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## Germline Determinants of Esophageal Adenocarcinoma

Minyi Lee, B.S.<sup>1,2,†</sup>, George Eng, M.D., Ph.D.<sup>1,3,4,†</sup>, Anna Handte-Reinecker, B.S.<sup>1</sup>,  
MGH-MIT Gastrointestinal Cohorts Working Group,

Vikram S. Deshpande, M.B.B.S.<sup>5</sup>, Omer H. Yilmaz, M.D., Ph.D.<sup>3,4,6</sup>, Manish K. Gala, M.D.<sup>1,7,\*</sup>

<sup>1</sup>Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Boston, MA

<sup>2</sup>M.D.-Ph.D Program, Boston University School of Medicine, Boston, MA

<sup>3</sup>Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA

<sup>4</sup>David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA

<sup>5</sup>Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA

<sup>6</sup>Department of Biology, Massachusetts Institute of Technology, Cambridge, MA

<sup>7</sup>Clinical and Translational Epidemiology Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA

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The risk of progression for Barrett’s esophagus (BE) is estimated to range from 0.12% to 0.5% per year.<sup>1</sup> Identification of clinical risk factors such as age, sex, obesity, smoking, presence of hiatal hernia, and length of BE, are insufficient to wholly account for the few individuals who progress from BE to adenocarcinoma.<sup>2</sup> To explain some of the unaccounted risk, we hypothesized that a significant fraction of individuals with BE who progress to adenocarcinoma harbor pathogenic germline mutations in cancer predisposing genes.

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\* **Corresponding Author:** Dr. Manish K. Gala, MD, 55 Fruit Street, GRJ 708, Boston, MA 02114, mgala@mgh.harvard.edu, Phone: 617-726-7892, Fax: 978-882-6710.

† Denotes shared first authorship

# Author is recently deceased

**Author Contributions:** **Minyi Lee:** acquisition of data, analysis and interpretation of data, drafting of the manuscript, statistical analysis; **George Eng:** acquisition of data, analysis and interpretation of data, drafting of the manuscript; **Anna Handte-Reinecker:** acquisition of data, drafting of the manuscript; **Alvin Jeon:** acquisition of the data, analysis and interpretation of the data; **Eugeniu Stratulat:** acquisition of the data; **Rachid Zagani:** acquisition of the data; **Martin Taylor:** acquisition of the data, analysis and interpretation of the data, drafting of the manuscript; **Steffen Rickelt:** acquisition of the data; **Dmitriy Kedrin:** analysis and interpretation of the data; **Anupam Batra:** acquisition of the data; **Ardeshir Hashmi:** acquisition of the data, study supervision; **Norman Nishioka:** acquisition of the data; **Chin Hur:** acquisition of the data, drafting of the manuscript; **Lipika Goyal:** acquisition of the data; **Fateh Bazerbachi:** acquisition of the data, drafting of the manuscript; **Lawrence Zukerberg:** acquisition of the data, analysis and interpretation of the data; **Vikram Deshpande:** acquisition of the data, analysis and interpretation of the data; **Omer Yilmaz:** technical or material support, study supervision; **Manish Gala:** study concept and design, analysis and interpretation of data, statistical analysis, drafting of the manuscript, obtained funding, study supervision.

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We examined the prevalence of monoallelic, pathogenic germline mutations associated with moderate to high risk of cancer in 640 study participants with esophageal adenocarcinoma (EAC) enrolled in publicly available genomic cohorts that performed either whole genome sequencing (ICGC-ARGO) or whole-exome sequencing (TCGA Pan-Cancer Cohort, Broad Institute Esophageal Adenocarcinoma Cohort, and Memorial Sloan Kettering Prospective Clinical Cohort, Figure 1A).<sup>3–6</sup> Pathogenic germline mutations were discovered in 59 out of 640 individuals (9.2%, Figure 1B, Supplementary Table 1). *ATM* was the most frequently mutated gene, occurring in 10 individuals (1.6%) followed by *CHEK2* (1.25%). Five individuals (0.8%) harbored germline mutations in *TP53*. Two individuals (0.3%) harbored distinct, splice-donor mutations in *CDHI* at intron 10. Despite this prevalence, somatic coding mutations that represent likely loss-of-heterozygosity events, were only present in 3/60 tumors (5.0%, 1 *BRCA2*- and 2 *TP53* mutation carriers).

