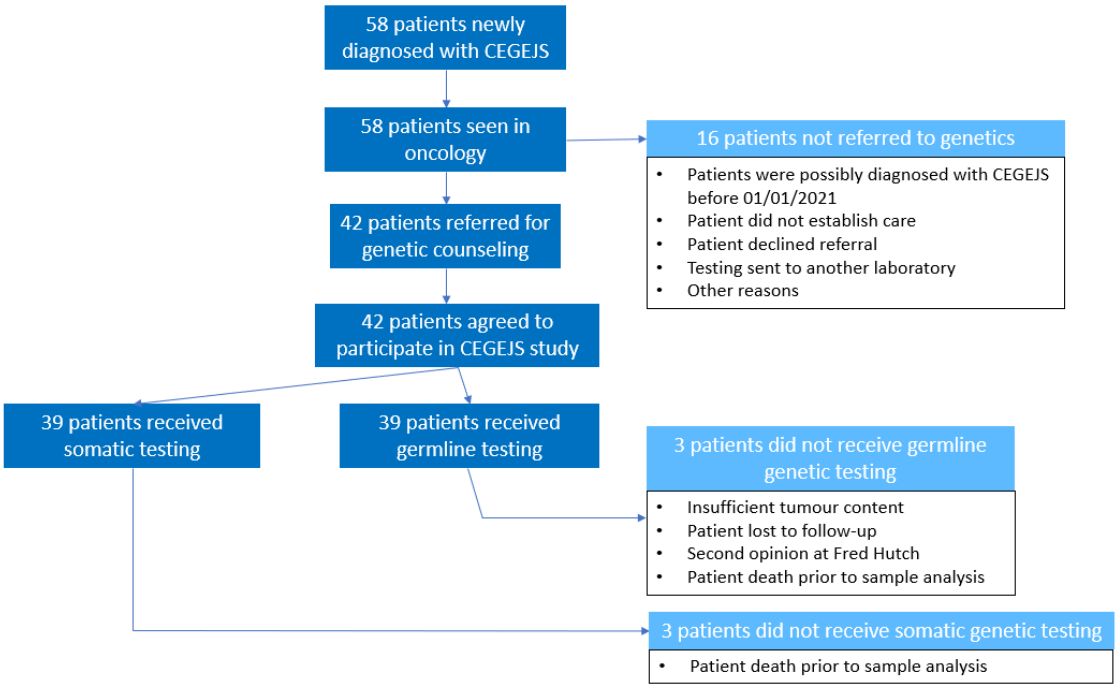

Figure 1 Consort diagram for the study on cancers of the oesophagus, gastro-oesophageal junction and stomach (CEGEJS).

Patient and public involvement

The IRB team of the University of Washington includes unaffiliated community members of the Seattle area. They reviewed the protocol for this study. Genetics results for each patient obtained during the study were shared with them, ample time for review and questions was provided. Results of the study will be shared with patients and their families after publication.

RESULTS

Characteristics of patients newly diagnosed with CEGEJS compared with patients diagnosed between 2015 and 2019 in the Puget Sound

Between 01 January 2021 and 31 December 2022, 58 patients completed an appointment at Fred Hutch for a new diagnosis of CEGEJS, see figure 1. 43 patients were referred to our cancer genetics service, and one patient, who was given a diagnosis of laryngeal cancer extending into the upper oesophagus, was excluded. Median age at diagnosis was 59.5 years (range, 33–81 years) with 21 patients (50.0%) aged 30–59 years; 27 patients (64.3%) were male sex compared with 67.4% in our registry from 2015 to 2019; 29 patients (69.0%) were reported of white or European ancestry and 8 patients of Asian descent (19.0%) compared with 79.3% and 9.0%, respectively, in our registry (see table 1 and online supplemental figures S1 and S2).

Of these 42 patients, 14 (33.3%) had oesophageal cancer, 21 (50.0%) had gastric cancer and 26 (61.9%) had stage 3 or 4 disease at time of diagnosis compared with 41.9% in our registry (see online supplemental figure S2). 12 patients (28.6%) had a prior *Helicobacter pylori* infection, and 10 (23.8%) had Barrett's oesophagus. 13 patients (31.0%) had a previous primary cancer diagnosis with breast cancer being the most common prior cancer. Of the 39 patients (92.9%) who had a family history of cancer, 35 patients (81.0%) met the NCCN guideline for genetic testing for HBOC and/or for Lynch syndrome, and 24 patients would not have received germline testing around the time of CEGEJS diagnosis if not referred to cancer genetics through this study, see online supplemental figure S3. 37 patients had Medicare/Medicaid or Tricare, and 30 had a commercial or another insurance. Area Deprivation Index was collected in our payor claims data but not for our prospective cohort as zip codes were not recorded. It was six or greater for 197 patients (35.4%) when most of the inhabitants of the Puget Sound region have an Area Deprivation Index of 3 or lower, see online supplemental figure S2. All patients in our prospective cohort received treatment compared with 424 of 556 patients (76.3%) who received treatment in our registry (online supplemental figure S2). By 1 January 2024, 18 patients (42.9%) had died of complications of CEGEJS.

