Information on Genetic Variants Does Not Increase Identification of Individuals at Risk of Esophageal Adenocarcinoma Compared to Clinical Risk Factors

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Author contributions:

ATK: study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; obtained funding.

MCG: acquisition of data; analysis and interpretation of data; statistical analysis; critical revision of the manuscript for important intellectual content.

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Abstract

We previously developed a tool that identified individuals who later developed esophageal adenocarcinoma (EAC; based on age, sex, body mass index, smoking status, and prior esophageal conditions) with an area under the curve of 0.80. In this study, we collected data from 329,463 individuals in the UK Biobank cohort who were tested for genetic susceptibility to EAC (a polygenic risk score based on 18 recognized genetic variants). We found that after inclusion of this genetic information, the area under the curve for identification of individuals who developed EAC remained at 0.80. Testing for genetic variants associated with EAC therefore seems unlikely to improve identification of individuals at risk of EAC.

Novel screening and risk-stratification methods are needed to improve early detection of esophageal adenocarcinoma as the vast majority of patients are currently diagnosed at a late stage when survival is poor[1] and population-wide endoscopy screening is unlikely to be cost-effective[2]. Established clinical risk factors may be useful in identifying individuals at a higher risk of esophageal adenocarcinoma[4-6]. Our findings within the UK Biobank indicated that a model including age, sex, body mass index (BMI), smoking status and prior esophageal conditions had an area under receiver operating characteristic curve (AUROC) for predicting esophageal adenocarcinoma within 5 years of 0.80 (95% CI 0.76-0.82)[3]. Whilst these results suggested that clinical risk factors may be useful as an inexpensive and non-invasive risk-stratification tool, additional robust, minimally-invasive follow-up risk-stratification methods will likely be required to identify individuals at sufficiently high risk to warrant endoscopy screening. Conversely, those at lowest risk could avoid unnecessary and invasive procedures.

Germline mutations associated with esophageal adenocarcinoma risk can be readily assessed using DNA derived from minimally invasively biological samples, thus their evaluation for the purpose of risk stratification in a routine setting is appealing. The clinical utility of assessing single nucleotide polymorphisms (SNPs) for esophageal adenocarcinoma risk-prediction has been investigated in a previous case-control study consortium[6], but remains to be investigated in large-scale prospective studies.

In this study, we analyzed prospective data from the UK Biobank to assess whether the addition of genetic factors to an established clinical risk prediction score enhances the ability to identify individuals at high risk of esophageal adenocarcinoma development within 5 years. A secondary aim was to validate the associations between SNPs and esophageal adenocarcinoma development identified in large genome-wide association studies (GWAS) [7-8].

Clinical factors were assessed using a touchscreen questionnaire, and BMI measurements and blood tests were taken at baseline[9] . The clinical risk-factors (age ,sex, BMI, smoking status and reflux diagnosis/symptoms) used for risk-prediction modelling were selected from a previous investigation

within the UK Biobank[3]. Germline mutations were genotyped using the Affymetrix UK BiLEVE and UK Biobank Axiom arrays (Santa Clara, CA, USA), followed by imputation methods to >90 million variants. Previous GWAS identified 24 genetic variants associated with esophageal adenocarcinoma[7-9]. Two SNPs were unavailable for analysis (rs9918259 and rs75783973), two SNPs were excluded due to linkage disequilibrium (Supplementary Table 1) and one SNP (rs9257809) was excluded as it was not in Hardy-Weinberg equilibrium (P<0.01). Polygenic risk scores were calculated by summing the positive risk allele counts for 18 genetic variants weighted by their odds ratios from previous GWAS [8-9], and dividing the total by 18[6].

Diagnostic accuracy was quantified for the combined genetic and clinical risk factor models using the AUROC curve with 95% confidence interval (CI). A points-based model, created from our previous investigation of established clinical risk factors[3], was used to assign points to tertiles of the polygenic risk score. Sensitivity, specificity, Youden's index (sensitivity + specificity -1) and net reclassification index[10] were assessed for individuals above each points based cut-off threshold when using the established risk factor model alone and when combined with the genetic factors. Further details are outlined in the Supplementary Methods.

Of 502,640 participants in UK Biobank, the following exclusions were applied: 117,891 were aged under 50 years, 30,665 had a history of cancer (or cancer within 6 months of baseline), 8,515 had missing clinical or genetic data, two participants withdrew consent, 772 had insufficient genetic data quality and 15,152 reported a non-White ethnicity. A total of 329,643 (65.6%) were eligible for inclusion, among whom 214 individuals were diagnosed with esophageal adenocarcinoma within 5 years. Individuals diagnosed with esophageal adenocarcinoma were more likely to be older, male, smoke (current or former), have a higher BMI, have an existing esophageal condition, and have a higher polygenic risk score than non-cases (Supplementary Table 2). Mean follow-up time was 4.8 (standard deviation 0.6) years.

