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GOG 8020/210: Risk Stratification of Lymph Node Metastasis, Disease Progression and Survival Using Single Nucleotide Polymorphisms in Endometrial Cancer: an NRG Oncology/ Gynecologic Oncology Group study

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I have no conflicts of interest to report.

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Abstract

Objectives: The ability to stratify a patient's risk of metastasis and survival permits more refined care. A proof of principle study was undertaken to investigate the relationship between single nucleotide polymorphisms (SNPs) in literature based candidate cancer genes and the risk of nodal metastasis and clinical outcome in endometrioid endometrial cancer (EEC) patients.

Methods: Surgically-staged EEC patients from the Gynecologic Oncology Group or Washington University School of Medicine with germline DNA available were eligible. Fifty-four genes represented by 384 SNPs, were evaluated by Illumina Custom GoldenGate array. Association with lymph node metastases was the primary outcome. Progression-free survival (PFS) and overall survival (OS) was also evaluated.

Results: 361 SNPs with high quality genotype data were evaluated in 337 patients with outcome data. Five SNPs in CXCR2 had an odds ratio (OR) between 0.68 and 0.70 ($p\text{-value} \leq 0.025$). The A allele rs946486 in ABL had an OR of 1.5 ($p\text{-value}=0.01$) for metastasis. The G allele in rs7795743 in EGFR had an OR for metastasis of 0.68 ($p\text{-value}=0.02$) and hazard ratio (HR) for progression of 0.66 ($p\text{-value}=0.004$). Importantly, no SNP met genome wide significance after adjusting for multiple test correcting and clinical covariates. The A allele in rs2159359 SNP in NME1 and the G allele in rs13222385 in EGFR were associated with worse OS. Both exhibited genome wide significance; rs13222385 remained significant after adjusting for prognostic clinical variables.

Conclusion: SNPs in cancer genes including rs2159359 SNP in NME1 and rs13222385 in EGFR may stratify risk in EEC and are prioritized for further investigation.

INTRODUCTION

As the most common gynecologic malignancy and the fourth most common cancer affecting US women, endometrial cancer (EC) affects a large number of women, and it is estimated that 63,230 women will be diagnosed and 11,350 women will die of this disease in 2018 [1]. The presence of nodal disease is a strong predictor of patient outcomes. Approximately 15% of EC cases that appear to be early-stage have occult metastases found upon complete surgical staging, and five-year survival in patients with microscopic nodal disease is only 55% in some studies [2, 3]. The role of routine staging lymphadenectomy in apparent early-stage EC remains controversial in gynecologic oncology and has been challenged by two recent randomized prospective trials which failed to demonstrate an improvement in survival associated with lymphadenectomy [4, 5]. However, knowing that a patient has nodal

metastases enables tailored treatment to decrease recurrence and improve patient outcomes through the incorporation of adjuvant chemotherapy and/or radiation [6–8].

Lymph node assessment is an essential part of EC staging to stratify the risk of recurrence and guide adjuvant treatment. However, surgical lymphadenectomy increases procedure time and is associated with lymphocele formation, as well as the development of lymphedema in up to 38% of patients undergoing open surgery [9]. Although the role of sentinel lymph node dissection has also been an expanding approach, this procedure also requires additional expertise and carries with it similar risks. The ability to identify the subset of patients at highest risk for the presence of metastases preoperatively would allow physicians to restrict lymph node assessment to a smaller number of patients at the time of staging. This could present a significant advance in the care of patients with EC.

Metastatic disease accounts for approximately 90% of all cancer-related deaths [10]. While tumor size correlates with the risk of having metastases, the genetic background of the host in which a tumor arises may play an essential role in enhancing or suppressing the potential for a tumor to metastasize [11–13]. Inherited germline factors may provide a significant contribution to cancer's metastatic behavior and an individual's likelihood to develop metastases. Hunter *et al.* recently evaluated the effect of genetic background on metastatic behavior by crossing mice transfected with a highly metastatic polyoma mammary tumor into a variety of inbred strains. Significant variation of tumor metastases was observed between mice despite the fact that each animal had the same tumor-related transgene. This data indicates that the genetic differences between the mice lead to the differences in metastatic behavior of the tumor, and supports that germ-line polymorphisms may influence the different metastatic behavior of similar cancers in different individuals [11]. Additionally, gene expression microarray analysis of high and low metastatic phenotypes found gene expression differences between individuals, supporting that genetic background of an individual may be significant in how cancer behaves once it develops [3, 10, 11, 13].

