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Supplementary information

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# Integrated epidemiological and molecular data inform the relationship between precancer and cancer states of esophageal adenocarcinoma

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Supplemental Information for manuscript entitled, "Integrated epidemiological and molecular data inform the relationship between precancer and cancer states of oesophageal adenocarcinoma"

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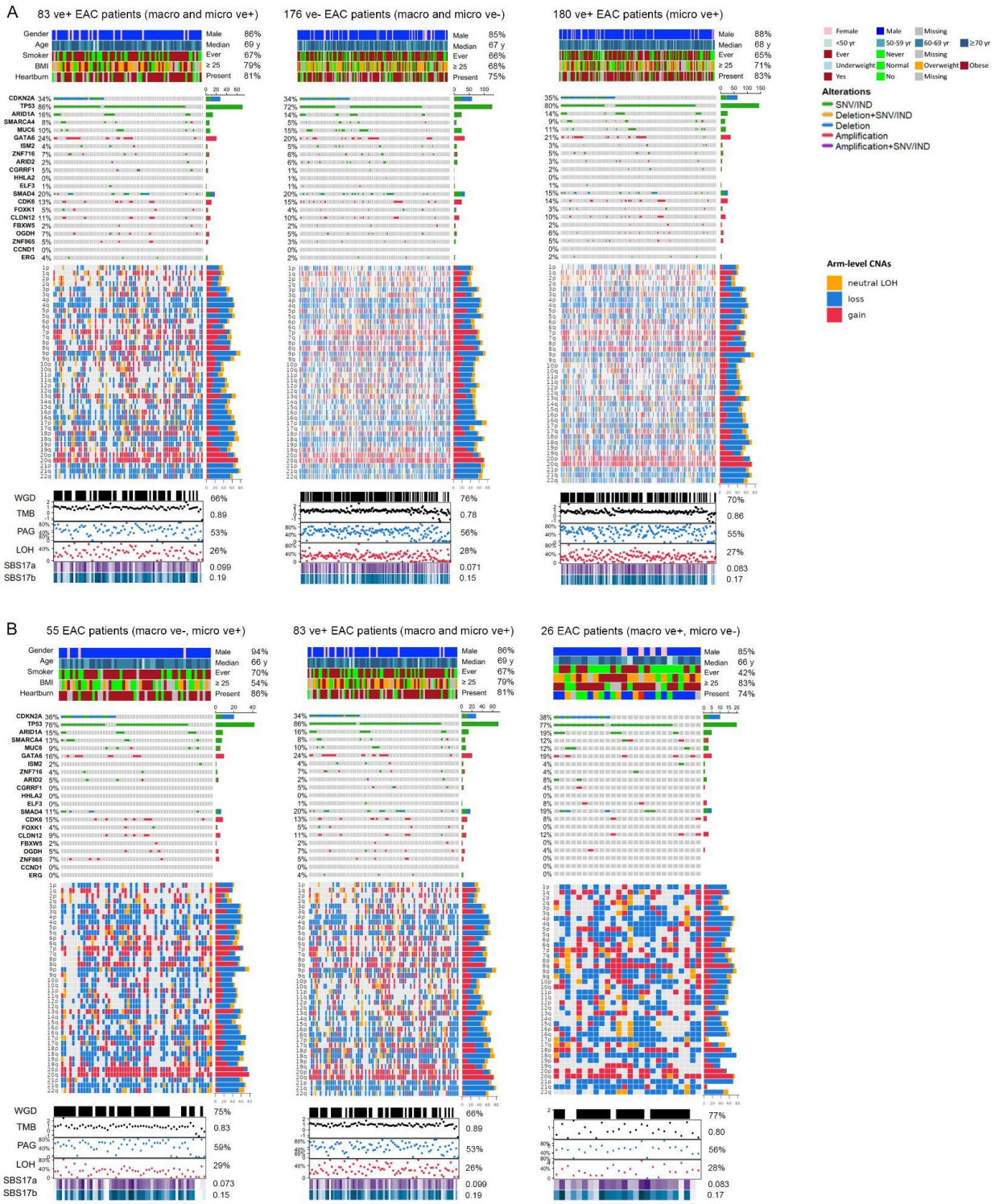
**Supplementary Table 1**. Exact statistical values for each pairs of comparison of oesophageal adenocarcinoma phenotypes that presented in Figure 5A.

