MiRNA-related SNPs and risk of esophageal adenocarcinoma and Barrett's esophagus:

Post genome-wide association analysis in the BEACON consortium

Matthew F. Buas

A thesis
submitted in partial fulfillment of the
requirements for the degree of

Master of Science

University of Washington

2013

Committee:
Thomas L. Vaughan
Ulrike Peters

Program Authorized to Offer Degree:
Public Health-Epidemiology

ACKNOWLEDGEMENTS

I would like to thank Tom Vaughan for his generous and supportive mentorship throughout my time at the UW. His critical insights, flexible thinking, and natural curiosity continue to enrich my research endeavors. I am grateful for our many interesting conversations about science and careers, and for his genuine interest in the futures of those he mentors. I would also like to acknowledge Riki Peters for her helpful scientific critiques and constructive input. I thank Dave Levine and Lynn Onstad for their behind-the-scenes contributions in data preparation and management.

University of Washington

Abstract

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Matthew F. Buas

Chair of the Supervisory Committee:

Professor Thomas L. Vaughan
Department of Epidemiology

The incidence of esophageal adenocarcinoma (EA) has increased significantly in recent decades. Although several major risk factors have been identified for EA and its precursor, Barrett's esophagus (BE), including reflux, Caucasian race, male gender, obesity, and smoking, only limited knowledge exists regarding the role of inherited genetic variation and its interplay with environmental factors. The Barrett's and Esophageal Adenocarcinoma Consortium (BEACON) recently completed a genome-wide association study (GWAS) of 1,517 EA cases, 2,416 BE cases, and 2,187 controls. Using this dataset, we examined single nucleotide polymorphisms (SNPs) that potentially affect the biogenesis or biological activity of microRNAs (miRNAs), a class of small non-coding RNAs that regulate post-transcriptional gene expression and are deregulated in many cancers, including EA. Polymorphisms in three classes of genes were evaluated for their association with risk of EA or BE: 1) miRNA biogenesis genes (157 SNPs / 21 genes), 2) miRNA gene loci (234 SNPs / 210 genes), and 3) miRNA-targeted mRNAs (179 SNPs / 158

genes). 28 SNPs were nominally associated (P<0.05) with EA, and 34 with BE, compared to 29 expected by chance for each condition. A polymorphism located in a predicted miRNA-138 binding site of the DNA repair gene *XRCC1* (rs1799782) was the top hit identified in either analysis (per allele OR 0.68 for EA, 95% CI 0.55-0.83, p=0.00025), with a p-value approaching but not reaching the Bonferroni threshold for significance (α=8.8 x 10^{-5}) after correction for multiple comparisons. This analysis provides the most extensive assessment to date of miRNA-related SNPs in relation to risk of EA and BE. While chance alone may account for the reported findings, follow-up studies are underway using an expanded sample set with an additional 1,000 EA cases and 1,000 controls to further evaluate whether genetic variation in this pathway correlates with disease susceptibility and interacts with known risk factors.

Introduction

Esophageal adenocarcinoma (EA) is a rare but often lethal disease that represents a growing public health problem (1). EA typically arises from a squamous-to-columnar metaplastic precursor lesion known as Barrett's esophagus (BE). While gastroesophageal reflux disease (GERD), Caucasian race, male gender, obesity, and smoking are established risk factors for EA and BE (2), less is known about the role of inherited genetic variation and its interplay with environmental factors. Past studies based on candidate-gene approaches have linked altered risk of EA or BE to DNA polymorphisms in genes implicated in a wide range of biological pathways: inflammation (*COX-2, IL-18, TNF-β*), detoxification (*GSTM1, GSTT1, GSTP1*, *NQO1*), DNA repair (*MGMT*), angiogenesis (*VEGF, MMP1, MMP3*), and apoptosis (*CASP7*, *CASP9*) (3-13). Interactions have also been described between smoking and variants of *GSTM1, GSTT1* and *VEGF* (7, 11), and between GERD and variants of *MMP1 and MMP3* (12), in relation to risk of EA. These studies, however, have been limited by small sample sizes (100-300 subjects per case group) and lack of validation in independent study populations. Two large-scale genome-wide association studies of BE and EA recently identified several polymorphisms significantly associated with disease risk (14, 15). These hits included variants located in three transcription factors, a transcriptional co-activator, and the major histocompatability complex locus, none of which were previously implicated by candidate-based studies.

MiRNAs are small non-coding RNAs that function in post-transcriptional gene regulation (16). MiRNA gene loci are transcribed by RNA polymerase II to generate primary (pri-) miRNA transcripts, which are then cleaved by the nuclear RNAse DROHSA complex to form stem-loop precursor (pre-) miRNAs. Following nuclear export to the cytoplasm, pre-miRNAs undergo further processing by the DICER complex to generate mature miRNAs, which typically bind to the 3' untranslated region of target mRNAs and mediate translational repression or RNA degradation. A large body of work has established that miRNAs act as oncogenes or tumor suppressors in a variety of tissues, and their deregulation can lead to neoplasia (17). MiRNAs have also been implicated in inflammatory pathways (18, 19), which are likely to play an important role in BE/EA. Changes in miRNA expression have been detected at multiple stages in the development of EA, and specific miRNA signatures may predict progression or prognosis (20-29).

Many studies have reported associations between miRNA-related SNPs and risk of multiple cancers (30). These SNPs may reside in a) miRNA biogenesis genes, b) miRNA gene loci, or c) miRNA-targeted mRNAs. Functional miRNA-related SNPs may affect global miRNA expression levels, processing or expression of individual miRNAs, and miRNA target gene specificity. A case-control study of 346 esophageal cancer cases (86% EA) and an equal number of matched controls reported that seven miRNA-related SNPs from a panel of 41 total SNPs tested were associated with altered risk of esophageal carcinoma, with the association of one SNP in the pre-miRNA-423 region remaining statistically significant after correction for multiple comparisons (31). These SNPs have not been validated in independent study populations or evaluated for potential associations with BE, and it is currently unknown whether additional SNPs in the miRNA pathway may modulate risk of these conditions. Using data from a recent genome-wide association study (GWAS) conducted on several thousand subjects pooled from multiple studies included in BEACON (15), we selected a total of 157 biogenesis pathway SNPs, 234 miRNA SNPs, and 179 mRNA target SNPs and assessed their associations with risk of EA or BE.

Methods

Study population and SNP genotyping

The Barrett's and Esophageal Adenocarcinoma Genetic Susceptibility Study included EA cases, BE cases, and controls pooled from 14 individual studies conducted in Western Europe, Australia, and North America over the past twenty years. Histological confirmation was carried out for all EA and BE cases, with minor variations across studies in the endoscopic criteria used for the definition of BE. Population controls were drawn from the included BEACON studies to serve as a comparison group for both EA and BE subjects. These controls included 100% of controls from BE-only studies, 100% of controls from paired EA/BE studies with shared control groups, and randomly selected non-Hispanic Caucasian controls (within strata of sex and 5-year age group) from EA-only studies in a 2:1 ratio of controls to cases. Data for EA/BE risk factors such as age, sex, race, BMI, and smoking history were collected by all of the included studies via standardized questionnaires, usually through personal interviews.

DNA specimens were shipped to the Fred Hutchinson Cancer Research Center for processing. Genotyping of buffy coat or whole blood DNA was performed using the Illumina Omni1M Quad platform. We performed standard quality control procedures as described previously (32). We excluded samples that had either unacceptable quality or questionable identity. We looked for plate- and batch-specific effects and missing call rate differences in cases and controls. We identified samples that showed unexpected relatedness and used this information to ensure no more than one family member was used in any association test. We used duplicate sample pairs to assess genotyping quality and accuracy.

We performed Principal Component Analysis (PCA) as a two-step process using the SNPRelate software (http://cran.r-project.org/web/packages/SNPRelate/index.html). First, we used PCA to define a homogeneous set of European ancestry samples. We did this by running PCA on a set of 6,249 unrelated (except for six two-person families) subjects, each of which was an EA case, a BE case, or a control. A majority of these subjects (~97%) self-identified their race as "White", and a scatterplot of all subjects along the axes of the first two eigenvectors showed the majority of samples formed a tight cluster (data not shown). Therefore, we computed the means and standard deviations (SD) of the first two eigenvectors and defined any sample that fell within a two SD rectangle of both eigenvector means to be of homogeneous European ancestry (n=6,126). After selection of unrelated subjects with a missing genotyping call rate < 2%, the final study population included 1,517 EA cases, 2,416 BE cases, and 2,187 controls. Three of the 2,187 controls were excluded from the BE analysis due to familial relation to cases.

Selection of miRNA-related SNPs

SNPs selected for this study are located in (or in proximity to) a) miRNA biogenesis genes (+/- 2kb), b) miRNA gene loci (+/- 25bp), or c) predicted or verified mRNA targets of miRNAs (Figure S1). We excluded SNPs from consideration that failed Illumina quality measures or standard quality control procedures (32). Specifically, SNPs were excluded if any of the following criteria were satisfied: i) Illumina GenTrain score < 0.6 or cluster separation < 0.4; ii) >5% missing call rate over samples; iii) discordant genotype calls in any pair of duplicate study samples; iv) Mendelian error in either one of the HapMap QC trios or a small number of families identified in the BEACON data; v) significant departure from Hardy-Weinberg Equilibrium ($P<10^{-4}$); vi) minor allele frequency (MAF) < 1%.