The ability to predict who is at risk of nodal metastases, recurrence, progression, and death permits more individualized care for patients, providing the option of selectively performing lymphadenectomies in the subset at risk for metastasis and deploying the best adjuvant treatments for that patient's disease. The current research was designed as a proof of principle study to evaluate genetic polymorphisms as a predictor of nodal metastases in a case-control study of well-defined EC patients. We developed a list of candidate genes with published evidence of a role in metastasis and cancer development in EC or other cancers, selected tagging SNP in these genes, and analyzed their associations with metastasis, patient outcomes, and clinical characteristics. This was done with the intent to identify hypothesis generating SNPs for further evaluation in follow-up trials.

METHODOLOGY

PATIENTS AND SAMPLES

This study was designed as a case-control study using a combined cohort of patients with well-characterized endometrioid endometrial cancer (EEC) from an internal tumor bank maintained through the Washington University School of Medicine Division of Gynecologic

Oncology (WashU) and the NRG/Gynecologic Oncology Group (GOG) 210/8020 protocol. The Washington University, Human Research Protection Committee, approved this study. The GOG cases were accrued 2003–2007 and the Washington University cases accrued 1993–2010.

Controls were defined as patients with EEC confined to the uterus that demonstrated myometrial invasion (1988 FIGO stage IB-IC) with lymph nodes negative for metastases. Cases were defined as patients with at least one positive lymph node, but no other evidence of metastatic disease (1988 FIGO stage IIIC). As our primary aim was to identify predictors of specifically nodal metastases, stage IV patients were not included. Only patients with endometrioid histology were included, to minimize heterogeneity in the type of EC being evaluated. To be included in the study as a control (Stage IB-IC), the patient was required to have undergone surgical staging with lymphadenectomy. For cases, once the nodal disease was diagnosed in patients with stage IIIC disease, a full staging lymphadenectomy was not required. The race was limited to Caucasian to avoid any confounding population substructure factors. The combined GOG-210 and Washington University cohorts did not include sufficient numbers of non-Caucasian patients to conduct similar association analyses in racial groups other than Caucasians.

Samples were utilized from two sources. The GOG-210 protocol was an extensive molecular and surgical-pathological study of EC that opened in 2004. All patients were required to be surgically and pathologically staged, consistently evaluated, treated as appropriate, and followed for ten years. GOG-210 cases were augmented with an identical population but separate group of patients meeting the same criteria from an internal bank at Washington University School of Medicine. Samples were roughly matched between the Washington University and GOG-210 cohorts by stage, grade, and age of diagnosis within five years when possible. Samples were on separate patients, and no duplicate samples were used. Quality control analyses were performed to ensure that the patient characteristics and sample quality did not vary significantly between the Washington University cohort and the GOG cohort (see Supplemental Figures). The study flow is shown in Figure 1.

Development of the candidate gene list

A candidate gene approach was taken. An extensive literature review was performed of studies published on pubmed.gov to identify candidate genes and SNPs associated with metastases, the risk of cancer and cancer outcomes, EC pathways, and treatment response. We reviewed approximately 200 papers to make a preliminary list of genes. Weight was placed on genes with functional or nonsynonymous SNPs. Fifty-four such genes were identified, involved in cell cycle regulation, DNA repair, the metalloproteinase family, well-known tumor suppressor genes, and other vital processes. (Table 2). From the published literature, 166 specifically described SNPs were identified. To optimize gene coverage, this list was supplemented with a carefully designed set of tagging SNPs to cover the gene of interest, and were identified using HapMap data, the SNAGGER algorithm, and processed through Illumina’s design team for the probability of successful assay on the golden gate custom platform. Each gene was also expanded 3 KB upstream and downstream to cover the promoter and gene regulatory regions. 218 tagging SNPs were combined with 166

functional and literature reported SNPs to complete the list of 384 SNPs. The number of SNPs varied from 1–45 based on the size, complexity, and functional significance placed on the gene. This design also took into account consideration for linkage disequilibrium.

GENOTYPING

An Illumina Goldengate custom array was designed to interrogate 384 SNPs in our 54 candidate genes. Germline DNA was extracted from either blood or uninvolved/normal uterine tissue using standard methods. 368 patient samples were plated on four 96 well trays with four negative controls per tray and were randomly allocated across each tray to account for batch effects and control between the institution (WashU vs. GOG 210). Seven patients were later excluded for the wrong histology or stage, leaving a total of 361 patients included in the original statistical analysis. The Washington University School of Medicine Genome Institute core facility performed the custom Illumina Goldengate genotyping.