Tumour profiling and germline genetic results

Through our study, 39 out of 42 patients received tumour genetic testing, see table 2.

Six CEGEJS (14.3%) had microsatellite instability-high (MSI-H) and 28 (66.7%) were microsatellite stable

Table 1 Demographics and risk factors for gastro-oesophageal cancer in patients newly diagnosed with CEGEJS

Variable	N, population	% Population
Age (years)		
30–39	2	4.8%
40–49	7	16.7%
50–59	12	28.6%
60–69	10	23.8%
70–79	8	19.0%
80 and older	3	7.1%
Sex		
Female	15	35.7%
Male	27	64.3%
Race		
White/European	29	69.0%
African American/black	1	2.4%
Asian	8	19.0%
American Indian/Alaskan Native	0	0.0%
Native Hawaiian/Pacific Islander	0	0.0%
Other	2	4.8%
Unknown	1	2.4%
Declined to answer	1	2.4%
Ethnicity		
Hispanic/Latino	7	16.7%
Non-Hispanic/Latino	33	78.6%
Unknown	2	4.8%
Cancer type		
Oesophageal, ICD-10 Code C15	14	33.3%
Gastro-oesophageal Junction, ICD-10 Code C16.0	7	16.7%
Gastric, ICD-10 Code C16.1–9	21	50.0%
Stage		
I	4	9.5%
II	12	28.6%
III	5	11.9%
IV	21	50.0%
Past cancer diagnosis		
Yes	13	31.0%
No	29	69.0%
BMI at diagnosis		
BMI <25	18	42.9%
BMI 25–30	17	40.5%
BMI >30	7	16.7%
Smoking history		
Never	26	61.9%
Current	2	4.8%
Former	14	33.3%
Alcohol use		
Yes	20	47.6%
No	22	52.4%
Gastro Intestinal (GI) medical conditions		
Helicobacter pylori Infection	12	28.6%
Inflammatory condition	0	0.0%
Polyps	12	28.6%
Barrett's oesophagus	10	23.8%
Comorbidities	38	90.5%
Family history of cancer		
Yes	39	92.9%
Patients who met NCCN guidelines	34	81.0%
Patients identified by Oncology team without the study	10	23.8%
No	3	7.1%

BMI, body mass index; CEGEJS, cancers of the oesophagus, gastro-oesophageal junction and stomach; ICD, International Classification of Diseases; NCCN, National Comprehensive Cancer Network.

(MSS). Of the 6 CEGEJS with MSI-H, 3 patients had documented hypermethylation of the *MLH1* promoter, one had somatic biallelic inactivation of *MLH1*, one with somatic biallelic inactivation of *MSH6* and hypermethylation studies were cancelled at patient death for the last patient. All six had negative germline genetic testing. Six CEGEJS (14.3%) had a high Tumour Mutational Burden (TMB >5), TMB for them was between 9 mutations/Mb and 50 mutations/Mb. All 6 of them had concurrent MSI-H. We had no reported MSI status and TMB for 8 and 9 patients, respectively. Reasons for missing tumour profiling data included insufficient tumour content, lost to follow-up, second opinion at Fred Hutch and patient death. A CPS score was documented for 31 of the 42 CEGEJS (73.8%); 26 tumours had a CPS score >1 and 5 had a CPS score ≤1.

Most common somatic pathogenic variants (PVs) identified were in the gene *TP53* (53.1%, n=17) followed by *KRAS, GRAS* and *NRAS* grouped together (n=8, 25.0%), *HER2* (n=6, 18.8%) and *MLH1* promoter hypermethylation (n=5, 15.6%). Interestingly, 3 patients had a somatic PV in *PIK3CA*. One patient had gastro-oesophageal junction cancer and a *PIK3CA* c.1634 A>G (p.E545G) along with somatic biallelic inactivation of *PTEN* and *KRAS* c.175G>A (p.A59T). Two patients had gastric cancer, one with *PIK3CA* c.3140A >G (p.H1047R) and one with *PIK3CA* c.323G>A (p.R108H) and *KRAS* c.38G>A (p.G13D). Five patients (11.9%) had an amplification of *CCND1*, one in *CCNE1* and one in *CCND2*. No patients received a *KRAS* inhibitor such as sotorasib (Lumakras)