| group1 | group2 | chisq_stat | df | p_value | p_adj_fdr | p_adj_bonferroni |
|---|---|---|---|---|---|---|
| Full_BE (?) | Full_BE+ve | 46.74 | 3 | 0.00 | 0.00 | 0.00 |
| Full_BE (?) | Full_BE-ve | 12.27 | 3 | 0.01 | 0.01 | 0.10 |
| Full_BE (?) | Surveillance_BE (?) | 28.95 | 3 | 0.00 | 0.00 | 0.00 |
| Full_BE (?) | Surveillance_BE+ve | 145.88 | 3 | 0.00 | 0.00 | 0.00 |
| Full_BE (?) | Surveillance_BE-ve | 2.15 | 3 | 0.54 | 0.58 | 1.00 |
| Full_BE+ve | Full_BE-ve | 97.27 | 3 | 0.00 | 0.00 | 0.00 |
| Full_BE+ve | Surveillance_BE (?) | 8.79 | 3 | 0.03 | 0.05 | 0.48 |
| Full_BE+ve | Surveillance_BE+ve | 68.77 | 3 | 0.00 | 0.00 | 0.00 |
| Full_BE+ve | Surveillance_BE-ve | 0.15 | 3 | 0.99 | 0.99 | 1.00 |
| Full_BE-ve | Surveillance_BE (?) | 48.84 | 3 | 0.00 | 0.00 | 0.00 |
| Full_BE-ve | Surveillance_BE+ve | 207.80 | 3 | 0.00 | 0.00 | 0.00 |
| Full_BE-ve | Surveillance_BE-ve | 5.21 | 3 | 0.16 | 0.20 | 1.00 |
| Surveillance_BE (?) | Surveillance_BE+ve | 5.71 | 3 | 0.13 | 0.17 | 1.00 |
| Surveillance_BE (?) | Surveillance_BE-ve | 2.85 | 3 | 0.42 | 0.48 | 1.00 |
| Surveillance_BE+ve | Surveillance_BE-ve | 12.33 | 3 | 0.01 | 0.01 | 0.09 |

**Supplementary Table 2**. List of variables included for the analysis from the OCCAMS study. Bold indicates variables which were selected for further analysis using the selection process.

| Domain | Variable |
|---|---|
| **Demographics** | **Age at diagnosis** |
| | **Sex** |
| | Ethnicity |
| **Risk factor exposures** | **BMI at baseline; *kg/m²*** |
| | BMI five years prior to diagnosis; *kg/m²* |
| | BMI difference (prior to baseline); *kg/m²* |
| | **Cigarette smoking status** |
| | Number of cigarettes smoked per day |
| | Years of smoking cigarettes |
| | Number of pack-years of smoking |
| | Heavy alcohol drinking status |
| | Units of alcohol intake per week |
| **Anti-inflammatory medications** | Aspirin use status |
| | Years of aspirin use |
| | NSAID use status |
| | Years of NSAID use |
| | **Any use of aspirin or NSAID** |
| **Reflux symptoms & acid suppressant medications** | Frequency of reflux symptoms |
| | Duration since reflux symptoms began |
| | Currently taking acid suppressant medications |
| | Currently symptomatic for reflux while on acid-suppressants |
| | PPI medication use status |
| | Years of PPI medication use |
| | OTC acid suppressant medication use status |
| | Years of OTC medication use status |
| | H2RA medication use |
| | Years of H2RA medication use |
| | Use of any acid suppressant/reducing medications status |
| | **Derived heartburn symptom status** |
| **Clinical factors** | Tumor length, *cm* |
| | Tumor growth (T stage pre-op to T stage post-op) |
| | **TNM** |


A
83 ve+ EAC patients (macro and micro ve+)
176 ve- EAC patients (macro and micro ve-)
180 ve+ EAC patients (micro ve+)
Gender
Age
Smoker
BMI
Heartburn
Male
Median
Ever
≥ 25
Present
Female
Missing
<50 yr
50-59 yr
60-69 yr
≥70 yr
Never
Normal
Overweight
Obese
Yes
No
Alterations
SNV/IND
Deletion+SNV/IND
Deletion
Amplification
Amplification+SNV/IND
Arm-level CNAs
neutral LOH
loss
gain
WGD
TMB
PAG
LOH
SBS17a
SBS17b
B
55 EAC patients (macro ve-, micro ve+)
83 ve+ EAC patients (macro and micro ve+)
26 EAC patients (macro ve+, micro ve-)

**Supplementary Figure 1.** Subgroup analysis of epigenetic and BE-relevant genomic phenotypes comparing combinations of endoscopic (macro) and pathological (micro) Barrett's oesophagus (BE) diagnosis.