STUDY DESIGN AND STATISTICAL ANALYSIS

This case-control study was designed to generate proof of principal evidence to prioritize candidates for further investigation. SNPs with poor genotyping quality were removed before analysis; SNPs with a Hardy-Weinberg Equilibrium (HWE) $p < 0.001$, missing-ness < 0.05, and minor allele frequency < 0.05 were excluded. Tests for SNP associations with metastases and risk of recurrence (low versus high) were performed by logistic regression. SNPs were coded as an additive, with the variable encoding SNP status equal 0 for no copies of the minor allele, 1 for one copy, and 2 for two copies of the minor allele. The Bonferroni *q-value* correction for multiple testing was used. Progression-free survival (PFS) was evaluated using Cox regression modeling. Survival distributions for 1–2 vs. 0 minor alleles were compared using log-rank testing. A pre-study power analysis was performed to evaluate 368 SNPs genotyped in 163 cases and 198 controls, from Caucasian women diagnosed with EEC. The study was designed with an 80% power to detect an increase in the odds of metastatic disease for the minor allele of 2.4, 2, and 1.85, with minor allele frequencies of 0.10, 0.20, and 0.30 respectively. The study also had an 80% power to detect an increase in the risk of an event of 4.4, 2.9, and 2.5, respectively, for minor allele frequencies of 0.10, 0.20, and 0.30.

RESULTS

PATIENTS AND OUTCOMES

There were 337 eligible women were analyzed after excluding those for wrong stage or race (7) and those with insufficient clinical outcome data available (24). The clinical characteristics of these patients are presented in Table 1. Median patient age was 64 years old. One-hundred fifty cases had metastatic disease and were compared to 187 controls with no lymph node metastases. A total of 73 women in this cohort experienced recurrence or progression (41/150 with stage IIIC disease and 32/187 with stage I disease) at the time the dataset was locked for analysis.

GENOTYPING RESULTS

Of the initial 384 SNPs, eight were excluded based on HWE (*p-value*<0.001), three SNPs were dropped due to missing-ness (*p-value*<0.05), and 15 removed for minor allele frequency < 0.05. There were 361 evaluable tagging SNPs in 54 cancer genes included in the analysis (Table 2). The total genotyping rate for the eligible patients and SNPs was over 99%.

ASSOCIATIONS WITH METASTASIS

Logistic regression analysis for metastasis was performed, and SNPs were ranked based on their association with metastasis. There were 10 SNPs associated with metastasis with a *p*-value<0.05 (Table 3) including SNPs in epidermal growth factor receptor (EGFR), Abelson murine leukemia viral oncogene homolog 1 (ABL1), nucleoside diphosphate kinase 1 (NME1) and interleukin eight receptor B (CXCR2). None met genome wide significance after multiple test correcting and other clinical covariates. The SNP with the strongest association with metastases was rs1558544 in an intronic region of EGFR. The presence of the minor allele in rs1558544 was associated with a 46% lower risk of lymph node metastases (odds ratio [OR] 0.54, 95% confidence interval 0.37–0.79, q value 0.484). SNP rs7795743 in the EGFR had a consistent association with metastasis (OR 0.68, 95% CI 0.49–0.94), though not statistically significant (q > 0.05). Five SNPs in CXCR2 were associated with a consistent ~30% reduction in the risk of nodal metastases (OR 0.68–0.71, 95% CI ranged from 0.49–0.96, q value > 0.05). In contrast, the SNP rs946486 in ABL1 was associated with an increased risk of nodal metastases (OR 1.51, 95% CI 1.10–2.07, q value > 0.05). There were two SNP in NME1 with a non-statistically significant association with metastasis. The rs3760469 in NME1 had an OR of 1.45 (95% CI 1.05–1.99) whereas the rs16949649 had an OR of 0.71 (95% CI 0.52–0.97). The Bonferroni *q*-value for each of the SNP relationships with metastasis exceeded 0.05.

ASSOCIATIONS WITH PATIENT OUTCOMES

Cox regression modeling was used to rank the candidate SNPs based on their association with outcome. There were 124 disease recurrence or progression events, and 106 deaths. There were four SNPs that showed a trend towards increased risk of disease progression, though none met genome wide significance after adjusting for multiple test correcting and other clinical covariates. (Table 4). This included rs1322385 in EGFR (hazard ratio [HR] 1.62, 95% CI 1.24–2.11), rs845558 in EGFR (HR 1.49, 95% CI 1.14–1.95), rs4638843 in MSH2 (HR 1.69, 95% CI 1.19–2.39) and rs2159359 in NME1 (HR 1.50, 95 CI 1.12–2.01) (All q values > 0.05) There were also four other tagging SNPs in EGFR including rs845561, rs884225, rs884904, rs7795743 with a HR ranging from 0.38 to 0.66 (95% CI ranging from 0.21–0.87, q value > 0.05). The rs3168175 and rs3740640 in FGF4 were both associated with an HR of 0.45 (95% CI for both was 0.28–0.71). The Bonferroni *q*-value for each of the SNP relationships with PFS exceeded 0.05.