Table 2 Tumour and germline genetic testing results

Record ID	Organ type	Somatic mutations	MSI	TMB	Germline mutations	Follow-up testing
10	Oesophagus	FGFR2-TACC2 fusion, TP53 c.824G>A (p.C275Y), JAK3 c.475C>T (p.Q159*)	Stable	Low	Negative	No
16	Oesophagus	CSF3R c.1640G>A (p.W547*), ERBB2 and EGFR amplification	Stable	Low	Negative	No
17	Oesophagus	COG7-PLK1 and MRPS15-CSF3R rearrangements, deletion in CDKN2A	Stable	Low	Negative	No
20	Oesophagus	N/A	High	High	Negative	No
24	Oesophagus	TP53 c.422G>T (p.C141F), ARID1A c.5131_5132del (p.K1711Efs*16)	Stable	Low	Negative	No
25	Oesophagus	KRAS, ETV6, and CCND2 amplification, TP53 c.844C>T (p.R282W)	Stable	Low	Negative	No
29*	Oesophagus	2 PV in FANCA(1. exon 15-17del, and 2. c.1505dup (p.Y503Vfs*40)), TP53 c.949C>T (p.Q317*), CDKN2A c.247C>T (p.H83Y)	Stable	Low	FANCA(1. exon 15-17del, and 2. c.1505dup (p.Y503Vfs*40))	Yes
31	Oesophagus	N/A	Stable	N/A	Negative	No
34	Oesophagus	TP53 c.1024C>T (p.R342*), APC c.4666dup (p.T1556Nfs*3)	Stable	Low	Negative	No
37†	Oesophagus	BRCA2 c.9076C>T (p.Q3026*), TP53 c.637C>T (p.R213*), CDKN2A, CDKN2B, MTAP deletion, APC [1. c.7744G>T (p.E2582*), and 2. 65 bp del at exon 7-intron seven boundary)	Stable	Low	BRCA2 c.9076C>T (p.Q3026*)	Yes
40	Oesophagus	KRAS c.38_40dup (p.G13dup), TP53 c.797G>A (p.G266E), AXIN2 c.2406–2A>G, ANKRD26	Stable	Low	Negative	Yes
41	Oesophagus	N/A	N/A	N/A	Negative	Yes
42	Oesophagus	KRAS amplification, ERCC2 c.1972C>T (p.R658C), CCND1 amplification, MET, TP53 c.586C>T (p.R196*)	Stable	Low	Negative	Yes
43	Oesophagus	ERB2, ARID1B c.1543–2A>G, MPL, CDK12	Stable	Low	Negative	Yes
12	GEJ	KRAS and MYC amplification, ARID1A c.1459C>T (p.Q487*)	Stable	Low	VUS: CTNNA1 c.1726A>G (p.T576A)	No
15‡	GEJ	ATM mutation c.103C>T (p.R35*), MTOR c.6959A>T (p.Y2320F), CCND1 amplification	Stable	Low	ATM c.103C>T (p.R35*)	Yes
18§	GEJ	KRAS c.182A>T (p.Q61L), CDKN2A c.247C>T (p.H83Y) and BRCA1 c.68_69del (p.E23Vlfs*17), MDM2 amplification	Stable	Low	BRCA1 c.68_69del (p.E23Vlfs*17), ATM c.901+1G>T (splicing)	No
26	GEJ	TP53 c.438G>A (p.W146*), ARID1A c.1636C>T (p.Q546*), CCND1 amplification	Stable	Low	Negative	No

Continued

Table 2 Continued

Record ID	Organ type	Somatic mutations	MSI	TMB	Germline mutations	Follow-up testing
27	GEJ	NF1 c.4733C>T (p.S1578F), STK11 c.408_425del (p.M136_S142delinsI), TP53 c.155_164del (p.Q52Lfs*68); EGFR and KRAS amplification	Stable	Low	Negative	Yes
28	GEJ	N/A	N/A	N/A	Negative	No
30	GEJ	ERBB2 copy number gain	Stable	Low	Negative	No
1	Stomach	CDH1 (1. c.539C>T (p.S180F), and 2. c.689T>G (p.L230R)), FGFR2 amplification, JAK2 amplification, CDKN2A focal copy loss	Stable	Low	Negative	No
3	Stomach	PIK3CA c.3140A>G (p.H1047R)	High	High	Negative	No
4	Stomach	CDH1 c.1944_1952del (p.E648_I651delinsD)	N/A	N/A	Negative	No
5	Stomach	PMS2 c.1239dup (p.D414Rfs*44), ASXL2 c.2255C>A (p.P752H), MUTYH c.85C>T (p.Q29*), DICER1 c.5186C>T (p.P1729L)	High	High	Negative	No
6	Stomach	TP53 (42 bp deletion in exon 7)	N/A	N/A	Negative	No
7	Stomach	TP53 c.524G>A (p.R175H), RB1 c.1072C>T (p.R358*), MUTYH c.1187G>A (p.G396D)	Stable	Low	MUTYH c.1187G>A (p.G396D)	No
8	Stomach	TGFBR2 c.1658G>A (p.R553H)	Stable	Low	Negative	No
9	Stomach	CCND1 amplification	Stable	Low	VUS: ATM c.7375C>G (p.R2459G)	No
11	Stomach	HER2 amplification, TP53 c.844C>T (p.R282W)	Stable	Low	Negative	No
13	Stomach	PRKACA-DNAJB1 fusion, VUS: PMS2 c.755G>T (p.C252F)	Stable	Low	Negative	No
14	Stomach	TP53 c.638G>A (p.R213Q), MYC amplification	Stable	Low	VUS: STK11 c.608C>T (p.P203L)	Yes
19	Stomach	HER2 c.2524G>A (p.V842I)	High	High	Negative	No
21	Stomach	CDH1 (1. c.1008+1G>A 2. c.1320G>T) and TP53 c.844C>T (p.R282W), CCND1 amplification	Stable	Low	Negative	Yes
22	Stomach	CTNNA1, ARID1A (1. c.4624G>T (p.E1542*) and 2. c.5221G>T (p.E1741*)), TP53 c.782+1G>A	Stable	Low	Negative	No
23¶	Stomach	KRAS c.38G>A (p.G13D), FANCA c.216_217del (p.L72Ffs*7), PIK3CA c.323G>A (p.R108H), VUS: FANCI c.839 A>G (p.K280R)	High	High	FANCA c.216_217del (p.L72Ffs*7)	Yes
32	Stomach	N/A	N/A	Unknown	VUS: PDGFRA c.470C>T (p.T157I)	No
33	Stomach	BAP1 c.178C>T (p.R60*)	Stable	Low	Negative	No