As validation, we performed germline WES on prospective cohorts at Massachusetts General Hospital that encompass BE progressors who developed high-grade dysplasia or intramucosal carcinoma (102 individuals), BE without progression to dysplasia over 10+ years (75 individuals), and healthy nonagenarians without any prior known history of gastrointestinal neoplasia (100 individuals). Again, germline *ATM* mutations were the most frequent pathogenic alteration, occurring in 2% and 2.7% of progressors and non-progressors (short-segment BE), respectively. Despite the lack of enrichment of *ATM* carriers among progressors, immunohistochemistry demonstrated loss of ATM staining among progressors and retained expression among non-progressors, implying epigenetic mechanisms for LOH (Supplementary Figure 1A).

Across all HGD/EAC cohorts, the prevalence of germline mutations in genes associated with monoallelic cancer predisposition within the Fanconi Anemia pathway (*BRCA2*, *PALB2*, *BRIP1*, *RAD51C*, *FANCA*, *FANCC*, *FANCM*) demonstrated enrichment over the carrier rate for all Fanconi Anemia genes in the general population (overall 2.3% vs. 0.6%). The age at diagnosis of those with high-grade dysplasia or adenocarcinoma did not differ between those with or without any germline mutations (Figure 1C).

Given this enrichment of pathogenic germline mutations in progressors, we examined if such germline alterations could influence the somatic mutanome. We examined the association of germline mutations with the development of pathogenic somatic *TP53* alterations, since such alterations have been associated with BE progression and genome doubling events (Figure 1D).<sup>7</sup> Pathogenic, somatic *TP53* mutations were detected among 75% of tumor exomes and 70% of tumor genomes. When stratified by somatic *TP53* mutant status, pathogenic germline mutations were present in 16.7% of cancer exomes with wild-type *TP53* versus 7.2% with *TP53* mutations (OR 2.6, 95% C.I. 0.9–6.8,  $P = 0.04$ , Fisher's exact test). Among cancer genomes, germline mutations were present in 15.6% of cases with wild-type *TP53* versus 6.1% of *TP53* mutants (OR 2.8, 95% C.I. 1.3–6.2,  $P = 0.004$  Fisher's exact test).

To examine if the overall enrichment of germline mutations among *TP53* wildtype tumors is driven by select genes, we stratified somatic *TP53* mutant status by each cancer-predisposing gene (Figure 1E). *ATM* germline mutations demonstrated 100% mutual exclusivity with pathogenic somatic *TP53* mutations (OR 0, 95% CI 0–0.2,  $P = 2.9 \times$

$10^{-6}$ , Fisher's exact test). We validated this mutual exclusivity with an independent cohort of 475 publicly available and non-redundant gastroesophageal adenocarcinomas previously sequenced on the MSK-IMPACT platform, with 7/7 *ATM* carriers harboring wild-type *TP53* (Supplementary Figure 1B). Exclusion of *ATM* carriers still demonstrated a persistent enrichment of germline mutations among *TP53* wildtype tumors, occurring in 10.1% and 12.1% of exomes and genomes, respectively.

Pathogenic germline *BRCA2* mutations also demonstrated a trend toward mutual exclusivity with *TP53* mutation (OR 0.2, 95% C.I. 0.2–1.4,  $P = 0.06$ , Fisher's exact test). Given the strong association of homologous recombination deficiency with somatic *TP53* mutations, we examined HRD status from tumor genomes utilizing the HRDetect algorithm. We observed HRD present in only 14/400 (3.5%) of tumor whole genomes, with only 1/4 *BRCA2* carriers demonstrating HRD (Supplementary Figure 1C). Among tumor exomes with either *BRCA2* or *PALB2* germline alterations, no samples demonstrated dominance by the single base substitution signature associated with HRD (Sig3, Supplementary Figure 1D).

Among 742 individuals with BE with HGD or EAC, we identified pathogenic germline mutations in monoallelic, cancer-predisposing genes among 9.0% of participants, compared to 2.7% of non-progressors. This overall enrichment suggests that these mutations facilitate the progression of Barrett's esophagus to adenocarcinoma. The ages of onset for those with germline mutations did not cluster among earlier-onset cases but occurred throughout the age spectrum, implying that these inherited mutations may require the development of BE and additional environmental factors as prerequisites to promote esophageal carcinogenesis.