Two of the SNPS that were associated with PFS was also significantly associated with OS. The Bonferroni q-values for each of these SNPs and OS was < 0.05. The A allele in rs2159359 in NME1 was also associated with worse OS (Figure 2A, *q-value=0.043)*. In addition, the G allele in rs13222385 in EGFR was incrementally associated with worse OS

(Figure 2B, *q-value< 0.0001).* The relationship between rs13222385 and OS remained significant after adjusting stage, grade, age, and lymphvascular space invasion (LVSI) with an HR of 1.89 (95% CI 1.36–2.62). An exploratory analysis was then performed in stage I controls without metastatic disease and stage IIIC cases with nodal metastases at diagnosis. The association between rs13222385 and OS appeared to persist in both groups (Figure 2C–D).

DISCUSSION

This study was designed using a candidate gene approach to interrogate SNPs in key genes associated with metastases, or with functional importance in EC pathogenesis, behavior, and response to treatment. This was intended as a hypothesis generating proof of principle investigation to evaluate if any SNPs may be interesting for future evaluation in validation studies. Tagging SNPs were used to broadly and thoroughly cover the genes of interest and evaluate potential associations between the minor allele and EC events. Although none of the SNPS evaluated met genome wide significance or multiple test correcting for lymph node metastases or disease progression, (all q values > 0.05), the top candidates are further described here below. Five SNPs in CXCR2 had an odds ratio (OR) between 0.68 and 0.70 (*p-value≤0.025*), and the A allele in rs946486 in ABL had an OR of 1.5 (*p-value=0.01*) for metastasis. The G allele in rs7795743 in EGFR had an OR for metastasis of 0.68 (*p-value=0.*02) and a hazard ratio (HR) for progression of 0.66 (*p-value=0.004*). The A allele in rs2159359 in NME1 had an HR for progression of 1.50 (*p-value=0.007*), and this SNP also passed Bonferroni correction for association with worse OS (*q-value=0.043).* Even more striking, the G allele in rs13222385 in EGFR was strongly associated with worse OS in an incremental manner, and this association persisted after correcting for multiple test correcting and clinical factors including age, stage, and the presence of LVSI. This study yields proof of principal evidence to support further study of these relationships with risk stratification in EEC patients.

The reduced risk of lymph node metastasis associated with five tagging SNPs in CXCR2 was consistent with reports demonstrating that this chemokine receptor regulates cell adhesion, Wnt signaling, PI3-Kinase and self-renewal/senescence [14]. It is involved in some upstream signaling pathways and is an important regulator of inflammation and the tumor microenvironment (14). Moreover, SNPs in CXCR2 have been associated with metastases in cancers of the ovary, colon, pancreas, prostate, and breast [15–18]. The observations that the A allele in rs946486 in ABL1 indicated an increased risk in lymph node metastasis is supported by evidence that this non-receptor tyrosine kinase controls cell differentiation, division, adhesion, and stress response [19]. SNPs in ABL1 have been shown to be associated with ovarian cancer risk, and age of onset of esophageal adenocarcinoma while mutations in ABL1 have been linked with risk of leukemia [20–22].

The G allele in rs7795743 in EGFR was associated with a consistent reduced risk of lymph node metastasis and disease progression. In contrast, the G allele in rs13222385 in EGFR was associated with increased risk of disease progression (HR 1.62, 95% CI 1.24–2.11). The G allele was also very strongly associated with OS in an incremental manner after adjusting for multiple test correcting and clinical factors including age, stage, and the presence of

LVSI. EGFR, a receptor tyrosine kinase, is linked to multiple cancers and is often (60–80%) overexpressed in endometrial cancer [23]. It is interesting to speculate that this EGFR SNP may influence endometrial cancer behavior through the AKT pathway. A recent TCGA evaluation of glioblastoma patients found an association between rs13222385 and expression of the lanthionine synthetase C-like protein (LanCL2) which is a positive regulator of AKT activation, an important pathway in EC [24, 25]. EGFR regulates proliferation, invasion, angiogenesis and the tumor microenvironment [26, 27]. Given the complex multi-functionality activity of this gene, it is not surprising that the some of the tagging SNPs in EGFR indicated good vs. poor outcome in EEC and required further investigation to determine the causal relationships with metastasis and disease progression.