After imposing the above filters, we first identified all available Omni1M SNPs (N=185) located within fourteen genes in the core biogenesis pathway (2kb flanking sequences for each gene were also included, using gene boundaries defined in hg19/GRChB37): *DDX20, DGCR8*, *DICER1*, *DROSHA*, *EIF2C1-4*, *GEMIN4*, *GW182*, *PRKRA*, *RAN*, *TARBP2*, *XPO5*. Similarly, we identified all available Omni1M SNPs (N=70) located within 1601 human miRNA precursor sequences (+/- 25bp) deposited in miRBase (version 19). Additional Omni1M SNPs (N=160) in pairwise linkage disequilibrium (LD) (r^2>0.8) with non-Omni1M dbSNP polymorphisms located within the 1601 miRNA precursors (+/-25bp) were also identified, using 1000 Genomes data for the CEU population via the SNP Annotation and Proxy (SNAP) website (http://www.broadinstitute.org/mpg/snap). In the case of multiple possible Omni1M proxy SNPs, selection was based on maximum r^2 coefficient followed by minimum distance (bp). Within these two categories of SNPs, LD-based pruning was carried out with pairwise LD data from SNAP, using an r^2 threshold of 0.8, to reduce the number of redundant tests (59 of 185 biogenesis SNPs and 1 of 230 miRNA SNPs were excluded).

To identify potentially functional SNPs in miRNA-targeted mRNAs, we used a recently published database of polymorphisms predicted to alter miRNA-mRNA regulation (33). Two filters were imposed to limit the set of SNPs to those most likely to be functional. First, we considered miRNA-mRNA interactions only for miRNAs shown to be expressed in the esophagus at some point in the disease progression from normal squamous epithelium to BE to EA. Based on the union of several published reports (23, 25-29), 135 expressed miRNAs were identified (Table S1). Second, after the previous exclusions, we only considered a subset of miRNA-mRNA interactions (≈3%) that were predicted to be most strongly affected by genetic variants in the target mRNA (ΔS>0.85, where S is the predicted regulation score for a given miRNA:mRNA pairing). LD-based pruning of these filtered SNPs was performed as described previously (30 of 177 SNPs were excluded).

A literature search was also conducted to identify all miRNA-related SNPs shown to be associated with susceptibility to any type of cancer (Table S2) (30, 34-56). Among these SNPs (biogenesis: N=51, miRNA: N=15, mRNA: N=39), those not already captured by our described selection process were added if available in the Omni1M dataset (biogenesis: N=23 of 36, miRNA: N=2 of 9, mRNA: N=25 of 39), or proxy SNPs were substituted where possible (biogenesis: N=11 of 36, miRNA:

N=4 of 9, mRNA: 7 of 39). The final set of polymorphisms included 157 biogenesis pathway SNPs, 234 miRNA SNPs, and 179 target mRNA SNPs (Table S3).

Statistical analysis

Unconditional multivariate logistic regression was used to compute odds ratios for risk of EA or BE associated with a given SNP variant, using an additive model (per-allele), while adjusting for age, sex, and multiple eigenvectors (ev) derived from PCA to account for population stratification by ancestry. We performed PCA on the pool of 6,126 subjects described previously. For the comparison of BE cases vs. controls, we included the first four eigenvectors from this analysis as covariates in the association test model since they were significantly correlated with case-control status, and a scree plot showed that the variance accounted for by each eigenvector flattened out after these four eigenvectors (data not shown). For the comparison of EA cases vs. controls, we included the first two eigenvectors as covariates in the association test model since only they were significantly correlated with case-control status. Age was included as a covariate only in the EA analysis, as this variable was correlated with case-control status for EA but not BE. The Bonferroni method was used to correct for multiple comparisons (570 SNPs), with a threshold of α=0.05/570=8.8 x 10^{-5} to assess statistical significance after correction. Stratified analyses by smoking history and body mass index (BMI) were also conducted, and evidence for interaction between these variables and SNP variants was assessed by including a product term in the logistic regression model. Smoking history and BMI were defined categorically in stratified analyses (smoking: ever/never, or pack-years: 0, >0 & <15, 15-29, 30-44, 45+; BMI: <25, 25-29, 30-34, 35+) or continuously (pack-years, BMI) to test for interaction. The cumulative effect of SNPs that were found to be associated with risk of EA or BE was evaluated by counting the number of unfavorable alleles in each subject, and categorizing subjects into risk groups based on the distribution of unfavorable alleles in controls. Odds ratios for risk of EA or BE were calculated for four risk groups using the lowest-risk group as the reference. Statistical analyses were conducted using STATA/SE version 12.0 (College Station, TX).

Results

Subject characteristics

The distribution of demographic characteristics among controls, EA cases, and BE cases is shown in Table 1. EA cases were somewhat older (64.6 years) and more likely to be male (88%) relative to controls (61.7 years, 79% male) and BE cases (61.7 years, 76% male). The percentage of subjects reporting ever having smoked cigarettes was higher among EA (75%) and BE (66%) cases compared to controls (59%). Heavy smoking (45+ pack years) was more prevalent among EA cases (21%) than among controls (14%) or BE cases (14%), while obesity (BMI 30+) was more prevalent among EA (30%) and BE (37%) cases relative to controls (20%).

Associations of individual SNPs with risk of EA or BE

Of the 157 biogenesis pathway SNPs, 234 miRNA SNPs, and 179 mRNA target SNPs evaluated in this study (Table S3), 28 were nominally associated (P<0.05) with risk of EA (Table 2A/S4A: 1, 14, and 13 SNPs in the respective classes), and 34 with risk of BE (Table 2B/S4B: 9, 18, and 7 SNPs in the respective classes). A SNP within a predicted miRNA-138 binding site in the DNA repair gene *XRCC1* (rs1799782 G>A) was the top hit identified overall in either analysis (per allele OR 0.68 for EA, 95% CI 0.55-0.83, P=0.00025), but did not reach statistical significance after (stringent) correction for multiple comparisons (Bonferroni $\alpha=8.8 \times 10^{-5}$). The *XRCC1* polymorphism was not associated with risk of BE (per allele OR 0.94, P=0.47, Table S4B/S5B). Four SNPs were associated with risk of both EA and BE. One of these (rs12534337 G>A), located immediately adjacent to the pre-miR-4467 sequence, was the top hit for BE (P=0.00328) and was associated with approximately 30% increased risk for both conditions. A second polymorphism (rs12461701 G>A) in LD with two SNPs in pre-miR-3188 was associated with ≈10% risk reduction for EA and BE. The other two shared hits (*ZNF17* rs2023761 G>A and *E2F2* rs2075993 T>C) are located in the 3'UTRs of mRNAs with predicted miRNA binding sites. Both SNPs were associated with increased risk of EA (per allele 22% and 12%, respectively) and BE (per allele 19% and 9%, respectively). The minor allele for rs2075993 was "C" in the EA analysis and "T" in the BE analysis. Additional polymorphisms in Tables 2A/2B were associated with risk of EA or BE with per allele ORs ranging from 0.74-1.44. None of the associations remained significant after applying the Bonferroni correction.

Stratified analyses were conducted to determine if the most significant associations (P<0.02) in Table 2 are modified by smoking history (Table 3) or BMI (Table 4). The inverse association of *XRCC1*

rs1799782 with EA risk was stronger in magnitude in subjects who smoked heavily (per allele OR 0.44 in 45+ pack-year smokers versus 0.82 in non-smokers) or were obese (per allele OR 0.43 for BMI 35+ versus OR 0.80 for BMI<25). The inverse association of this SNP with EA risk remained significant (P<0.05) among ever-smokers (P=0.0007), 45+ pack-year smokers (P=0.0093), and obese subjects (BMI 30-34.9: P=0.0057 and BMI 35+: P=0.0459), but not among never-smokers, those who had smoked under 45 pack-years, or non-obese subjects. An interaction was observed between rs1799782 and pack-year smoking history (P=0.02), but not between rs1799782 and BMI (P=0.11).

Discussion

Using genotyping data from a recent consortium-based GWAS, we evaluated the association of 570 miRNA-related SNPs with risk of EA or BE. 28 SNPs were found to be nominally associated (P<0.05) with risk of EA, and 34 with risk of BE. A polymorphism in a predicted miRNA-138 binding site of the DNA repair gene *XRCC1* was the top hit overall and was associated with a 32% reduced risk of EA, which approached, but did not reach, significance after Bonferroni correction for multiple comparisons.

Aberrant expression of miRNAs has been reported in many cancers, and several studies have described miRNA expression changes at specific stages in the development of EA, which may be associated with progression or prognosis (22-24, 26, 27). Inherited genetic variation in the miRNA pathway has been linked to altered susceptibility to a variety of cancers, but few studies have focused on esophageal cancer, and in particular, EA (as opposed to esophageal squamous cell carcinoma) (30, 31, 57, 58). The largest previous study was conducted by Wu and colleagues (31) and identified seven SNPs significantly associated with risk of esophageal cancer, five of which were also associated with EA. A SNP in the pre-miR-423 region remained significant after adjustment for multiple comparisons.