Continued

Table 2 Continued

Record ID	Organ type	Somatic mutations	MSI	TMB	Germline mutations	Follow-up testing
35	Stomach	N/A	N/A	Unknown	Negative	No
36	Stomach	N/A	N/A	Unknown	Negative	Yes
38	Stomach	N/A	N/A	Unknown	Negative	Yes
39	Stomach	KRAS c.175G>A (p.A59T), PIK3CA c.1634A>G (p.E545G), PTEN (1. c.188del (p.N63Tfs*36) and 2. c.1034T>C (p.L345P))	High	High	Negative	No

*Deidentified details on cancer diagnosis and treatment course can be shared on request. Genetic testing revealed two germline *FANCA* pathogenic variants, one of which is well-characterised as a disease-causing variant in other patients with Fanconi Anemia.
†Deidentified details on cancer diagnosis and treatment course can be shared on request. Tumour profiling results were released after adjuvant treatment decision and were significant for biallelic inactivation of the gene *BRCA2* with one pathogenic variant of germline origin. The patient has no evidence of disease at 2 years.
‡Deidentified details on cancer diagnosis and treatment course can be shared on request. Pathogenic variant in the gene *ATM* was associated with loss of heterozygosity in the tumour. The University of Washington (UW) laboratory included this tumour sample in the validation of their assay measuring a homologous repair damage deficiency (dHRD) score by assessment of genome-wide burden of loss of heterozygosity.[43] dHRD score in the pretreated cancer was 21%, 5% above the laboratory's current threshold of 16% for a positive dHRD score suggesting at least a partial causative role for *ATM*. Further studies measuring dHRD in gastrointestinal tumours are needed as chromosome losses and gains are common in gastro-oesophageal junction cancers, and this may manifest as an elevated LOH score in the absence of HRD deficiency.
§Deidentified details on cancer diagnosis and treatment course can be shared on request. The patient had no evidence of disease at the 3-year mark, and screening for other cancers was negative. The University of Washington (UW) laboratory included this tumour sample in the validation of the assay described above. As the patient had a near complete response from neoadjuvant therapy, there was insufficient tumour content for HRD score analysis.
¶Deidentified details on cancer diagnosis and treatment course can be shared on request. The patient was found to have a germline *FANCA* pathogenic variant and a variant of uncertain significance in the gene *FANCI* with a Variant Allele Fraction of 49% on tumour profiling test. The patient died of progression of disease without further germline confirmation testing. We do not know the significance of results given that there are limited studies on the risk of developing solid malignancies in adults with Fanconi Anemia.
GEJ, gastro-oesophageal junction; HER2, Human Epidermal Growth Factor 2; MSI, microsatellite instability; TMB, Tumour Mutational Burden; VUS, variants of uncertain significance.

or a PIK3CA inhibitor such as alpelisib (Piqray). One was prescribed the CDK4/6 inhibitor abemaciclib (Verzenio) that was denied by the insurance. One patient was found to have an incidental PV in the gene *CSF3R* at variant allele fraction (VAF) of 37% that was suspected but not confirmed germline. *CSF3R* encodes the receptor for granulocyte-colony stimulating factor (G-CSF), is involved in myeloid cell differentiation, and this variant has been associated with lower *CSF3R* messenger RNA, receptor and response to G-CSF.[42] The patient received 5'florouracil based chemotherapy, required G-CSF when his absolute white count nadired below 0.5 and mounted a normal white blood cell count response.