The A allele in rs2159359 in NME1 was associated with worse PFS and OS. Moreover, the relationship between this SNP and worse OS passed Bonfferoni *q-value* correction. NME1 appeared to be a metastases suppressor gene and decreased mRNA levels have been demonstrated in highly metastatic cell lines [28]. NME1 supplies GTP for G protein synthesis plays a role in cell signal transduction and regulates the c-Myc oncogene. Overexpression of NME1 appears to be protective in some studies including breast cancer and melanoma, and is a negative factor in colon cancer and neuroblastoma, suggesting a tissue-specific effect [29–31]. Additional studies will be required to investigation the relationship between the intronic rs2159359 SNP in NME1 and worse PFS and OS in a large series of EEC patients.

We also showed that two tagging SNPs in FGF4 with a reduced risk of disease progression. FGF4 is a signaling molecule with broad mitogenic and cell survival activities, including cell growth, morphogenesis, tissue repair, growth, and invasion. Amplification of the FGFR4 receptor has been associated with nodal metastases in breast and gynecologic cancers [32]. In contrast, the C allele in rs4638843 was associated with an increased risk of disease progression. Mutations in MSH2 are linked to HNPCC and the development of endometrial cancer and remains a pathway of interest [33].

The completion of the HapMap project and development of lower cost high throughput genotyping has allowed identification of millions of polymorphisms. Many have been associated with differences amongst individuals in drug metabolism, disease development, treatment response, and patient outcomes. However, specific polymorphisms often fail to hold up in validation studies, and proving a causal relationship can be challenging [34]. We designed this study using tagging SNPs to cover and evaluate candidate genes of interest, understanding that the SNPs identified may not be the causal SNP but instead travel in LD with the causal SNP yet to be identified. Additionally, each SNP may be a surrogate marker for another SNP that travels with it in linkage disequilibrium [35]. Therefore, emphasis should be placed on the association with the genes of interest more than specific SNPs.

There are limitations to this study. First, the samples were obtained from two different cohorts, approximately half from the GOG 210 study, and approximately half from a single institution introducing potential imbalance with respect to staging pathology interpretation, and patient care factors. Quality control measures were performed, demonstrating similar patient outcomes (See supplemental Figures 1–3), yet it is possible that unmeasured

differences may have existed in the patient populations or treatment received. More cases were obtained from the GOG cohort, and more controls were obtained from Wash U, introducing potential selection bias [36]. Importantly, none of the SNPs evaluated in this study met genome wide statistical significance after adjusting for multiple test correcting and clinical factors. Another limitation is the limited power to detect association and type I error given our modest sample size and the number of tests performed. Although a power analysis was done, our sample size calculation was done before genotyping, thus after excluding samples with poor genotyping quality adequate power may not have been present to detect a difference. Moreover, the Bonferroni correction was used in our analysis, assuming that our associations are all independent, though since many of our tagging SNPs may have been in linkage disequilibrium with each other, this may have been overly stringent. Another limitation is that our cohort is restricted to Caucasian women with stage IB, IC or IIIC EEC which represents a subset of the actual patient population, and therefore is not applicable to the population as a whole. SNPs in the genes studied here, or in other genes, could explain in part the differences in outcomes for white and black endometrial cancer patients. As genomic analyses become routine in cancer patient care it may be possible to use clinical testing results to evaluate SNP associations in non-Caucasian endometrial cancer cohorts. Nonetheless, this is a straight-forward evaluation of a carefully chosen list of candidate genes based on extensive literature review. Our study has one of the largest and best-characterized populations of endometrial cancer patients evaluated using a candidate gene approach reported to date. The results identify a list of genes implicated in metastasis and available for further study in clinically relevant models of endometrial cancer metastasis and recurrence, following the Oncotype Dx® model in breast cancer where literature supported candidate genes were evaluated with high throughput technology to identify genomic factors, both germline and somatic to stratify risk in cancer patients.