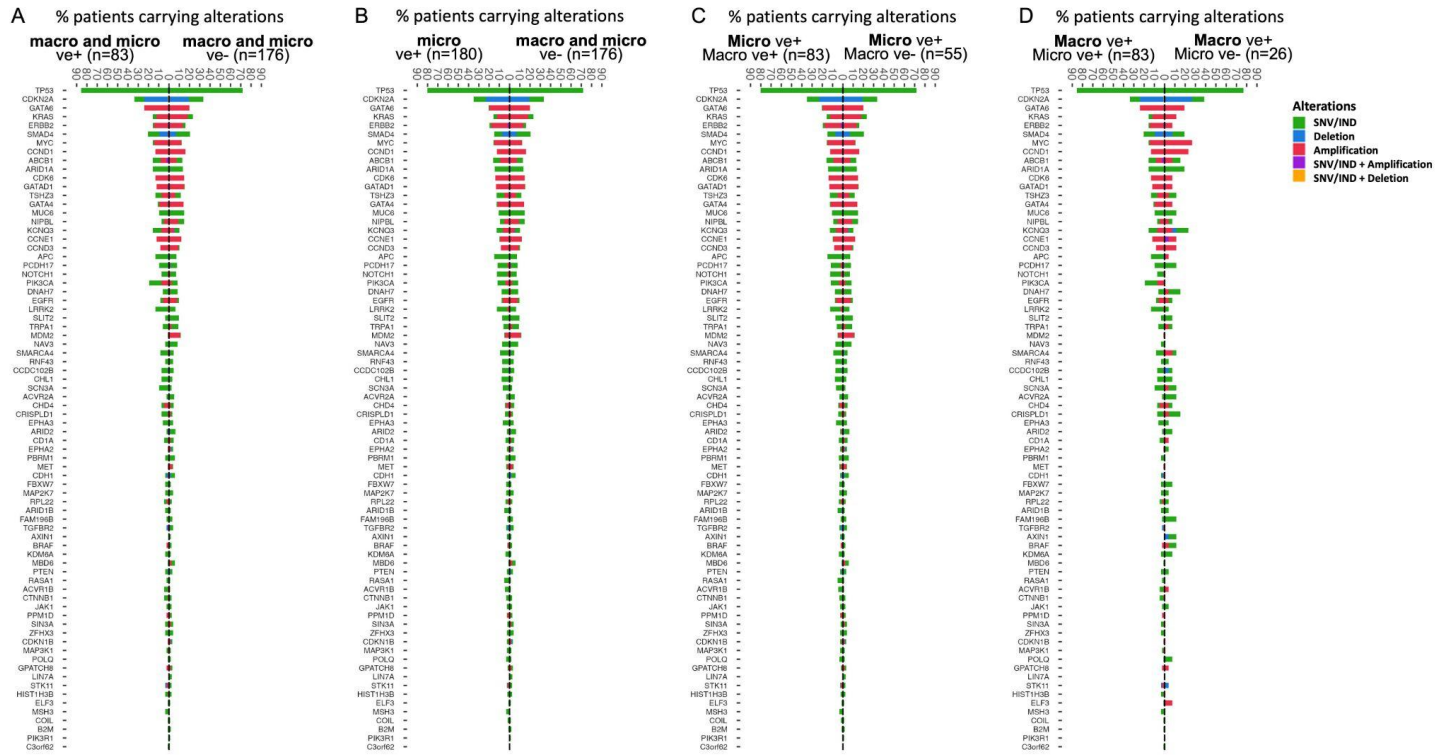
No statistically significant differences were found across any subgroup comparisons.

A1 vs. A2 (Most stringent comparison, same criteria as Table R1): Comparison of cases with both endoscopic and pathological evidence of BE (n=83) versus cases with no evidence by either method (n=176).

A3 vs. A2 (Micro +ve vs. fully negative, same criteria as Table R2): Comparison of all pathology-positive cases regardless of endoscopy (n=180) versus fully negative cases (n=176).