Of 42 patients, 39 (92.8%) received germline genetic testing and three died prior to providing a sample. Six PVs were identified, 2 patients had PVs in genes associated with autosomal recessive conditions, 4 (9.5%) had one or more variants of uncertain significance (VUS), and 29 (69.0%) had negative results. Four patients had germline alterations in the homologous recombination DNA damage/repair pathway with PV in *BRCA2, ATM, BRCA1* and biallelic *FANCA*. One patient with oesophageal cancer before the age of 50 years had a tumour PV in the gene *ERCC2* called c.1972C>T (p.R658C) with loss of heterozygosity. There was no history of Xeroderma pigmentosum. One patient with gastric cancer had a PV in the gene *FANCA* called c.216_217del (p.L72Ffs*7) and a VUS in the gene *FANCI* called c.839 A >G was identified at VAF 49% on tumour testing. The finding in *FANCI* was not confirmed to be germline in origin. One patient with gastric cancer before the age of 50 years and their father with a history of gastric cancer shared the same VUS in the *PDGFRA* called c.470C>T (p.T157I), gene for which there are no functional assays to help clarify its significance. One patient with gastric oesophageal junction cancer had 3 VUSs, one in *CTNNA1* called c.1726A >G (p.T576A) which is at a highly evolutionarily conserved position but with limited population and functional data, one splice site variant in the gene *USP7* called c.1839+5G>A and one in the gene *FBXW7* called c.1076A >G (p.H359R). The gene *FBXW7* is a tumour suppressor gene known to be downregulated in gastric cancers. It is being evaluated as a marker for poor prognosis.[43] Of the 3 patients who couldn't receive paired testing, one patient was diagnosed with metastatic diffuse gastric adenocarcinoma with signet ring cells before the age of 40 years. Their tumour was sent to a tumour-only commercial laboratory, and an in-frame deletion in the gene *CDH1* called c.1747_1749del (p.L583del) was identified at 47.8% VAF and classified as a VUS. Given the high suspicion for hereditary diffuse gastric cancer syndrome, multiple attempts were made to follow-up without success.

Treatment and targeted therapies

Most patients received surgery alone or neoadjuvant chemotherapy and radiation before surgery when they were eligible. Molecular tumour profiling unlocked access to at least one adjuvant targeted therapy approved by the US Food and Drug Administration for 32 of the 42 patients (76.2%). Targeted therapy was known to be beneficial for 17 patients (40.5%) and potentially beneficial for 21 patients (50.0%) as efficacy was not established yet in gastro-oesophageal cancer but reported in other cancer types. An example of this was having an *FGFR2* amplification or a fusion with the potential benefit of erdafitinib (Balversa). Of the 42 patients, 31 patients (61.3%) had a CPS score documented. 19 of them received adjuvant immunotherapy; the tumours of 16 of the 26 patients (61.5%) had a CPS score >1, and 3 had a CPS score ≤1. 11 CEGEJS did not have a CPS score documented, and 6 patients (54.5%) received immunotherapy anyway. Overall, 24 patients (57.1%) received at least one targeted therapy such as pembrolizumab (Keytruda), nivolumab (Opdivo), trastuzumab (Herceptin) and ramucirumab (Cyramza) as part of their first line treatment. Should they need further therapy, 17 patients (40.5%) would be eligible for future clinical trials with a regimen containing a WEE1 kinase inhibitor given *TP53* tumour alterations.

DISCUSSION

In our study, we report on the clinical utility of paired normal-tumour genetic testing when performed for all patients newly diagnosed with CEGEJS. In 2021, the NCCN guideline encouraged screening CEGEJS with multiple biomarker tests for eligibility for targeted therapies as part of the standard of care for patients with an advanced diagnosis.[17 22] Biomarker testing included testing for HER2 overexpression to prompt considering treatment with trastuzumab,[44] testing for MSI or MMR deficiency and PD-L1 to prompt eligibility for adjuvant immune checkpoint inhibitors[45] and testing with next generation sequencing panel, when possible, for eligibility to receive novel tyrosine kinase inhibitors. More than 75% of patients who received testing in our study were eligible for a targeted therapy regardless of their stage at diagnosis. Six patients who received trastuzumab had HER2 overexpression in their tumours. Almost three quarters of CEGEJS cases were submitted for a CPS score. 26 patients had a CPS score >1 and only 16 patients received immunotherapy. For the remaining 9 patients, the benefit of immunotherapy was unknown given absent CPS score or CPS score ≤1. Furthermore, a quarter of our patients were found eligible for a novel targeted therapy based on our paired testing that went beyond what is recommended by the NCCN guidelines. Neither CDK4-CDK6 inhibitors nor PIK3CA inhibitors have approval for CEGEJS today. Our data highlight the importance of improving access and utilisation of normal-tumour genetic testing for every CEGEJS to guide treatment decision making[21] and to identify better treatment options in the future.