In conclusion, this study generated promising proof of principal evidence indicating that tagging SNPs in CXCR2 and FGF4 were consistently associated with a reduced risk of lymph node metastasis or disease progression, respectively, though in a non-statistically significant fashion. The A allele in rs2159359 in NME1 and G allele in rs13222385 in EGFR were both associated with worse PFS and OS. The relationships with OS remained significant after *Bonferroni q*-value correction. The rs13222385 in EGFR also remained significant after adjusting for prognostic clinical variables including age, stage, grade, and LVSI. Additional studies are needed to assess these variants further and evaluate for the ability for these or other related SNPS to advance risk stratification in EEC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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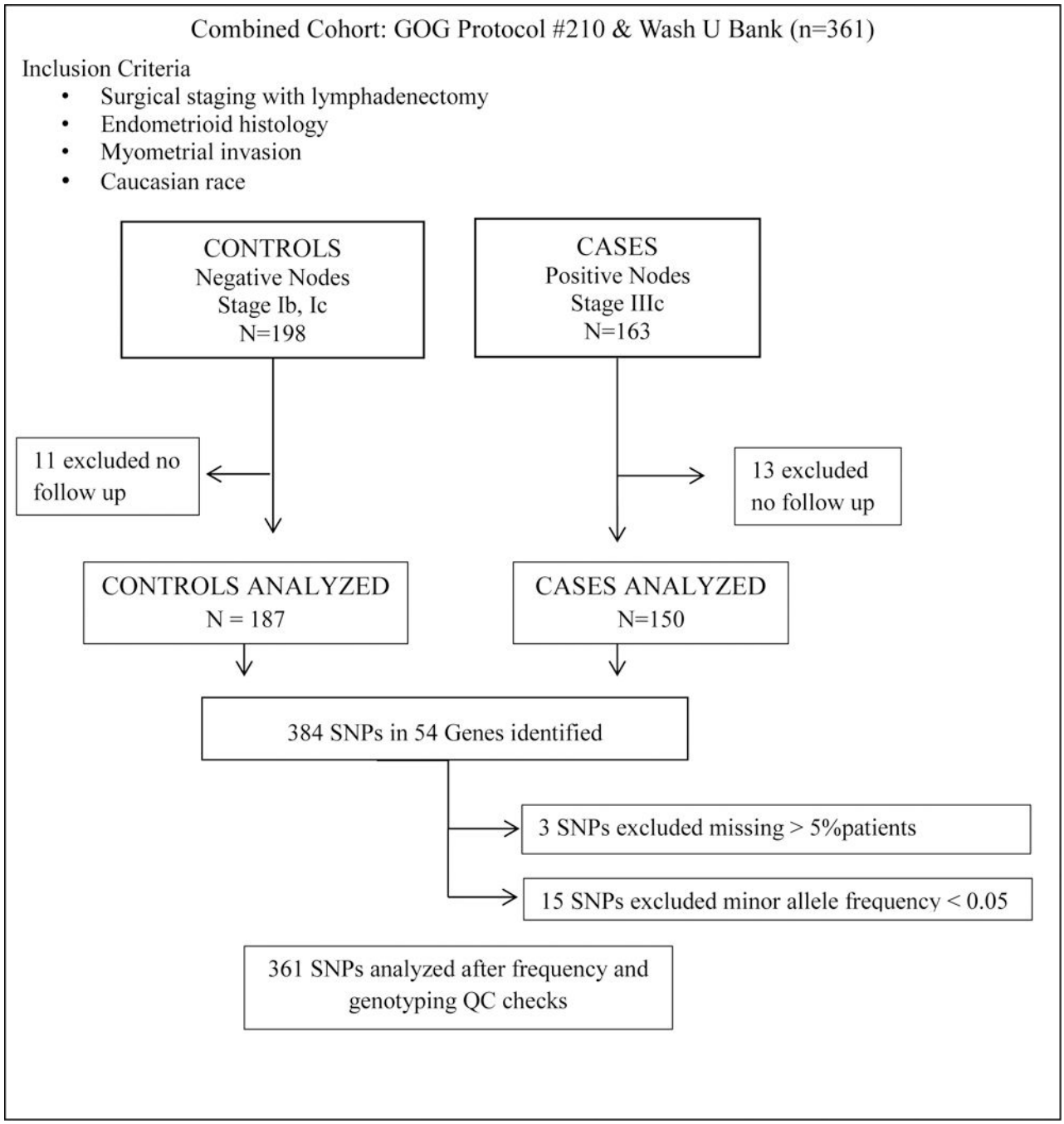