Of particular interest in our analysis is the top hit identified overall, a SNP located in *XRCC1* (rs1799782 G>A). *XRCC1* (X-ray repair cross-complementing group 1) encodes a scaffold protein involved in the base-excision DNA repair pathway (59). The *XRCC1* rs1799782 variant results in substitution of arginine 194 with tryptophan. In-vitro DNA repair studies have provided preliminary evidence that this polymorphism may result in enhanced repair activity (60, 61). A recent study by Nicoloso et al. further showed that rs1799782, while located in the coding region of *XRCC1*, lies within a

predicted binding site for miR-138 (36). Surprisingly, while the C>T variant increases the base complementarity between miR-138 and the XRCC1 mRNA, luciferase reporter assays suggested increased stability of the variant transcript relative to the wildtype transcript in the presence of miR-138. The authors speculated on a possible non-canonical stabilizing role for miR-138 in binding the XRCC1 variant mRNA. Consistent with potentially enhanced repair activity of the XRCC1 variant observed in-vitro, a comprehensive meta-analysis reported that rs1799782 Arg194>Trp was associated with reduced overall cancer risk, using a dominant model (OR 0.89, 95% CI 0.81-0.98) (62). A non-significant inverse association (OR 0.86, 95% CI 0.72-1.03) was observed specifically for gastroesophageal cancer (a mixed category of gastric adenocarcinoma and esophageal squamous cell carcinoma).

Our analysis also suggested an inverse association between *XRCC1* rs1799782 G>A and risk of EA, which was more pronounced among subjects with the longest pack-year smoking history (45+) or in the highest categories of BMI (30+). Given the established mutagenicity of tobacco smoke, it is striking that the *XRCC1* variant (which may have enhanced repair activity) is associated with the strongest apparent protective effect in heavy smokers. The *XRCC1* polymorphism was similarly found to be associated with reduced risk of lung cancer in smokers with the longest pack-year history (63). SNPs in additional genes involved in DNA repair have also been linked previously to altered risk of EA (64-66).

Obesity is a second established risk factor for EA, and the specific association of visceral adiposity with disease risk has led to speculation over the role of inflammatory signaling that may contribute to susceptibility, potentially in part through increased oxidative stress and associated DNA damage (67). Interestingly, we also observe a stronger inverse association of rs1799782 with EA risk in obese subjects, although the interaction was only borderline significant (P=0.11). In theory, any potential impact of the *XRCC1* polymorphism on DNA repair activity could result from (1) the Arg194>Trp amino acid substitution in the XRCC1 protein (which could affect scaffold function) and/or (2) altered miR-138 binding to the XRCC1 C>T mRNA (which could influence XRCC1 mRNA/protein levels). Further studies are required to evaluate these potential consequences of the polymorphism, ascertain expression levels of miR-138 in esophageal tissue, and substantiate the reported effects of the variant on DNA repair activity.

Given that BE is an established risk factor for and the only known precursor of EA, it was of interest to compare the list of SNPs associated with risk of each condition. In theory, a SNP causally associated with increased risk of EA could act by increasing either the risk of BE, or the risk of progression from BE to EA, or both (though less plausible, a polymorphism that increased risk of BE but decreased risk of progression, or vice versa, could be associated with elevated, reduced or unchanged risk of EA overall). In general, SNPs causally associated with increased risk of BE would be expected to increase risk of EA as well, unless associated with reduced risk of progression of equal or greater magnitude. Of the 28 SNPs associated at $P<0.05$ with risk of EA and the 34 associated with risk of BE, four SNPs (pre-miR-4467 rs12534337 G>A, rs12461701 G>A [in LD with pre-miR-3188 rs7247237 & rs7247767], *ZNF17* rs2023761 G>A, and *E2F2* rs2075993 T>C) were shared hits for both EA and BE. For each of these SNPs, the direction and magnitude of the OR was very similar for BE and EA, suggesting that the association with risk of BE may account for the association with risk of EA. However, given our use of a single control group for comparison to both the EA and BE case groups, a certain number of shared associations could be expected from chance alone, and these overlapping hits should be interpreted cautiously. When EA and BE cases were combined into a single case group and compared to the same set of controls, p-values for the four shared hits were smaller than observed in the individual analyses (Table S6), but did not reach significance using the Bonferroni threshold ($\alpha=8.8 \times 10^{-5}$).

The first of these four shared hits, rs12534337 G>A, is located in the precursor of miR-4467, identified by deep sequencing of the small RNA transcriptome of normal and malignant B cells, and paired normal and tumor breast tissue (68, 69). Expression levels of miR-4467 have not been assessed in the esophagus, and no studies have examined potential functional effects of this miRNA. Of potential interest, the proto-oncogene jun B mRNA is predicted by the TargetScan algorithm to be one of 23 conserved targets of miR-4467, but experimental validation has not been reported. Whether this SNP, which is situated two nucleotides downstream of the predicted miRBase precursor sequence, has an impact on the biogenesis or processing of miR-4467 remains to be determined. *ZNF17* rs2023761 G>A and E2F2 rs207599 T>C are located within the 3'UTRs of putative miRNA targets. ZNF17, a zinc-finger transcription factor, belongs to a class of regulatory proteins that includes both oncogenes and tumor suppressors (70, 71). The rs2023761 G>A polymorphism results in the creation of a predicted binding site

for miR-101 (33), a miRNA upregulated in the esophagus in the transition from low-grade to high-grade dysplasia (27). Further studies are required to determine the expression pattern and function of ZNF17 within esophageal tissue, and to assess the effect of this variant on ZNF17 abundance. E2F2 is a transcription factor that can exert either pro- and anti-proliferative effects (72). The *E2F2* 3'UTR SNP (rs207599) is located in proximity to predicted binding sites for miR-663, a potentially oncogenic miRNA upregulated by almost 5-fold in EA relative to paired normal tissue (27, 73). Rs207599 has also been shown to be associated with increased risk of ovarian cancer (37). The expression pattern and function of E2F2 in esophageal tissues has not been characterized, and it is unknown if this polymorphism results in altered E2F2 expression levels. The fourth shared hit (rs12461701 G>A), is located ≈3kb upstream from and (according to 1000 Genomes data) in LD (r^2>0.8) with two variants in pre-miR-3188 (rs7247237, rs7247767). MiR-3188 was identified by deep sequencing of the melanoma miRNAome (74), but its functional effects and range of expression have not been described.

Several other SNPs nominally associated (P<0.05) with risk of EA or BE are located within or in proximity to genes that contain polymorphisms previously linked to altered cancer susceptibility (*XPO5*, *miR-492*, *EIF2C2*, *DDX20*, *LIN28*, *miR-938*) (31, 37, 40, 43, 46, 75, 76), while additional hits are within or in proximity to genes functionally implicated in carcinogenesis (*miR-196-a*, *APSN*, *SCUBE2*, *ADAMTS1*, *TARBP2*, *EIF2C3*, *miR-559*, *TAP1*) (77-84). Interestingly, a missense variant in the *XPO5* gene (kgp1460594 G>A/Ser241Asn), which encodes a protein involved in miRNA transport to the cytoplasm, was associated with ≈20% increased risk of EA, while an *XPO5* 3'UTR SNP 44kb downstream was associated with elevated EA risk in a past study (31).

Five of the seven SNPs reported to be associated with risk of esophageal cancer by Ye et al. (31) were included in our analysis of genotyped SNPs from the BEACON GWAS, including the single SNP (pre-miR-423 rs6505162) that Ye et al. reported as significant after correction for multiple comparisons. Three of these five SNPs were shown to be associated with EA (miR-423 rs6505162, miR-196a-2 rs11614913, and RAN rs14035), while two reached borderline significance (XPO5 rs11077 and pri-miR-219-1 rs213210). In our study, none of these five SNPs were found to be associated (P<0.05) with either EA (Table S7A) or BE (Table S7B), and nearly all ORs were very close to 1. Ye et al. evaluated three different genetic models (additive, recessive, dominant) and reported the best-fitting model in their

analysis, in contrast to our approach of assessing exclusively the additive model. After re-analysis of these five SNPs using the specified models, one polymorphism (pri-miR-219-1 rs213210 T>C) was associated with elevated risk of EA in our dataset (dominant model, OR 1.22, 95% CI 1.01-1.47, P=0.038), consistent with the previously published results (OR 1.61, P=0.058). Conversely, Ye et al. did not evaluate any of the 58 hits identified in our analysis. Multiple factors could account for discrepancies between studies. First, many hits in association studies may be false positives, and replication in large, independent populations is critical (our EA analysis included over four times as many cases as the previous study). Second, different approaches were taken in adjustment for covariates. While both studies included only Caucasians, we also adjusted for population stratification via inclusion of several eigenvectors derived from principal components analysis, but chose not to adjust for smoking status.

Strengths of our study included the use of pooled data from the BEACON GWAS, which provided the largest sample size to date in the evaluation of miRNA-related SNPs and risk of EA or BE. Inclusion of both BE and EA cases allowed for a comparison of the genetic variation associated with risk of a neoplastic precursor lesion and the cancer that arises from it. The availability of covariate data for smoking history and BMI further enabled us to evaluate gene-environment interactions for two established risk factors for these conditions. Our assessment of 570 polymorphisms significantly expands upon past efforts to examine genetic variation in this pathway in relation to risk of esophageal adenocarcinoma or Barrett's esophagus. While chance findings cannot be ruled out, follow-up studies are underway using an expanded sample set with an additional 1,000 EA cases and 1,000 controls. Further replication in independent study populations, coupled with experimental validation of the effects of specific polymorphisms on gene expression or function, will be needed to substantiate any variants as true modifiers of disease risk.

