Cancer Genetics 216-217 (2017) 111–119

Cancer Genetics

ORIGINAL ARTICLE

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

Thomas Slavin [a,b,*], Susan L. Neuhausen [c], Christina Rybak [a,1], Ilana Solomon [a], Bita Nehoray [a], Kathleen Blazer [a], Mariana Niell-Swiller [a,2], Aaron W. Adamson [c], Yate-Ching Yuan [d], Kai Yang [a], Sharon Sand [a], Danielle Castillo [a], Josef Herzog [a], Xiwei Wu [d], Shu Tao [d], Tanya Chavez [a], Yanghee Woo [e], Joseph Chao [b], Pamela Mora [f], Darling Horcasitas [g], Jeffrey Weitzel [a,b]

[a] *Division of Clinical Cancer Genomics, City of Hope National Medical Center, 1500 E. Duarte Rd., Bldg 173, Duarte, CA 91010;* [b] *Department of Medical Oncology & Therapeutics Research, City of Hope National Medical Center, Duarte, CA 91010;* [c] *Department of Population Sciences, Beckman Research Institute of City of Hope, City of Hope National Medical Center, 1500 E. Duarte Rd., Duarte, CA 91010;* [d] *Department of Molecular Medicine, Beckman Research Institute of City of Hope, City of Hope National Medical Center, 1500 E. Duarte Rd., Duarte, CA 91010;* [e] *Division of Surgical, City of Hope National Medical Center, 1500 E. Duarte Rd., Duarte, CA 91010;* [f] *Instituto Nacional de Enfermedades Neoplásticas, Av. Angamos Este 2520, Surquillo 15038, Peru;* [g] *New Mexico Cancer Center, 4901 Lang Avenue NE, Albuquerque, NM 87109*

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

Received May 17, 2017; received in revised form August 4, 2017; accepted August 6, 2017.

* Corresponding author.
E-mail address: tslavin@coh.org

[1] Ms. Rybak's present address is Human Longevity Inc. 4570 Executive Dr. San Diego, CA 92121.

[2] Mrs. Niell-Swiller's present address is Dyson Center for Cancer Care 45 Reade Pl. Poughkeepsie, NY 12601.

2210-7762/$ - see front matter © 2017 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.cancergen.2017.08.001

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) (Supplementary Table S1) (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) (Supplementary Table S1) (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 Health Insurance Portability and Accountability Act (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 (Supplementary Table S1). 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 100×. 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