Figure 1:
Flow Diagram for the Candidate SNP Study

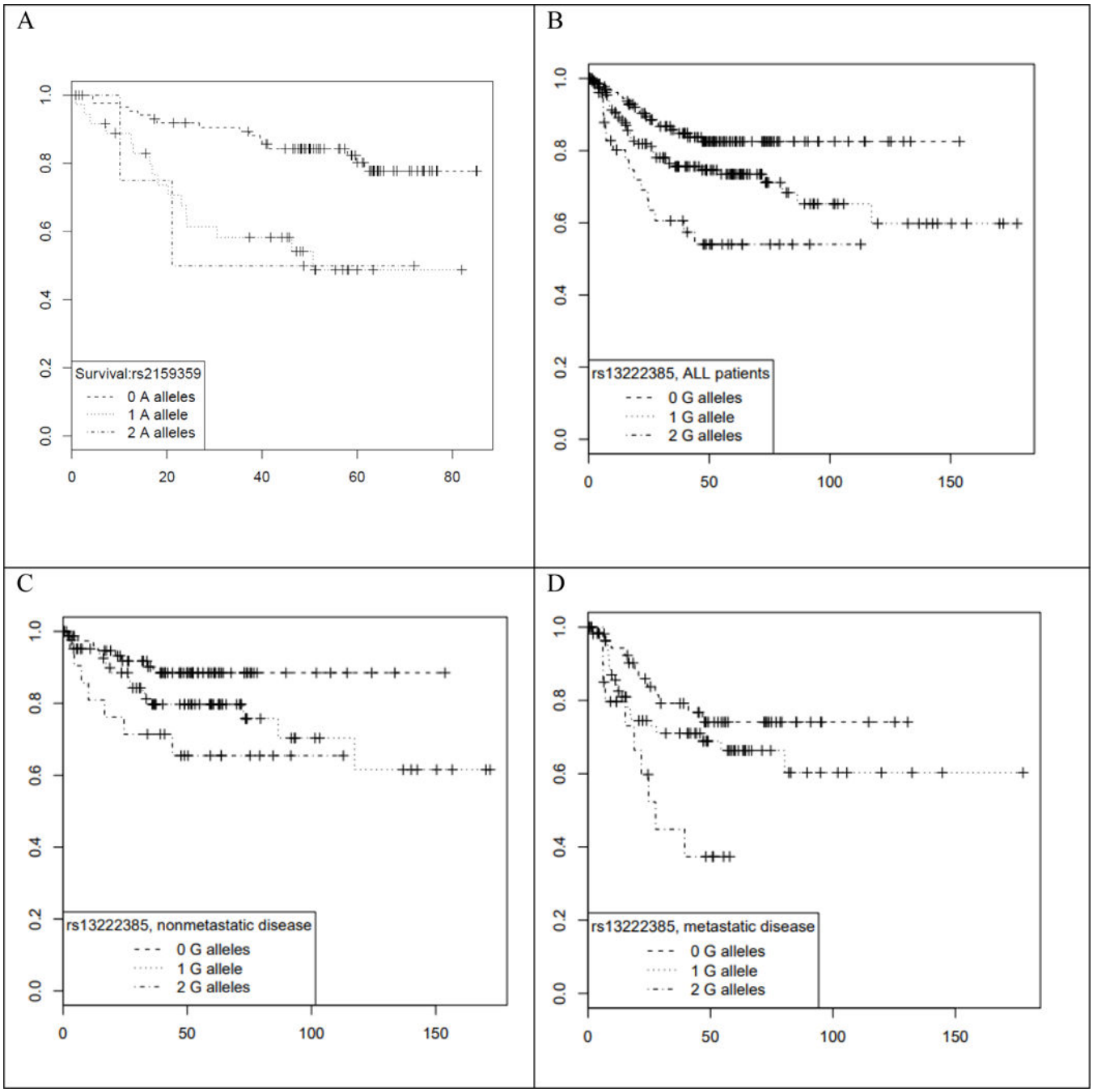

Figure 2:

Survival differences for the number of A alleles in rs2159359 or G alleles in rs13222385 (B-D). Survival distributions were compared using log rank testing and adjusted for multiple testing. **A**. The A allele in rs2159359 (NME1) is associated with decreased OS (q =0.043). **B**. The G allele in EGFR SNP rs13222385 is associated with worse OS in all patients (q < 0.0001). This effect persisted after adjusting for clinical prognostic variables including age, stage, and the presence of LVSI. **C-D**. An exploratory subset analysis of EGFE SNP rs13222385 in stage I controls without metastatic disease and stage IIIC cases with lymph

node metastases at diagnosis was performed and indicated a persistent relationship with survival in both subsets.

Table 1.

Patients characteristics (n=337).