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Table 1. Characteristics of controls, EA cases, and BE cases

		Controls * (n=2187)		EA (n=1517)		BE (n=2416)	
		n	(%)	n	(%)	n	(%)
Age, years	mean (SD)	61.7	11.1	64.6	10.7	61.7	12.2
Sex	Male	1718	78.6	1338	88.2	1836	76.0
	Female	469	21.4	179	11.8	580	24.0
Smoking status#	Never	889	40.9	348	24.6	801	33.8
	Ever	1286	59.1	1067	75.4	1570	66.2
Smoking (pack-years)#	0	889	41.2	348	32.6	801	44.6
	>0 & <15	358	16.6	156	14.6	320	17.8
	15-29	327	15.2	162	15.2	232	12.9
	30-44	273	12.7	174	16.3	198	11.0
	45+	310	14.4	226	21.2	244	13.6
BMI#	<25	787	36.3	246	24.6	426	20.7
	25-29.9	944	43.5	458	45.8	883	42.8
	30-34.9	308	14.2	201	20.1	522	25.3
	35+	130	6.0	95	9.5	230	11.2

*3 subjects were excluded from the control group for comparison to BE cases due to relatedness
Numbers do not add to total subjects due to missing data

Table 2A. MiRNA-related SNPs and risk of esophageal adenocarcinoma

Biogenesis pathway

			Controls		EA cases				
SNP	**Gene**	**Alleles†**	**N**	**MAF‡**	**N**	**MAF‡**	**OR***	**95% CI**	**P**
kgp1460594	XPO5	A/G	2187	0.08	1517	0.09	1.23	(1.04-1.46)	0.01512

miRNA genes

			Controls		EA cases				
SNP	**Gene**	**Alleles†**	**N**	**MAF‡**	**N**	**MAF‡**	**OR***	**95% CI**	**P**
rs9842591	miR-5186	A/C	2187	0.46	1517	0.49	1.16	(1.05-1.27)	0.00246
rs895819	miR-27a	G/A	2174	0.33	1506	0.36	1.15	(1.04-1.27)	0.00703
rs12461701	miR-3188	A/G	2187	0.28	1517	0.26	0.87	(0.79-0.97)	0.01374
rs10899620	miR-5579	C/T	2180	0.2	1512	0.18	0.86	(0.76-0.97)	0.01528
rs17880825	miR-4725	C/T	2187	0.02	1516	0.03	1.44	(1.06-1.97)	0.02066
rs1378940	miR-4513	G/T	2187	0.34	1516	0.32	0.89	(0.80-0.98)	0.02109
rs12534337	miR-4467	A/G	2186	0.04	1517	0.05	1.3	(1.04-1.63)	0.02364
rs13005714	miR-3129	A/G	2180	0.08	1510	0.07	0.82	(0.68-0.98)	0.02618
rs17023366	miR-492	T/C	2186	0.06	1517	0.04	0.78	(0.63-0.98)	0.02893
rs3787547	miR-4756	A/G	2186	0.43	1517	0.41	0.9	(0.82-0.99)	0.02916
rs718079	miR-196a-1	T/C	2187	0.29	1517	0.31	1.12	(1.01-1.24)	0.03041
rs10849785	miR-4700	A/G	2187	0.05	1517	0.04	0.79	(0.63-0.99)	0.04143
kgp10521113	miR-4519	T/C	2183	0.38	1517	0.4	1.11	(1.00-1.22)	0.04191
rs12926295	miR-4519	G/A	2185	0.38	1515	0.4	1.1	(1.00-1.22)	0.04551

Table 2A [continued]. MiRNA-related SNPs and risk of esophageal adenocarcinoma

			mRNA targets						
			Controls		EA cases				
SNP	Gene	Alleles†	N	MAF‡	N	MAF‡	OR*	95% CI	P
rs1799782	XRCC1	A/G	2187	0.07	1517	0.05	0.68	(0.55-0.83)	0.00025
rs1644730	RDH8	T/A	2185	0.48	1517	0.44	0.85	(0.78-0.94)	0.00097
rs3174352	ASPN	T/C	2187	0.51	1515	0.47	0.88	(0.80-0.96)	0.0058
rs3746794	TBC1D20	A/G	2185	0.48	1517	0.45	0.88	(0.80-0.97)	0.01016
rs3209160	ZDHHC21	G/C	2182	0.13	1513	0.15	1.18	(1.03-1.36)	0.01415
rs2075993	E2F2	C/T	2186	0.49	1517	0.51	1.12	(1.01-1.23)	0.02362
rs11169571	ATF1	C/T	2187	0.39	1516	0.42	1.11	(1.01-1.22)	0.02795
rs1914321	CPLX4	T/A	2179	0.18	1516	0.16	0.87	(0.76-0.99)	0.03013
rs1367	SCUBE2	G/A	2187	0.08	1516	0.06	0.82	(0.68-0.99)	0.03665
rs12140	ADAMTS1	G/A	2175	0.06	1509	0.07	1.22	(1.01-1.48)	0.04202
rs13835	DET1	A/C	2187	0.43	1516	0.45	1.1	(1.00-1.21)	0.04409
rs2023761	ZNF17	A/G	2187	0.06	1516	0.07	1.22	(1.00-1.48)	0.04921
rs9804386	MORN4	C/T	2187	0.19	1517	0.21	1.13	(1.00-1.27)	0.04955

† Minor/major alleles, ‡ Minor allele frequency, * OR adjusted for age, sex, ev1, ev2, using additive model (per-allele)

Table 2B. MiRNA-related SNPs and risk of Barrett's esophagus

Biogenesis pathway

			Controls		BE cases				
SNP	**Gene**	**Alleles†**	**N**	**MAF‡**	**N**	**MAF‡**	**OR***	**95% CI**	**P**
rs595055	EIF2C1	G/A	2182	0.15	2416	0.13	0.85	(0.75-0.96)	0.00825
rs8192593	TARBP2	A/G	2182	0.04	2414	0.03	0.74	(0.60-0.93)	0.00922
rs11247946	LIN28	G/A	2184	0.35	2415	0.33	0.9	(0.83-0.99)	0.02173
rs2944760	EIF2C2	C/A	2182	0.2	2413	0.19	0.89	(0.80-0.99)	0.02509
rs538779	DDX20	A/G	2184	0.22	2416	0.2	0.89	(0.81-0.99)	0.0302
rs12741800	LIN28	A/G	2179	0.46	2406	0.44	0.92	(0.85-1.00)	0.03951
rs673019	DROSHA	G/A	2183	0.11	2413	0.09	0.87	(0.75-1.00)	0.04432
rs4351606	EIF2C3	A/G	2182	0.06	2415	0.05	0.83	(0.69-1.00)	0.04638
rs639174	DROSHA	T/C	2183	0.26	2416	0.24	0.91	(0.82-1.00)	0.04962

miRNA genes

			Controls		BE cases				
SNP	**Gene**	**Alleles†**	**N**	**MAF‡**	**N**	**MAF‡**	**OR***	**95% CI**	**P**
rs12534337	miR-4467	A/G	2183	0.04	2415	0.06	1.34	(1.10-1.63)	0.00328
rs3785722	miR-1269b	T/C	2183	0.45	2416	0.42	0.89	(0.81-0.96)	0.00435
rs10862193	miR-617	G/A	2180	0.43	2412	0.4	0.89	(0.82-0.97)	0.00757
rs10906086	miR-548ak	C/A	2183	0.47	2414	0.5	1.12	(1.03-1.22)	0.00821
rs4369899	miR-4431	C/T	2184	0.34	2414	0.31	0.89	(0.81-0.97)	0.00869
rs12416605	miR-938	T/C	2183	0.25	2415	0.28	1.13	(1.03-1.24)	0.00919
rs9911968	miR-4520a/b	A/G	2181	0.49	2415	0.46	0.9	(0.83-0.97)	0.01025
rs7000768	miR-3686	G/A	2183	0.3	2416	0.32	1.12	(1.03-1.23)	0.01196
rs10953326	miR-4653	T/C	2183	0.18	2409	0.16	0.87	(0.78-0.98)	0.01603
rs9907126	miR-548at	C/T	2184	0.26	2413	0.29	1.12	(1.02-1.22)	0.01629
rs2037128	miR-944	T/C	2183	0.14	2416	0.12	0.86	(0.76-0.97)	0.01656
rs724714	miR-1343	G/A	2184	0.19	2416	0.21	1.13	(1.02-1.25)	0.02061
rs17036544	miR-559	G/A	2182	0.08	2413	0.07	0.83	(0.71-0.98)	0.02336
rs7188539	miR-5189	G/A	2183	0.33	2416	0.35	1.1	(1.01-1.20)	0.02886
rs12461701	miR-3188	A/G	2184	0.28	2415	0.26	0.9	(0.82-0.99)	0.03247
rs2297333	miR-4642	C/T	2183	0.15	2416	0.13	0.88	(0.78-0.99)	0.03926
rs7211449	miR-548h-3	A/C	2183	0.22	2416	0.2	0.9	(0.81-0.99)	0.0395
rs17252270	miR-548x-2	T/C	2183	0.14	2414	0.15	1.12	(1.00-1.26)	0.04819