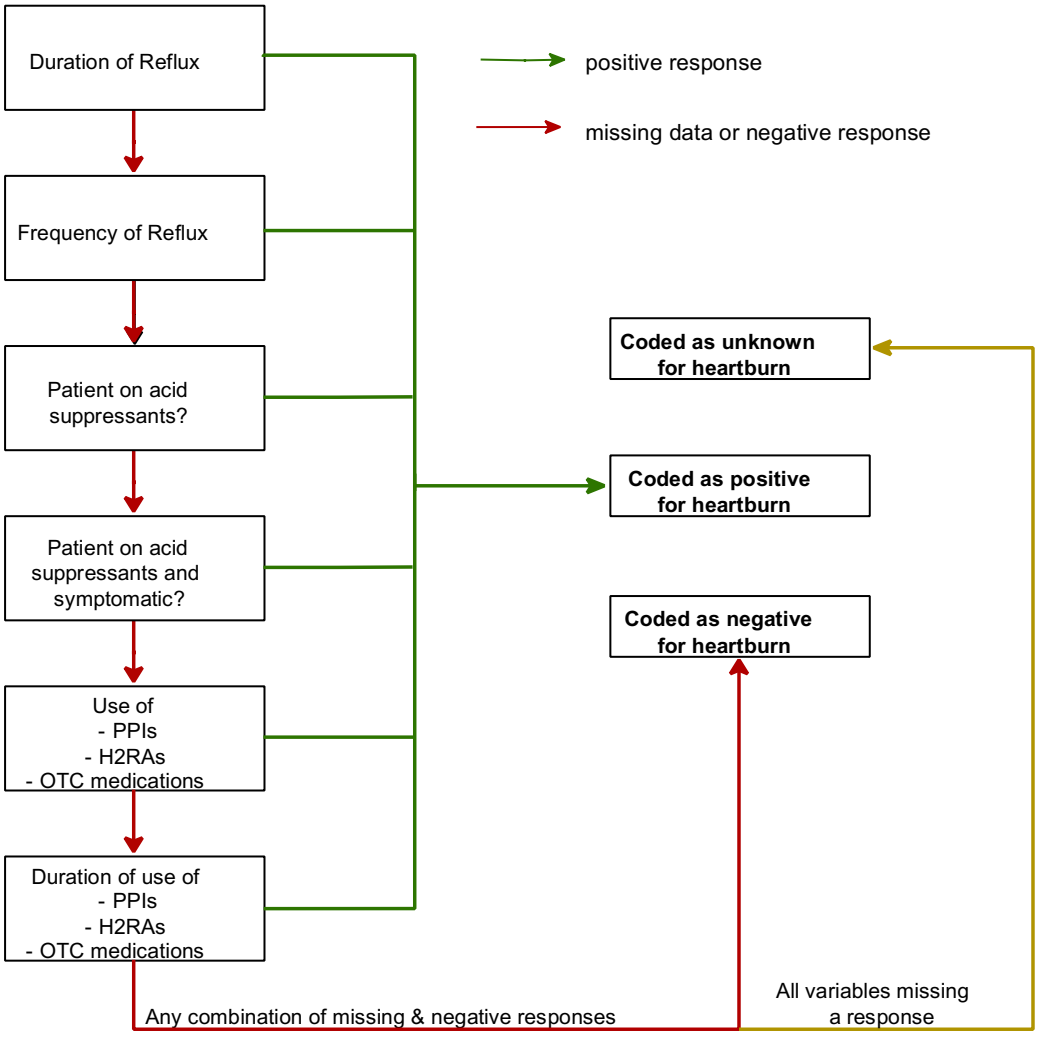
B1 vs. B2 (Internal comparison within pathology/microscopically positive cases, same criteria as Table R3): 53 cases with pathology-positive but endoscopy-negative findings versus 83 cases positive by both methods.

B3 vs. B2 (Internal comparison within endoscopy/macroscopically positive cases, same criteria as Table R4): 26 cases with endoscopy-positive but pathology-negative findings versus 83 cases positive by both methods.




**Supplementary Figure 2.** Subgroup analysis of oesophageal adenocarcinoma (EAC) driver gene variations comparing combinations of endoscopic (macro) and pathological (micro) Barrett's oesophagus (BE) diagnosis.

Overall no significant differences were found. Panels A–D correspond respectively to the grouping criteria in Table R1-R4: (A) the most stringent comparison, (B) micro-positive vs. fully negative, (C) internal comparison within pathology-positive cases, and (D) internal comparison within endoscopy-positive cases.




**Supplementary Figure 3.** Schematic for deriving the heartburn variable using a combination of reflux-related variables.

Abbreviations: PPIs, proton pump inhibitors; H2RAs, histamine H2-receptor antagonists; OTC, over the-counter.

**Source data legends**

**Source data Figure 2**. Comparison of Barrett's driver gene mutations and arm-level copy number alterations between Barrett's oesophagus (BE) and oesophageal adenocarcinoma (EAC), and between BE positive and negative phenotypes of EAC.

**Source data Figure 3A**. Comparison of oesophageal adenocarcinoma (EAC) driver gene mutations between Barrett's oesophagus (BE) positive and negative phenotypes of EAC.

**Source data Figure 4A.** Phylogenetic trees for all patients, ordered by clusters. Phylogenetic trees were generated from multi-regional whole exome sequencing (WES) cohort. The trees are displayed according to their BE associated phenotype and evolutionary clusters. The trunks typically display more driver genes in more advanced cases, but the driver gene events and overall tree shape are very similar between phenotypes.

**Source data Figure 4C**. Comparison of clonal-to-subclonal ratios for driver genes in the multiregional whole-exome sequencing cohort.

**Source data Figure 5B**. The raw images of spatial transcriptomics data.