The strength of associations for each individual SNP were broadly in line with expectations [7-9], though modest and not statistically significant, in either crude or multivariable analyses (Supplementary Table 3). The strongest adjusted associations with esophageal adenocarcinoma within 5 years were for SNPs at, or near, the genes *CFTR* (rs17451754; OR 1.21, 95% CI 0.80-1.83) and *BARX1* (rs11789015; OR 1.20, 95% CI 0.96-1.50).

The association between polygenic risk score and risk of esophageal adenocarcinoma within 5 years was modest and not statistically significant (Adjusted OR $_{\text{middle versus bottom tertile}}$ 1.09, 95% CI 0.77-1.55; Adjusted OR $_{\text{top versus bottom tertile}}$ 1.38, 95% CI 0.99-1.92).

The AUROC for the clinical risk factor model was 0.80 (95% CI 0.76-0.82), as previously published[3], and was unchanged when polygenic risk score categories were added to the model (0.80, 95% CI 0.77-0.83) (Figure 1) and in a secondary analyses adding the 18 SNPs to the model (0.81, 95% CI 0.78-0.83).

A points-based model, used points created from a previous investigation of established clinical risk factors[3], i.e. age (55-60 years: 1.5; 60-65 years: 2.5; 65+ years: 3.5), sex (males: 4), smoking status (former: 2; current: 3.5), BMI (>25-30: 1; 30-<35: 1.5; 35+: 2.5), history of esophageal conditions or treatment (1.5). Points for polygenic risk score categories were assigned (middle category=0 points, top category=1 point) by dividing their coefficient when added to the clinical risk factor model by the smallest coefficient in the previous model (0.40 for BMI of 25-<30 kg/m^2) and rounding to the nearest 0.5 to allow easier to interpret cut-offs. When comparing the combined clinical and polygenic risk score model to the original clinical points based model, changes in net reclassification index[10] and Youden's index[11] were modest at any of the cut-off points, as modest improvements in sensitivity were offset by modest reductions in specificity (Table 1).

The results from this large UK cohort study with prospective follow-up data suggest that SNPs previously implicated in esophageal adenocarcinoma susceptibility do not aid risk-prediction alone, or in conjunction with known clinical risk factors. The lack of predictive ability occurred regardless of method used to derive the polygenic risk score and seems unlikely to be improved by additional

statistical power as AUROC confidence intervals were narrow. These results validate findings from a previous case-control study[6], despite the weaker association between polygenic risk scores and oesophageal adenocarcinoma in the current study. Similar lack of improvements were apparent when stratified by certain demographic features (Supplementary Table 4). Future genetic analyses of families with a history of esophageal adenocarcinoma may be required to identify a polygenic risk score that accounts for a larger proportion of the heritability of esophageal adenocarcinoma than the SNPs identified using GWAS studies to date.

Nevertheless, the results provide modest support for a potential role of some genes in esophageal adenocarcinoma development. In particular, genetic variants on or near the CFTR gene (rs17451754) related to cystic fibrosis, which displays phenotypic overlap with reflux[12], and the *BARX1* gene (rs11789015) related to differentiation of esophageal epithelia[13].

To our knowledge, this is the first prospective assessment of genetic susceptibility combined with known clinical risk factors in esophageal adenocarcinoma risk prediction. Potential limitations include reduced statistical power to detect significant associations for low frequency SNPs; lack of information for two SNPs previously identified in GWAS or family history of esophageal adenocarcinoma and; criticism of the UK Biobank's generalizability[14].

Thus, testing for currently recognized germline mutations is unlikely to improve stratification of individuals at risk of esophageal adenocarcinoma at a population level. Future studies should examine other novel screening methods, biomarkers, or epigenetic studies to achieve earlier detection of this tumor.

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Table 1. Performance statistics of the combined (clinical and polygenic risk scores) and clinical only points-based esophageal adenocarcinoma risk-prediction models at different points based cut-offs

Points cut-off		Patients deemed high-risk (%)	Sensitivity	Specificity	Youden's index	NRI[1]
7+	Combined	146,042 (44.3%)	86.4%	55.7%	0.42	-0.04
	Original	134,550 (40.8%)	85.0%	59.2%	0.44	-
8+	Combined	110,358 (33.5%)	81.3%	66.5%	0.48	0.01
	Original	98,203 (29.8%)	77.1%	70.2%	0.47	-
9+	Combined	78,215 (23.7%)	69.2%	76.3%	0.46	0.05
	Original	68,383 (20.8%)	63.6%	79.2%	0.43	-
10+	Combined	52,439 (15.9%)	57.9%	84.1%	0.42	0.07
	Original	44,350 (13.5%)	51.9%	86.5%	0.38	-

[1] Net Reclassification index: positive values indicate that a larger proportion of individuals with events were moved up to the high-risk group than individuals without events when changing from the original to combined model.

Figure legend

Figure 1. Receiver operating characteristic curve for the clinical model alone (•) and when combined with polygenic risk score categories (+) for predicting risk of esophageal adenocarcinoma within 5 years.