Table 2B [continued]. MiRNA-related SNPs and risk of Barrett's esophagus

			mRNA targets						
			Controls		BE cases				
SNP	Gene	Alleles†	N	MAF‡	N	MAF‡	OR*	95% CI	P
rs1043681	THAP3	G/A	2184	0.32	2415	0.3	0.89	(0.81-0.97)	0.00957
rs3198005	TAP1	T/C	2184	0.05	2416	0.06	1.24	(1.03-1.50)	0.02171
rs1423380	ST8SIA4	G/A	2183	0.36	2416	0.38	1.11	(1.01-1.20)	0.02323
rs1043641	ACBD3	T/C	2184	0.17	2416	0.15	0.88	(0.79-0.98)	0.02552
rs1043420	CAPN5	C/T	2184	0.22	2416	0.24	1.11	(1.01-1.23)	0.0334
rs2023761	ZNF17	A/G	2184	0.06	2415	0.07	1.19	(1.00-1.42)	0.04616
rs2075993	E2F2	T/C	2183	0.51	2415	0.49	0.92	(0.85-1.00)	0.04693

† Minor/major alleles, ‡ Minor allele frequency, ** OR adjusted for sex, ev1-ev4, using additive model (per-allele)

Table 3A (i). MiRNA-related SNPs and risk of esophageal adenocarcinoma stratified by smoking history

SNP	Never smokers OR* (95% CI) P	Ever smokers OR* (95% CI) P	P-int#
kgp1460594	1.25 (0.90-1.73) 0.1831	1.30 (1.05-1.60) 0.0141	0.6228
rs9842591	1.17 (0.98-1.40) 0.0853	1.16 (1.04-1.31) 0.0102	0.9275
rs895819	1.04 (0.86-1.26) 0.71	1.17 (1.03-1.32) 0.0129	0.2758
rs12461701	0.83 (0.68-1.02) 0.0758	0.91 (0.79-1.03) 0.1427	0.4917
rs10899620	0.89 (0.71-1.12) 0.3152	0.86 (0.74-1.00) 0.0504	0.7573
rs1799782	0.82 (0.57-1.20) 0.3125	0.63 (0.49-0.82) 0.0007	0.2354
rs1644730	0.84 (0.70-1.01) 0.063	0.87 (0.77-0.98) 0.0192	0.8137
rs3174352	0.96 (0.81-1.15) 0.6772	0.81 (0.72-0.91) 0.0005	0.1329
rs3746794	0.82 (0.68-0.99) 0.0394	0.88 (0.78-0.99) 0.0289	0.7743
rs3209160	1.29 (1.00-1.66) 0.0522	1.16 (0.99-1.37) 0.0733	0.584

* OR adjusted for age, sex, ev1, ev2, using additive model (per-allele), # P-value for coefficient of product term included in the logistic model

Table 3A (ii). MiRNA-related SNPs and risk of esophageal adenocarcinoma stratified by pack-year smoking history

	Pack-years					
	0	>0 & <15	15-29	30-44	45+	
SNP	OR* (95% CI) P	OR* (95% CI) P	OR* (95% CI) P	OR* (95% CI) P	OR* (95% CI) P	P-int#
kgp1460594	1.25 (0.90-1.73) 0.1831	1.84 (1.11-3.03) 0.0171	1.06 (0.66-1.71) 0.7973	1.00 (0.63-1.58) 0.995	1.15 (0.72-1.82) 0.5645	0.4168
rs9842591	1.17 (0.98-1.40) 0.0853	1.25 (0.96-1.62) 0.1001	1.01 (0.77-1.33) 0.953	1.06 (0.81-1.39) 0.6735	1.42 (1.10-1.83) 0.0066	0.12
rs895819	1.04 (0.86-1.26) 0.71	1.34 (1.01-1.78) 0.0443	1.17 (0.87-1.55) 0.2971	1.33 (1.00-1.77) 0.0492	0.98 (0.76-1.28) 0.907	0.6972
rs12461701	0.83 (0.68-1.02) 0.0758	0.94 (0.70-1.27) 0.7066	0.76 (0.54-1.05) 0.0947	0.93 (0.68-1.26) 0.6416	0.89 (0.66-1.19) 0.4306	0.3778
rs10899620	0.89 (0.71-1.12) 0.3152	0.93 (0.66-1.32) 0.6966	0.77 (0.54-1.11) 0.163	0.80 (0.57-1.14) 0.2195	0.87 (0.62-1.21) 0.4063	0.6506
rs1799782	0.82 (0.57-1.20) 0.3125	0.59 (0.30-1.15) 0.122	0.76 (0.43-1.36) 0.3609	0.67 (0.34-1.34) 0.2629	0.44 (0.23-0.81) 0.0093	0.0231
rs1644730	0.84 (0.70-1.01) 0.063	0.88 (0.67-1.16) 0.3673	0.84 (0.64-1.11) 0.2254	0.91 (0.70-1.19) 0.508	0.85 (0.66-1.09) 0.2071	0.987
rs3174352	0.96 (0.81-1.15) 0.6772	0.75 (0.57-0.99) 0.0431	0.76 (0.58-1.00) 0.0479	0.70 (0.53-0.92) 0.0105	0.86 (0.67-1.11) 0.2416	0.3087
rs3746794	0.82 (0.68-0.99) 0.0394	0.94 (0.72-1.24) 0.6813	0.82 (0.63-1.07) 0.1458	0.76 (0.58-1.00) 0.0541	0.88 (0.68-1.13) 0.3166	0.8423
rs3209160	1.29 (1.00-1.66) 0.0522	1.66 (1.16-2.36) 0.0051	1.43 (0.96-2.13) 0.077	0.79 (0.53-1.19) 0.2601	1.04 (0.73-1.49) 0.834	0.176

Table 3B (i). MiRNA-related SNPs and risk of Barrett's esophagus stratified by smoking history

	Never smokers	Ever smokers	
SNP	**OR[**] (95% CI) P**	**OR[**] (95% CI) P**	**P-int[#]**
rs595055	0.80 (0.66-0.98) 0.0335	0.87 (0.75-1.02) 0.0893	0.5517
rs8192593	0.56 (0.38-0.81) 0.0022	0.91 (0.68-1.21) 0.5093	0.0369
rs12534337	1.45 (1.03-2.04) 0.0316	1.26 (0.99-1.61) 0.0646	0.5672
rs3785722	0.87 (0.76-0.99) 0.0399	0.90 (0.81-1.01) 0.0649	0.6692
rs10862193	0.80 (0.70-0.92) 0.0017	0.94 (0.85-1.05) 0.2863	0.0626
rs10906086	1.07 (0.93-1.23) 0.3312	1.14 (1.02-1.27) 0.0169	0.5231
rs4369899	0.97 (0.84-1.13) 0.7024	0.85 (0.76-0.95) 0.0059	0.1544
rs12416605	1.10 (0.94-1.29) 0.2352	1.16 (1.03-1.30) 0.0139	0.5949
rs9911968	0.90 (0.79-1.03) 0.1419	0.90 (0.81-0.99) 0.0398	0.8895
rs7000768	1.14 (0.98-1.33) 0.0825	1.11 (0.99-1.24) 0.075	0.7874
rs10953326	0.93 (0.77-1.11) 0.4138	0.84 (0.73-0.97) 0.0139	0.3999
rs9907126	1.05 (0.91-1.22) 0.5143	1.16 (1.03-1.31) 0.0123	0.2485
rs2037128	0.75 (0.62-0.92) 0.0051	0.93 (0.79-1.09) 0.3434	0.112
rs1043681	0.88 (0.77-1.02) 0.0975	0.90 (0.80-1.01) 0.0786	0.7798

[**] OR adjusted for sex, ev1-ev4, using additive model (per-allele), [#] P-value for coefficient of product term included in the logistic model

Table 3B (ii). MiRNA-related SNPs and risk of Barrett's esophagus stratified by pack-year smoking history