We identified six germline PVs in high-risk genes that would change patients' eligibility for clinical trials and screening and early detection for their at-risk relatives. Five additional findings were suspicious but lacked either functional data or further work up (*CSF3R, CTNNA1,*

PDGFRA, *FANCI* and *CDH1*). More than 80% of patients in our cohort met the HBOC and/or the Lynch syndrome guideline for germline genetic testing. We expected that more patients with CEGEJS would meet the NCCN guidelines for genetic testing for Lynch syndrome, given it is associated with a stronger risk of upper gastrointestinal malignancy compared with HBOC. Of those meeting criteria, less than a third would have been offered germline genetic testing at CEGEJS diagnosis without this study. Still, the number of genetic tests ordered by oncologists was significantly higher than what was found in our retrospective payor data. Less than 2% of patients with CEGEJS, diagnosed between 2015 and 2019 in the Puget Sound region, had any claims for genetic counseling and/or testing. For those who did, they all met the eligibility criteria based on the documented personal or family history. Receipt of genetic counseling in CEGEJS was likely significantly under-reported in the claims data, given that: (1) many patients with CEGEJS do not need to see a genetic counselor to obtain genetic testing through their oncologist or a research study and (2) genetic counseling is not always billable or billed as a service. Findings from this cohort align with other research, showing that 1 in 6 patients with CEGEJS have an actionable hereditary cancer syndrome.[46] As more data highlight the prevalence of inherited cancer predispositions for patients with CEGEJS, the NCCN guidelines have updated their recommendations for germline genetic testing. Adding broader guidance on the appropriateness of germline genetic testing for each organ or listing the high-yield and actionable genes in each cancer type may help increase testing uptake. Point-of-care genetic testing may also accelerate the timely identification of patients and relatives with an actionable hereditary cancer syndrome and guide screening for at-risk relatives when they are in a window of opportunity for risk reduction or early detection.

Lastly, it is difficult to know for sure whether the hereditary genetic testing we provide for CEGEJS today is comprehensive. We assume that all cancers develop mutations in the same DNA repair, growth factors and cell cycle pathways. It is possible, however, that inherited alterations in pathways that repair damage caused by alcohol or immunodeficiency that prevent healing from chronic inflammation play a role in carcinogenesis for CEGEJS. The BROCA panel test, for example, did not cover the gene *RHBDF2* known to cause autosomal dominant tylosis with oesophageal cancer syndrome, making even this expert test an incomplete genetic evaluation for CEGEJS. Gain-of-function PVs in *RHBDF2* are associated with sustained *EGFR* signalling and dysregulated wound healing in the epidermis and non-keratinised epithelium of the upper gastrointestinal tract.[47 48] No patients in our study presented with characteristic features of palmoplantar keratoderma, oral lesions or recurring oesophageal strictures, lowering the probability of detection and causing us to miss this extremely rare diagnosis. Understanding interactions between genetic predispositions affecting chronic healing or repair from environmental exposures would bring powerful insights for cancer treatment and early detection in the future.

Limitations of our project include studying a small sample at one large cancer centre, a short study period during the COVID-19 pandemic, many patients being of white or European ancestry and our claims and SEER data including 13 but not all 39 counties of the state of Washington. It is possible we would have identified additional genetic, personal or environmental risk factors if the study was performed in a broader group of patients of Chinese or Japanese ancestry. Further studies are also needed to understand novel monogenic causes vs polygenic risk markers for CEGEJS, along with the interaction between genetic factors and environmental exposures that increase the risk of developing CEGEJS. A subset of patients with a new CEGEJS eligible for the study was not offered participation. Reasons for why 16 patients were not referred to our study are unknown. We hypothesise that they were not included because: they were diagnosed before 01 January 2021 and came for follow-up care without updated diagnosis codes (from diagnosis of cancer to history of cancer); they had a second opinion but did not establish care; they declined a referral or died before being scheduled; they had testing already or the biopsy was sent to another laboratory for tumour testing among other reasons. We noticed that patients with CEGEJS were referred more often by our main campus oncologists (88.1%, n=37) compared with our community oncologists (11.9%, n=5). Finally, many patients came to the clinic with advanced stage, poor nutritional status and many died before being able to complete their genetic test. Having the ability to store a patient's DNA in a Clinical Laboratory Improvement Amendments-certified biobank for the future would permit completion of clinical hereditary testing later for the benefit of at-risk relatives.

CONCLUSION

Our study highlights the yield and downstream impact of paired normal-tumour genetic testing in patients with CEGEJS. Identifying biomarkers unlocked targeted therapeutic options for most of our patients and we hope they will derive improved survival outcomes from these therapies. Uncovering a hereditary cancer syndrome in patients with CEGEJS also allowed for cascade testing, tailored screening, risk reduction and early detection for a broad range of cancers for family members. Further research is needed in the stratification of the risk to develop CEGEJS, genetic modifiers of risk, response to targeted therapy and novel blood-based disease recurrence surveillance tools.

X Marianne E Dubard-Gault @DrDubardGault

Acknowledgements We want to thank all the patients and families who participated in this study. We also want to thank Catherine Fedorenko and Winona Wright from HICOR and Lauren Santos, MSIM, for helping coordinate the implementation of this project in 2021.

Contributors KT, SC, MYL and MED-G designed the concept for study. LVN, MYL and MED-G prepared our site for this project. SC, LVN, CLH, VS, BO, WMG, BS, EL, LF and MED-G helped with patient care and consent. QS performed the primary analysis from the SEER data for the Puget Sound region. AJ, EQK and CP analysed genetic test results and AJ, EQK, CP and MED-G reviewed and interpreted all genetic results. KT and MED-G completed data analysis. All authors participated in developing the manuscript; all edited and approved the final version of the manuscript. MED-G is the guarantor for this study.