Somatic *TP53* alterations have been identified as a key driver in the progression of nondysplastic Barrett's esophagus to dysplasia, functioning as a checkpoint for genome doubling events and chromosomal instability.<sup>7</sup> Validating its role as a key driver of progression, we did observe an overrepresentation of germline *TP53* mutations (0.7% among progressors). However, 25–30% of esophageal adenocarcinomas lacked somatic alterations in *TP53*. We discovered that such *TP53* wild-type tumors were significantly enriched for pathogenic germline mutations compared to *TP53*-mutant cancers (overall 15.9% vs. 6.6%, OR 2.7, 95% CI 1.5–4.8,  $P = 4.2 \times 10^{-4}$ , Fisher's exact test). This enrichment implies an early and causative role for even heterozygous germline mutations in BE progression since they can obviate the selection pressures for the acquisition of somatic *TP53* coding mutations. Multiple studies have demonstrated that heterozygosity of cancer predisposing genes can promote genomic instability.<sup>8,9</sup> Genome-wide association studies have quantified moderate effects associated with rare, heterozygous germline mutations.

Genetic testing has been recommended for all individuals diagnosed with pancreatic adenocarcinoma, where the prevalence of germline mutations is 7–10% and second hit mutations are uncommon.<sup>10</sup> Given the similar prevalence in EAC, universal genetic testing should be considered.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Alvin Jeon, M.D.<sup>1</sup>, Eugeniu Stratulat, M.D.<sup>1</sup>, Rachid Zagani, Ph.D.<sup>1</sup>, Martin S. Taylor, M.D., Ph.D.<sup>2</sup>, Steffen Rickelt, Ph.D.<sup>3</sup>, Dmitriy Kedrin, M.D., Ph.D.<sup>1</sup>, Anupam Batra, M.D.<sup>4,5,6</sup>, Ardeshir Hashmi, M.D.<sup>4,7</sup>, Norman S. Nishioka, M.D.<sup>1</sup>, Chin Hur, M.D., M.P.H.<sup>1,8,9</sup>, Lipika Goyal, M.D.<sup>10</sup>, Fateh Bazerbachi, M.D.<sup>1,11</sup>, Lawrence Zukerberg, M.D.<sup>2#</sup>

1. Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Boston, MA
2. Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA
3. David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA
4. Massachusetts General Hospital Division of Senior Health and Harvard Medical School, Boston, MA
5. Department of Oncology, Cambridge Memorial Hospital, Cambridge, Ontario, Canada
6. Department of Oncology, Grand River Regional Cancer Center, Kitchener, Ontario, Canada
7. Center for Geriatric Medicine, Cleveland Clinic, Cleveland, OH, USA
8. Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY
9. Division of Digestive and Liver Diseases, Columbia University Vagelos School of Medicine, New York, New York
10. Division of Oncology, Massachusetts General Hospital Cancer Center and Harvard Medical School, Boston, MA
11. Interventional Endoscopy Program, CentraCare and St. Cloud Hospital, St. Cloud, MN
12. Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA
13. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA
14. Clinical and Translational Epidemiology Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA

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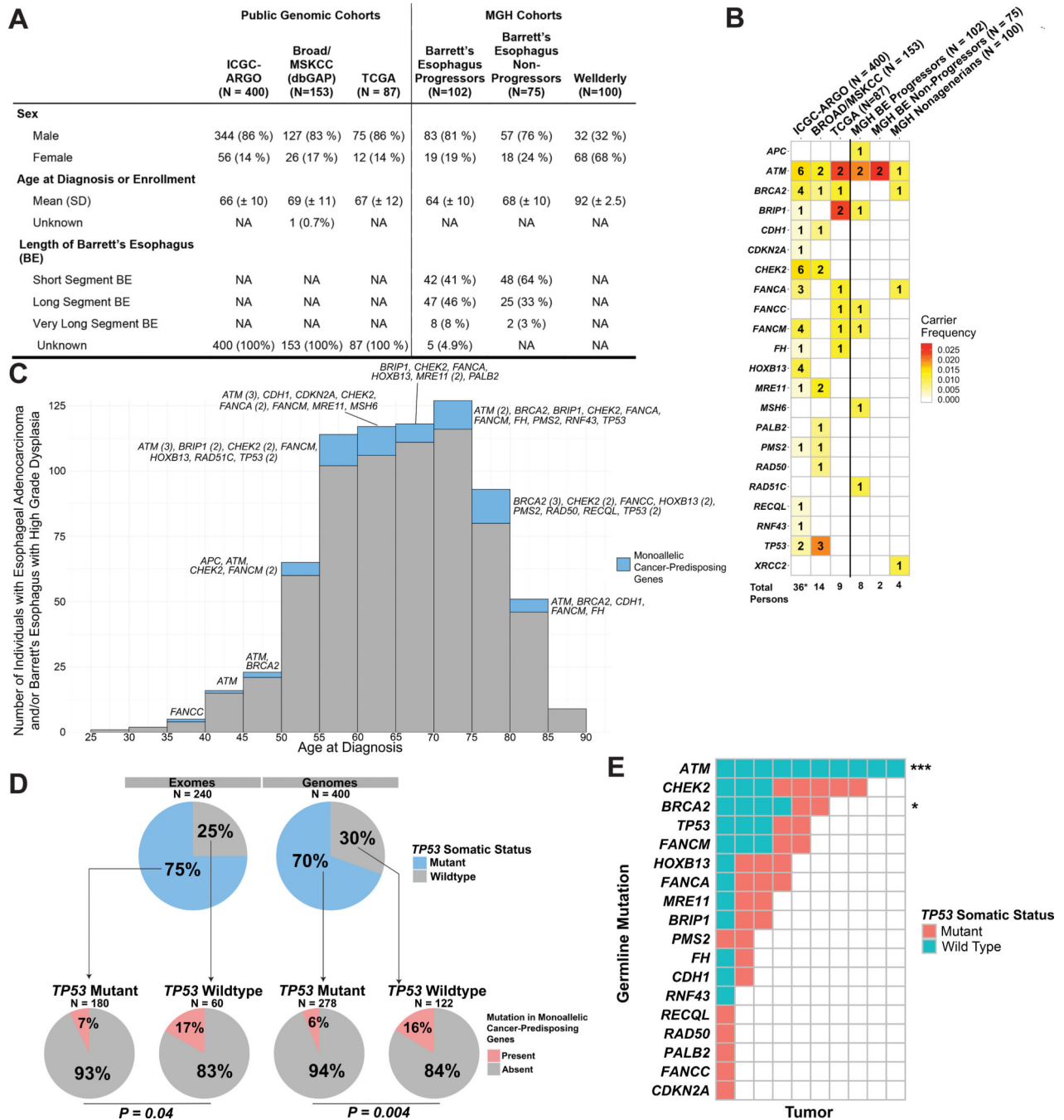
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### Abbreviations:

<b>BE</b>	Barrett's esophagus
<b>HGD</b>	high-grade dysplasia
<b>HRD</b>	homologous recombination deficient
<b>HRR</b>	homologous recombination repair
<b>MGH</b>	Massachusetts General Hospital

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**Figure 1: Germline Mutational Landscape Across Esophageal Adenocarcinoma.** (A) Clinical characteristics of study participants from public genomic and MGH cohorts. ICGC-ARGO refers to International Cancer Genome Consortium Project Accelerating Research in Genomic Oncology; Broad/MSKCC Cohort refers to the pooled public exomes of esophageal adenocarcinoma available on dbGAP; TCGA refers to The Cancer Genome Atlas; Welllderly refers to healthy nonagenarians without history of gastrointestinal neoplasia. (B) Number of pathogenic mutations itemized by cancer-predisposing genes across multiple cohorts. Color-coding of entries demonstrates carrier-frequency in their

respective cohorts. **(C)** Histogram showing the age at diagnosis of Barrett's esophagus with high-grade dysplasia or esophageal adenocarcinoma. Mutation carriers and non-carriers are color-coded by blue and gray, respectively. **(D)** Correlation of germline pathogenic mutations with somatic *TP53* status in tumors, segregated by exomes and genomes. **(E)** Correlations between individual genes mutated in the germline and somatic *TP53* status. \*\*\* designates  $P < 0.001$  and \* designates  $P = 0.06$ .