	Pack-years					
	0	>0 & <15	15-29	30-44	45+	
SNP	OR** (95% CI) P	OR** (95% CI) P	OR** (95% CI) P	OR** (95% CI) P	OR** (95% CI) P	P-int#
rs595055	0.80 (0.66-0.98) 0.0335	1.19 (0.87-1.62) 0.2785	0.81 (0.56-1.18) 0.2731	0.89 (0.60-1.34) 0.5876	0.64 (0.44-0.92) 0.0158	0.0579
rs8192593	0.56 (0.38-0.81) 0.0022	0.65 (0.37-1.16) 0.1461	1.73 (0.92-3.24) 0.0878	0.93 (0.44-1.97) 0.8462	1.00 (0.53-1.87) 0.989	0.2294
rs12534337	1.45 (1.03-2.04) 0.0316	1.33 (0.83-2.14) 0.2396	1.13 (0.62-2.04) 0.6917	1.13 (0.61-2.10) 0.7018	1.38 (0.78-2.44) 0.2687	0.5954
rs3785722	0.87 (0.76-0.99) 0.0399	0.90 (0.72-1.12) 0.3374	0.97 (0.76-1.24) 0.8078	0.78 (0.58-1.03) 0.0765	0.95 (0.74-1.22) 0.6874	0.3667
rs10862193	0.80 (0.70-0.92) 0.0017	0.92 (0.74-1.14) 0.4232	0.98 (0.77-1.25) 0.8915	1.18 (0.90-1.55) 0.2248	0.85 (0.66-1.10) 0.2211	0.1744
rs10906086	1.07 (0.93-1.23) 0.3312	1.13 (0.91-1.40) 0.2562	1.06 (0.83-1.36) 0.6351	1.47 (1.12-1.94) 0.0058	0.91 (0.71-1.16) 0.4322	0.3453
rs4369899	0.97 (0.84-1.13) 0.7024	0.79 (0.63-1.01) 0.0565	0.93 (0.72-1.22) 0.6185	0.75 (0.56-1.00) 0.0493	0.95 (0.73-1.24) 0.7227	0.5992
rs12416605	1.10 (0.94-1.29) 0.2352	1.32 (1.04-1.68) 0.0242	1.20 (0.91-1.58) 0.1972	1.00 (0.73-1.37) 0.9904	1.32 (1.01-1.73) 0.0387	0.543
rs9911968	0.90 (0.79-1.03) 0.1419	0.97 (0.78-1.20) 0.7606	0.89 (0.70-1.13) 0.3302	0.90 (0.68-1.18) 0.4309	0.84 (0.66-1.07) 0.1665	0.4176
rs7000768	1.14 (0.98-1.33) 0.0825	1.15 (0.91-1.44) 0.2332	1.11 (0.86-1.44) 0.4258	1.26 (0.94-1.69) 0.123	1.05 (0.81-1.35) 0.7269	0.8192
rs10953326	0.93 (0.77-1.11) 0.4138	0.83 (0.63-1.11) 0.2121	0.84 (0.61-1.14) 0.2642	0.66 (0.46-0.95) 0.0262	0.94 (0.69-1.30) 0.7179	0.568
rs9907126	1.05 (0.91-1.22) 0.5143	1.33 (1.04-1.69) 0.021	1.08 (0.82-1.41) 0.5798	1.09 (0.81-1.47) 0.5616	1.46 (1.12-1.90) 0.0057	0.0224
rs2037128	0.75 (0.62-0.92) 0.0051	0.95 (0.69-1.30) 0.7518	0.72 (0.48-1.07) 0.103	1.11 (0.76-1.63) 0.577	1.00 (0.70-1.42) 0.9843	0.3471
rs1043681	0.88 (0.77-1.02) 0.0975	0.88 (0.69-1.11) 0.2846	0.87 (0.66-1.13) 0.2994	0.70 (0.51-0.96) 0.0249	1.02 (0.79-1.32) 0.8915	0.7496

Table 4A. MiRNA-related SNPs and risk of esophageal adenocarcinoma stratified by BMI

	BMI				
	<25	25-29.9	30-34.9	35+	
SNP	OR* (95% CI) P	OR* (95% CI) P	OR* (95% CI) P	OR* (95% CI) P	P-int#
kgp1460594	1.59 (1.11-2.26) 0.0107	1.38 (1.03-1.84) 0.0323	0.90 (0.60-1.35) 0.6124	0.89 (0.43-1.86) 0.7617	0.1117
rs9842591	1.26 (1.03-1.54) 0.0274	1.15 (0.98-1.35) 0.0793	1.17 (0.90-1.51) 0.2462	1.08 (0.73-1.59) 0.7021	0.7626
rs895819	1.10 (0.89-1.36) 0.3729	1.30 (1.10-1.54) 0.0026	0.79 (0.59-1.06) 0.1109	1.38 (0.91-2.11) 0.1287	0.5814
rs12461701	0.90 (0.71-1.14) 0.3756	0.92 (0.76-1.10) 0.3326	0.92 (0.69-1.22) 0.5462	0.67 (0.44-1.04) 0.073	0.5051
rs10899620	0.93 (0.71-1.22) 0.617	0.86 (0.70-1.05) 0.1376	0.91 (0.65-1.29) 0.6033	0.72 (0.43-1.21) 0.2142	0.2518
rs1799782	0.80 (0.50-1.26) 0.3341	0.70 (0.49-1.01) 0.0575	0.37 (0.19-0.75) 0.0057	0.43 (0.18-0.98) 0.0459	0.1075
rs1644730	0.73 (0.59-0.90) 0.0026	0.88 (0.75-1.03) 0.1186	0.86 (0.66-1.11) 0.2503	0.89 (0.59-1.34) 0.5633	0.1781
rs3174352	0.83 (0.67-1.02) 0.0736	0.86 (0.73-1.01) 0.0619	0.89 (0.69-1.15) 0.3891	0.83 (0.57-1.20) 0.3246	0.9686
rs3746794	0.73 (0.59-0.91) 0.0044	0.90 (0.77-1.06) 0.2129	0.99 (0.76-1.28) 0.9192	0.86 (0.58-1.27) 0.4374	0.2096
rs3209160	1.03 (0.77-1.39) 0.8246	1.29 (1.03-1.62) 0.0271	1.31 (0.91-1.89) 0.1471	1.37 (0.78-2.40) 0.28	0.205

* OR adjusted for age, sex, ev1, ev2, using additive model (per-allele), # P-value for coefficient of product term included in the logistic model

Table 4B. MiRNA-related SNPs and risk of Barrett's esophagus stratified by BMI

	BMI				
	<25	25-29.9	30-34.9	35+	
SNP	OR** (95% CI) P	OR** (95% CI) P	OR** (95% CI) P	OR** (95% CI) P	P-int#
rs595055	0.70 (0.54-0.90) 0.0054	0.81 (0.67-0.98) 0.034	0.93 (0.69-1.25) 0.6286	2.13 (1.21-3.74) 0.0084	0.0001
rs8192593	0.79 (0.50-1.26) 0.3231	0.78 (0.55-1.11) 0.169	0.50 (0.29-0.86) 0.0123	1.04 (0.44-2.44) 0.9279	0.6257
rs12534337	0.97 (0.64-1.46) 0.874	1.35 (0.99-1.85) 0.0581	2.23 (1.30-3.82) 0.0035	1.12 (0.53-2.36) 0.7665	0.2495
rs3785722	0.87 (0.73-1.03) 0.1023	0.90 (0.79-1.03) 0.1302	0.80 (0.65-0.98) 0.0355	0.94 (0.70-1.28) 0.7028	0.4693
rs10862193	0.86 (0.73-1.02) 0.0802	0.81 (0.71-0.93) 0.0023	0.81 (0.66-1.00) 0.0541	1.23 (0.91-1.66) 0.1745	0.159
rs10906086	1.26 (1.07-1.50) 0.0072	0.96 (0.84-1.10) 0.5682	1.09 (0.88-1.35) 0.4384	1.08 (0.80-1.46) 0.6194	0.0648
rs4369899	0.78 (0.64-0.93) 0.0071	0.91 (0.79-1.05) 0.2	1.15 (0.91-1.45) 0.2446	0.94 (0.67-1.33) 0.7371	0.0306
rs12416605	1.17 (0.96-1.42) 0.1199	1.16 (1.00-1.35) 0.0461	1.15 (0.91-1.45) 0.2406	0.86 (0.62-1.20) 0.378	0.0807
rs9911968	0.95 (0.80-1.12) 0.5119	0.91 (0.80-1.04) 0.1596	0.99 (0.81-1.22) 0.9416	0.76 (0.56-1.04) 0.0831	0.3346
rs7000768	1.02 (0.85-1.23) 0.8252	1.15 (1.00-1.32) 0.0503	1.08 (0.86-1.34) 0.5105	1.18 (0.84-1.65) 0.3308	0.7797
rs10953326	0.75 (0.60-0.95) 0.0154	0.94 (0.80-1.12) 0.5148	0.94 (0.71-1.23) 0.6345	0.73 (0.48-1.12) 0.1449	0.4598
rs9907126	0.98 (0.82-1.18) 0.8677	1.19 (1.03-1.39) 0.0197	1.28 (1.02-1.60) 0.0344	1.00 (0.71-1.39) 0.9851	0.7038
rs2037128	0.86 (0.67-1.10) 0.2294	0.86 (0.71-1.04) 0.128	0.88 (0.66-1.19) 0.4139	0.99 (0.58-1.68) 0.9726	0.305
rs1043681	0.89 (0.74-1.07) 0.2111	0.82 (0.71-0.94) 0.0053	1.01 (0.81-1.28) 0.9036	1.19 (0.85-1.66) 0.307	0.0761

** OR adjusted for sex, ev1-ev4, using additive model (per-allele), # P-value for coefficient of product term included in the logistic model