Funding This work was funded by the Brotman Baty Institute for Precision Medicine.

Map disclaimer The depiction of boundaries on this map does not imply the expression of any opinion whatsoever on the part of BMJ (or any member of its group) concerning the legal status of any country, territory, jurisdiction or area or of its authorities. This map is provided without any warranty of any kind, either express or implied.

Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, conduct, reporting or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and the study was approved by the IRB of the University of Washington with IRB number: 11490. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Data requests should be submitted to the corresponding author.

ORCID iDs
Stacey Cohen http://orcid.org/0000-0002-6207-9856
Marianne E Dubard-Gault http://orcid.org/0000-0002-5735-1819

REFERENCES

1 SEER. Cancer of the esophagus - cancer stat facts. n.d. Available: https://seer.cancer.gov/statfacts/html/esoph.html
2 SEER. Cancer of the stomach - cancer stat facts. n.d. Available: https://seer.cancer.gov/statfacts/html/stomach.html
3 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020;70:7–30.
4 Blaydon DC, Etheridge SL, Risk JM, *et al*. RHBDF2 mutations are associated with tylosis, a familial esophageal cancer syndrome. *Am J Hum Genet* 2012;90:340–6.
5 Cercek A, Lumish M, Sinopoli J, *et al*. PD-1 Blockade in Mismatch Repair-Deficient, Locally Advanced Rectal Cancer. *N Engl J Med* 2022;386:2363–76.
6 Practice-changing keynote-177 results support first-line pembrolizumab monotherapy for MSI-H mCRC. Available: https://dailynews.ascopubs.org/do/10.1200/ADN.20.200206/full/?cid=DM5445 [Accessed 31 May 2020].
7 Le DT, Uram JN, Wang H, *et al*. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 2015;372:2509–20.
8 Overman MJ, McDermott R, Leach JL, *et al*. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol* 2017;18:1182–91.
9 Le DT, Durham JN, Smith KN, *et al*. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 2017;357:409–13.
10 Dudley JC, Lin MT, Le DT, *et al*. Microsatellite Instability as a Biomarker for PD-1 Blockade. *Clin Cancer Res* 2016;22:813–20.
11 Stadler ZK, Maio A, Chakravarty D, *et al*. Therapeutic Implications of Germline Testing in Patients With Advanced Cancers. *J Clin Oncol* 2021;39:2698–709.
12 Marabelle A, Le DT, Ascierto PA, *et al*. Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair-Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. *J Clin Oncol* 2020;38:1–10.
13 Wang Y, Zheng K, Huang Y, *et al*. PARP inhibitors in gastric cancer: beacon of hope. *J Exp Clin Cancer Res* 2021;40:211.
14 Kim T-Y, Yoon J, Lee D-W, *et al*. A phase II biomarker-oriented study to evaluate paclitaxel, olaparib, and durvalumab combination in patients with advanced gastric cancer. *JCO* 2023;41:404.
15 Samadder NJ, Riegert-Johnson D, Boardman L, *et al*. Comparison of Universal Genetic Testing vs Guideline-Directed Targeted Testing for Patients With Hereditary Cancer Syndrome. *JAMA Oncol* 2021;7:230–7.
16 Islami F, DeSantis CE, Jemal A. Incidence Trends of Esophageal and Gastric Cancer Subtypes by Race, Ethnicity, and Age in the United States, 1997-2014. *Clin Gastroenterol Hepatol* 2019;17:429–39.
17 National Comprehensive Cancer Network. Esophageal and esophagogastric junction cancer. Available: https://www.nccn.org/professionals/physician_gls/pdf/esophageal.pdf [Accessed 14 Aug 2020].
18 Ford JM. Hereditary Gastric Cancer: An Update at 15 Years. *JAMA Oncol* 2015;1:16–8.
19 Orloff M, Peterson C, He X, *et al*. Germline mutations in MSR1, ASCC1, and CTHRC1 in patients with Barrett esophagus and esophageal adenocarcinoma. *JAMA* 2011;306:410–9.
20 Tofani CJ, Gandhi K, Spataro J, *et al*. Esophageal adenocarcinoma in a first-degree relative increases risk for esophageal adenocarcinoma in patients with Barrett's esophagus. *United European Gastroenterol J* 2019;7:225–9.
21 Moore K, Colombo N, Scambia G, *et al*. Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. *N Engl J Med* 2018;379:2495–505.
22 National Comprehensive Cancer Network. Gastric cancer. Available: https://www.nccn.org/professionals/physician_gls/pdf/gastric.pdf [Accessed 14 Aug 2020].
23 van Nistelrooij AMJ, Dinjens WNM, Wagner A, *et al*. Hereditary Factors in Esophageal Adenocarcinoma. *Gastrointest Tumors* 2014;1:93–8.
24 van Nistelrooij AMJ, van Marion R, van Ijcken WFJ, *et al*. Germline variant in MSX1 identified in a Dutch family with clustering of Barrett's esophagus and esophageal adenocarcinoma. *Fam Cancer* 2018;17:435–40.
25 Masciari S, Dewanwala A, Stoffel EM, *et al*. Gastric cancer in individuals with Li-Fraumeni syndrome. *Genet Med* 2011;13:651–7.
26 Cunniff C, Djavid AR, Carrubba S, *et al*. Health supervision for people with Bloom syndrome. *Am J Med Genet A* 2018;176:1872–81.
27 Latham A, Srinivasan P, Kemel Y, *et al*. Microsatellite Instability Is Associated With the Presence of Lynch Syndrome Pan-Cancer. *J Clin Oncol* 2019;37:286–95.
28 Erratum: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2020;70:313.
29 GBD 2017 Stomach Cancer Collaborators. The global, regional, and national burden of stomach cancer in 195 countries, 1990-2017: a systematic analysis for the Global Burden of Disease study 2017. *Lancet Gastroenterol Hepatol* 2020;5:42–54.
30 van Zeeburg HJT, Snijders PJF, Wu T, *et al*. Clinical and molecular characteristics of squamous cell carcinomas from Fanconi anemia patients. *J Natl Cancer Inst* 2008;100:1649–53.
31 National Comprehensive Cancer Network. Gastric cancer and esophageal and esophagogastric junction cancer. Available: https://www.nccn.org/professionals/physician_gls/pdf/gastric.pdf [Accessed 14 Aug 2020].
32 Krumm N, Khasnavis NS, Radke M, *et al*. Diagnosis of Ovarian Carcinoma Homologous Recombination DNA Repair Deficiency From Targeted Gene Capture Oncology Assays. *JCO Precis Oncol* 2023;7:e2200720.
33 Kind AJH, Buckingham WR. Making Neighborhood-Disadvantage Metrics Accessible - The Neighborhood Atlas. *N Engl J Med* 2018;378:2456–8.