Figure S1. Selection of miRNA-related SNPs

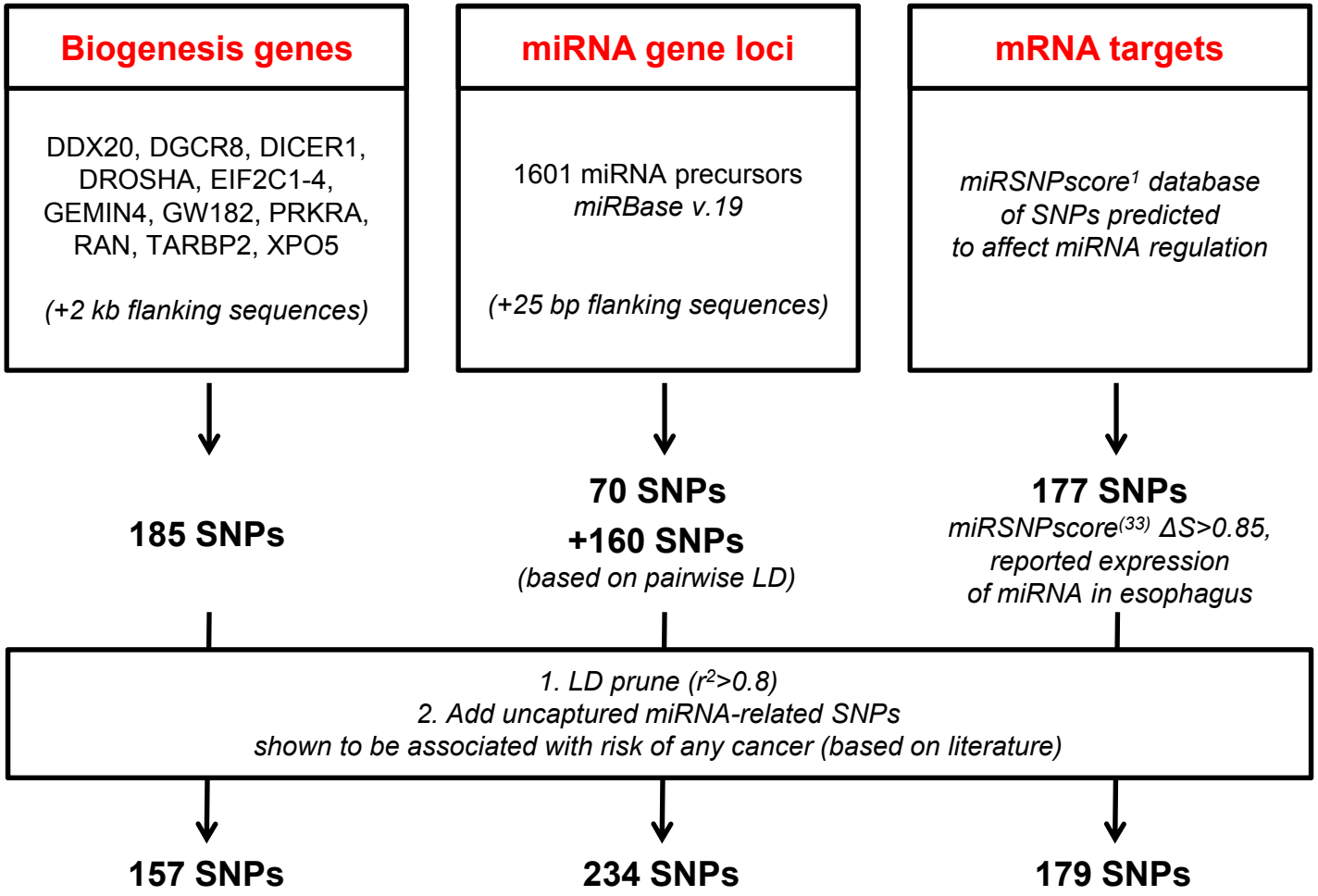

Table S1. miRNAs reported to be expressed in normal squamous epithelium of the esophagus, BE, dysplatic epithelium, or EA

1	let-7a	**32**	miR-30c	**63**	miR-148b	**94**	miR-215	**125**	miR-519d
2	let-7a-1	**33**	miR-30d	**64**	miR-149	**95**	miR-216a	**126**	miR-543
3	let-7a-2	**34**	miR-30e	**65**	miR-152	**96**	miR-216b	**127**	miR-548b-3p
4	let-7b	**35**	miR-31	**66**	miR-155	**97**	miR-219-5p	**128**	miR-557
5	let-7c	**36**	miR-32	**67**	miR-181a	**98**	miR-221	**129**	miR-560
6	let-7d	**37**	miR-33a	**68**	miR-181a-1	**99**	miR-222	**130**	miR-605
7	let-7f	**38**	miR-33b	**69**	miR-181a-2	**100**	miR-223	**131**	miR-615-3p
8	let-7i	**39**	miR-92-1	**70**	miR-181b	**101**	miR-224	**132**	miR-617
9	miR-7	**40**	miR-92a	**71**	miR-187	**102**	miR-326	**133**	miR-630
10	miR-10a	**41**	miR-93	**72**	miR-190	**103**	miR-330-5p	**134**	miR-636
11	miR-10b	**42**	miR-99a	**73**	miR-191	**104**	miR-338-3p	**135**	miR-663
12	miR-15b	**43**	miR-99b	**74**	miR-192	**105**	miR-338-5p		
13	miR-17	**44**	miR-100	**75**	miR-193a-3p	**106**	miR-342-3p		
14	miR-17-5p	**45**	miR-101	**76**	miR-193a-5p	**107**	miR-342-5p		
15	miR-20a	**46**	miR-103	**77**	miR-193b	**108**	miR-345		
16	miR-20b	**47**	miR-103-1	**78**	miR-194	**109**	miR-355-5p		
17	miR-21	**48**	miR-106a	**79**	miR-194-1	**110**	miR-369-3p		
18	miR-23a	**49**	miR-107	**80**	miR-195	**111**	miR-369-5p		
19	miR-23b	**50**	miR-125b	**81**	miR-196a	**112**	miR-370		
20	miR-24	**51**	miR-126	**82**	miR-197	**113**	miR-375		
21	miR-25	**52**	miR-135a	**83**	miR-199a*	**114**	miR-409-3p		
22	miR-26b	**53**	miR-140-3p	**84**	miR-199a-3p	**115**	miR-422b		
23	miR-27a	**54**	miR-140-5p	**85**	miR-199a-5p	**116**	miR-424		
24	miR-27b	**55**	miR-143	**86**	miR-199b-3p	**117**	miR-451		
25	miR-28-3p	**56**	miR-144	**87**	miR-199b-5p	**118**	miR-483-3p		
26	miR-28-5p	**57**	miR-145	**88**	miR-200a*	**119**	miR-494		
27	miR-29a	**58**	miR-146a	**89**	miR-200c	**120**	miR-497		
28	miR-29b	**59**	miR-146b-3p	**90**	miR-203	**121**	miR-509-3p		
29	miR-29c	**60**	miR-146b-5p	**91**	miR-205	**122**	miR-509-5p		
30	miR-30a	**61**	miR-147	**92**	miR-210	**123**	miR-513		
31	miR-30b	**62**	miR-148a	**93**	miR-214	**124**	miR-516a-5p		

Tables S2, S3, S4 available as Excel spreadsheets.

Table S5A. Association of SNPs in Table 2B [BE hits] with risk of esophageal adenocarcinoma

Biogenesis pathway

			Controls		EA cases				
SNP	**Gene**	**Alleles†**	**N**	**MAF‡**	**N**	**MAF‡**	**OR***	**95% CI**	**P**
rs595055	EIF2C1	G/A	2185	0.15	1517	0.14	0.94	(0.82-1.08)	0.37963
rs8192593	TARBP2	A/G	2185	0.04	1516	0.04	0.84	(0.66-1.08)	0.18102
rs11247946	LIN28	G/A	2187	0.35	1516	0.33	0.94	(0.85-1.04)	0.20245
rs2944760	EIF2C2	C/A	2185	0.2	1517	0.19	0.92	(0.81-1.03)	0.14782
rs538779	DDX20	A/G	2187	0.22	1517	0.22	0.99	(0.88-1.11)	0.81641
rs12741800	LIN28	A/G	2182	0.46	1515	0.46	0.98	(0.90-1.08)	0.7412
rs673019	DROSHA	G/A	2186	0.11	1517	0.1	0.95	(0.81-1.11)	0.51625
rs4351606	EIF2C3	A/G	2185	0.06	1515	0.05	0.85	(0.69-1.05)	0.13209
rs639174	DROSHA	T/C	2186	0.26	1517	0.25	0.97	(0.87-1.08)	0.56423

miRNA genes

			Controls		EA cases				
SNP	**Gene**	**Alleles†**	**N**	**MAF‡**	**N**	**MAF‡**	**OR***	**95% CI**	**P**
rs12534337	miR-4467	A/G	2186	0.04	1517	0.05	1.3	(1.04-1.63)	0.02364#
rs3785722	miR-1269b	T/C	2186	0.45	1516	0.42	0.93	(0.84-1.02)	0.11021
rs10862193	miR-617	G/A	2183	0.43	1516	0.42	0.94	(0.86-1.04)	0.23575
rs10906086	miR-548ak	C/A	2186	0.47	1516	0.47	1.02	(0.93-1.12)	0.64279
rs4369899	miR-4431	C/T	2187	0.34	1517	0.33	0.98	(0.88-1.08)	0.66276
rs12416605	miR-938	T/C	2186	0.25	1516	0.26	1.04	(0.93-1.16)	0.47194
rs9911968	miR-4520a/b	A/G	2184	0.49	1515	0.48	0.95	(0.86-1.04)	0.29253
rs7000768	miR-3686	G/A	2186	0.3	1517	0.32	1.1	(1.00-1.22)	0.05988
rs10953326	miR-4653	T/C	2186	0.18	1515	0.17	0.92	(0.82-1.05)	0.20773
rs9907126	miR-548at	C/T	2187	0.26	1516	0.28	1.05	(0.95-1.17)	0.33405
rs2037128	miR-944	T/C	2186	0.14	1517	0.14	0.98	(0.85-1.12)	0.73668
rs724714	miR-1343	G/A	2187	0.19	1516	0.2	1.04	(0.92-1.17)	0.56345
rs17036544	miR-559	G/A	2185	0.08	1517	0.08	1.01	(0.85-1.21)	0.87844
rs7188539	miR-5189	G/A	2186	0.33	1517	0.35	1.07	(0.97-1.18)	0.18526
rs12461701	miR-3188	A/G	2187	0.28	1517	0.26	0.87	(0.79-0.97)	0.01374#
rs2297333	miR-4642	C/T	2186	0.15	1517	0.13	0.91	(0.79-1.05)	0.18926
rs7211449	miR-548h-3	A/C	2186	0.22	1516	0.2	0.9	(0.80-1.02)	0.08907
rs17252270	miR-548x-2	T/C	2186	0.14	1517	0.14	1.01	(0.88-1.15)	0.93835

Table S5A [continued]. Association of SNPs in Table 2B [BE hits] with risk of esophageal adenocarcinoma

			mRNA targets						
			Controls		EA cases				
SNP	Gene	Alleles†	N	MAF‡	N	MAF‡	OR*	95% CI	P
rs1043681	THAP3	G/A	2187	0.32	1517	0.31	0.95	(0.86-1.05)	0.2898
rs3198005	TAP1	T/C	2187	0.05	1517	0.05	1.16	(0.94-1.44)	0.17567
rs1423380	ST8SIA4	G/A	2186	0.36	1517	0.36	1	(0.91-1.10)	0.98097
rs1043641	ACBD3	T/C	2187	0.17	1516	0.16	0.92	(0.81-1.04)	0.19195
rs1043420	CAPN5	C/T	2187	0.22	1517	0.23	1.05	(0.94-1.17)	0.4116
rs2023761	ZNF17	A/G	2187	0.06	1516	0.07	1.22	(1.00-1.48)	0.04921#
rs2075993	E2F2	C/T	2186	0.49	1517	0.51	1.12	(1.01-1.23)	0.02362#

† Minor/major alleles, ‡ Minor allele frequency, *OR adjusted for age, sex, ev1, ev2, using additive model (per-allele), # P<0.05

Table S5B. miRNA-related SNPs in Table 2A [EA hits] and risk of Barrett's esophagus

Biogenesis pathway

			Controls		BE cases				
SNP	**Gene**	**Alleles†**	**N**	**MAF‡**	**N**	**MAF‡**	**OR***	**95% CI**	**P**
kgp1460594	XPO5	A/G	2184	0.08	2414	0.08	1.05	(0.90-1.22)	0.53408

miRNA genes

			Controls		BE cases				
SNP	**Gene**	**Alleles†**	**N**	**MAF‡**	**N**	**MAF‡**	**OR***	**95% CI**	**P**
rs9842591	miR-5186	A/C	2184	0.46	2416	0.46	1.04	(0.96-1.13)	0.38952
rs895819	miR-27a	G/A	2171	0.33	2400	0.34	1.04	(0.95-1.14)	0.37257
rs12461701	miR-3188	A/G	2184	0.28	2415	0.26	0.9	(0.82-0.99)	0.03247#
rs10899620	miR-5579	C/T	2177	0.2	2408	0.2	1.01	(0.91-1.12)	0.82824
rs17880825	miR-4725	C/T	2184	0.02	2416	0.02	0.91	(0.67-1.23)	0.52848
rs1378940	miR-4513	G/T	2184	0.34	2416	0.33	0.96	(0.88-1.04)	0.3309
rs12534337	miR-4467	A/G	2183	0.04	2415	0.06	1.34	(1.10-1.63)	0.00328#
rs13005714	miR-3129	A/G	2177	0.08	2400	0.08	0.98	(0.84-1.13)	0.75141
rs17023366	miR-492	T/C	2183	0.06	2415	0.06	1.06	(0.89-1.26)	0.54096
rs3787547	miR-4756	A/G	2183	0.43	2416	0.42	0.93	(0.85-1.01)	0.07746
rs718079	miR-196a-1	T/C	2184	0.29	2415	0.3	1.06	(0.96-1.16)	0.2501
rs10849785	miR-4700	A/G	2184	0.05	2415	0.06	1.09	(0.91-1.30)	0.35292
kgp10521113	miR-4519	T/C	2180	0.38	2416	0.39	1.04	(0.96-1.13)	0.35851
rs12926295	miR-4519	G/A	2182	0.38	2412	0.39	1.04	(0.96-1.13)	0.36077

Table S5B [continued]. miRNA-related SNPs in Table 2A [EA hits] and risk of Barrett's esophagus

			mRNA targets						
			Controls		BE cases				
SNP	Gene	Alleles†	N	MAF‡	N	MAF‡	OR*	95% CI	P
rs1799782	XRCC1	A/G	2184	0.07	2416	0.06	0.94	(0.80-1.11)	0.47081
rs1644730	RDH8	T/A	2182	0.48	2414	0.46	0.93	(0.86-1.01)	0.09108
rs3174352	ASPN	C/T	2184	0.49	2414	0.5	1.03	(0.95-1.12)	0.42032
rs3746794	TBC1D20	A/G	2182	0.48	2414	0.46	0.94	(0.87-1.02)	0.14005
rs3209160	ZDHHC21	G/C	2179	0.13	2411	0.14	1.05	(0.93-1.18)	0.42871
rs2075993	E2F2	T/C	2183	0.51	2415	0.49	0.92	(0.85-1.00)	0.04693#
rs11169571	ATF1	C/T	2184	0.39	2415	0.4	1.03	(0.95-1.12)	0.46763
rs1914321	CPLX4	T/A	2176	0.18	2410	0.17	0.94	(0.84-1.04)	0.22958
rs1367	SCUBE2	G/A	2184	0.08	2415	0.07	0.92	(0.78-1.07)	0.28575
rs12140	ADAMTS1	G/A	2172	0.06	2408	0.06	1.16	(0.98-1.38)	0.08803
rs13835	DET1	A/C	2184	0.43	2416	0.43	1.01	(0.93-1.10)	0.85954
rs2023761	ZNF17	A/G	2184	0.06	2415	0.07	1.19	(1.00-1.42)	0.04616#
rs9804386	MORN4	C/T	2184	0.19	2416	0.21	1.1	(0.99-1.22)	0.07866

† Minor/major alleles, ‡ Minor allele frequency, **OR adjusted for sex, ev1-ev4, using additive model (per-allele), # P<0.05

Table S6. Four SNPs from the intersection of Tables 2A & 2B, and risk of esopheageal adenocarcinoma, Barrett's esophagus, or [EA/BE]

				EA*			BE**			EA/BE##		
	SNP	Gene	Alleles†	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
1	rs12534337	miR-4467	A/G	1.3	(1.04-1.63)	0.02364	1.34	(1.10-1.63)	0.00328	1.33	(1.11-1.59)	0.0023
2	rs12461701	miR-3188	A/G	0.87	(0.79-0.97)	0.01374	0.9	(0.82-0.99)	0.03247	0.89	(0.82-0.97)	0.00596
3	rs2075993	E2F2	T/C#	1.12	(1.01-1.23)	0.02362	0.92	(0.85-1.00)	0.04693	0.91	(0.85-0.98)	0.01596
4	rs2023761	ZNF17	A/G	1.22	(1.00-1.48)	0.04921	1.19	(1.00-1.42)	0.04616	1.21	(1.03-1.41)	0.01858

† Minor/major alleles

#Minor allele is "C" in the EA-only analysis

* OR adjusted for age, sex, ev1, ev2, using additive model (per-allele)

** OR adjusted for sex, ev1-ev4, using additive model (per-allele)

OR adjusted for age, sex, ev1-ev4 using additive model (per-allele)

Table S7A. Five significant miRNA-related SNPs from Ye et al. and risk of esophageal adenocarcinoma

			Controls		EA cases				
SNP	**Gene**	**Alleles†**	**N**	**MAF‡**	**N**	**MAF‡**	**OR***	**95% CI**	**P**
rs6505162	miR-423, -3184	C/A	2187	0.46	1517	0.45	0.98	(0.89-1.08)	0.72928
rs11614913	miR-196a-2	T/C	2186	0.41	1517	0.42	1.05	(0.95-1.15)	0.36125
rs14035	RAN	A/G	2187	0.31	1517	0.32	1.03	(0.93-1.14)	0.58301
rs213210	pri-miR-219-1	C/T	2184	0.07	1514	0.08	1.16	(0.97-1.38)	0.09716
rs11077	XPO5	G/T	2186	0.41	1517	0.42	1.03	(0.93-1.13)	0.59969

† Minor/major alleles, ‡ Minor allele frequency, * OR adjusted for age, sex, ev1, ev2, using additive model (per-allele)

Table S7B. Five significant miRNA-related SNPs from Ye et al. and risk of Barrett's esophagus

			Controls		BE cases				
SNP	**Gene**	**Alleles†**	**N**	**MAF‡**	**N**	**MAF‡**	**OR***	**95% CI**	**P**
rs6505162	miR-423, -3184	C/A	2184	0.46	2413	0.46	1.01	(0.93-1.10)	0.81798
rs11614913	miR-196a-2	T/C	2183	0.41	2413	0.41	0.99	(0.91-1.07)	0.76999
rs14035	RAN	A/G	2184	0.31	2415	0.31	0.99	(0.91-1.09)	0.90991
rs213210	pri-miR-219-1	C/T	2181	0.07	2414	0.07	0.95	(0.81-1.11)	0.51344
rs11077	XPO5	G/T	2183	0.41	2415	0.43	1.04	(0.96-1.13)	0.32589

† Minor/major alleles, ‡ Minor allele frequency, ** OR adjusted for sex, ev1-ev4, using additive model (per-allele)