34 Broca cancer risk panel. n.d. Available: https://testguide.labmed.uw.edu/view/BROCA
35 UW Oncoplex cancer gene panel. n.d. Available: https://testguide.labmed.uw.edu/view/OPX
36 Lin GA, Trosman JR, Douglas MP, *et al*. Influence of payer coverage and out-of-pocket costs on ordering of NGS panel tests for hereditary cancer in diverse settings. *J Genet Couns* 2022;31:130–9.
37 Grant P, Langlois S, Lynd LD, *et al*. Out-of-pocket and private pay in clinical genetic testing: A scoping review. *Clin Genet* 2021;100:504–21.
38 Salipante SJ, Scroggins SM, Hampel HL, *et al*. Microsatellite instability detection by next generation sequencing. *Clin Chem* 2014;60:1192–9.
39 Bartley AN, Washington MK, Colasacco C, *et al*. HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma: Guideline From the College of American Pathologists, American Society for Clinical Pathology, and the American Society of Clinical Oncology. *J Clin Oncol* 2017;35:446–64.
40 Sun J, Bai YK, Fan ZG. Role of FBXW7 expression in gastric cancer: Meta-analysis and bioinformatics analysis. *Oncol Lett* 2023;25:184.
41 Yamashita K, Iwatsuki M, Harada K, *et al*. Prognostic impacts of the combined positive score and the tumor proportion score for programmed death ligand-1 expression by double immunohistochemical staining in patients with advanced gastric cancer. *Gastric Cancer* 2020;23:95–104.
42 Liu Y-Z, Hodgson D, Locker G, *et al*. Olaparib plus paclitaxel sensitivity in biomarker subgroups of gastric cancer. *Ann Oncol* 2018;29:viii25–6.
43 Trottier AM, Druhan LJ, Kraft IL, *et al*. Heterozygous germ line CSF3R variants as risk alleles for development of hematologic malignancies. *Blood Adv* 2020;4:5269–84.
44 University of Wisconsin School of Medicine Public Health. 2015 area deprivation index v2.0. 2015. Available: https://www.neighborhoodatlas.medicine.wisc.edu/
45 Grant P, Cook CB, Langlois S, *et al*. Evaluation of out-of-pocket pay genetic testing in a publicly funded healthcare system. *Clin Genet* 2023;103:424–33.
46 Bannon SA, DiNardo CD. Hereditary Predispositions to Myelodysplastic Syndrome. *Int J Mol Sci* 2016;17:838.
47 Uson PLS Jr, Kunze KL, Golafshar MA, *et al*. Germline Cancer Testing in Unselected Patients with Gastric and Esophageal Cancers: A Multi-center Prospective Study. *Dig Dis Sci* 2022;67:5107–15.
48 Brooke MA, Etheridge SL, Kaplan N, *et al*. iRHOM2-dependent regulation of ADAM17 in cutaneous disease and epidermal barrier function. *Hum Mol Genet* 2014;23:4064–76.