Clinical Characteristics	No metastasis (control IB/IC) N=187 Number (%)	Metastasis (case IIIC) N=150 Number (%)	Median/ Total
Patient age (median)	66	63	64
FIGO 1988 Stage			
IB	60 (32%)	-	60
IC	127 (68%)	-	127
IIIC	-	150 (100%)	150
Grade			
1 well differentiated	41 (22%)	49 (33%)	90
2 moderately differentiated	44 (24%)	55 (37%)	99
3 poorly differentiated	102 (55%)	46 (31%)	148
Lymphvascular Space Invasion			
Absent	93 (50%)	27 (18%)	120
Present	87 (47%)	119 (79%)	206
Not specified	7 (3%)	4 (3%)	11

Characteristics of the 337 patients included in the final analysis, broken down by group (case *vs.* controls). All were endometrioid histology. Percentages broken down within their respective group (i.e. within cases *vs.* controls).

Table 2.

Candidate Genes Evaluated [number of SNPs per gene evaluated].

Cell Cycle Regulation	**DNA Repair**	**Proteinases**	**Steroid Metabolism**
MTOR [5] *AKT1* [3] *AKT2* [3] *CDC2* [3] *MAD1L1* [1] *MAD2L1* [10] *SIPA1* [6]	*MLH1* [2] *MSH2* [10] *MSH6* [8] *PMS2* [5] *BRCA1* [7] *ERCC1* [9] *MGMT* [3] *MUTYH* [2] *ATM* [5] *ATR* [9] *CHEK2* [1] *CKS1B* [1]	*MMP2* [7] *MMP3* [7] *MMP7* [3] *MMP8* [10] *MMP9* [7] *MMP12* [5] *MMP13* [7]	*CYP11A1* [2] *STAR* [1]
Cell Proliferation		**Cell Adhesion**	**Hormone Action**
FGF4 [5] *ABL1* [12] *NME1* [8]		*CDH1* [8] *APC* [4]	*ESR1 (ER-alpha)* [1]
Angiogenesis	**Signaling**	**Drug Efflux**	**Tumor Suppressor**
VEGFA [8]	*EGFR* [45] *ERBB2* [7] *FGFR2* [16] *FGFR4* [3] *KRAS* [8] *LRRFIP2* [1] *PIK3CA* [27]	*ABCB1* [12] *ABCC2* [8] *ABCG2* [9]	*PTEN* [9] *TP53* [2]
Apoptosis		**Chemokine**	**Inflammation**
MDM2 [1] *MDM4* [10] *FBXO11* [1]		*CXCR4* [1] *IL8* [2] *CXCR2* [5]	*COX2* [6]

Candidate genes are listed here by pathway or gene function, and the number of SNPS interrogated per gene that met quality control criteria is listed after the gene symbol in [ ]. Between 1–45 SNPs per gene were evaluated, including a 3 KB up- and downstream of gene to cover the promoter and regulatory regions within the gene.

Table 3.

Top Candidate SNPs Associated with Metastases

Gene Name	SNP	Region	Minor Allele	Odds Ratio*	95% Confidence Interval	*p*-value	Adjusted q-value$
Epidermal Growth Factor Receptor (EGFR)[a]	rs1558544	Intron	A	0.54	0.37 – 0.79	0.001	0.484
	rs7795743	Intron	G	0.68	0.49 – 0.94	0.020	1
Nucleoside Diphosphate Kinase 1/2 (NME1/2)[b]	rs3760469	Upstream 2KB	A	1.45	1.05 – 1.99	0.023	1
	rs16949649	Upstream 2KB	G	0.71	0.52 – 0.97	0.033	1
c-ABL Oncogene 1(ABL)[c]	rs946486	Intron	A	1.51	1.10 – 2.07	0.0102	1
Interleukin 8 Receptor-β (CXCR2) [d]	rs4674258	Intron upstream 2 KB	G	0.68	0.49 – 0.94	0.012	1
	rs4674259	Intron 5' UTR	G	0.68	0.50 – 0.92	0.014	1
	rs6723449	Intron	A	0.70	0.52 – 0.94	0.019	1
	rs1126579	3' UTR	A	0.70	0.52 – 0.94	0.019	1
	rs4674257	Upstream 2KB	A	0.70	0.52 – 0.96	0.025	1

*Odds ratio for metastases using an unadjusted additive model for allelic dosage, evaluating 1 or 2 minor alleles verses no minor alleles (reference group).

$Adjusted for stage (IIIC vs IB or IC), grade (3 vs 1 or 2), age at diagnosis, institution and LVSI (present vs absent) and multiple test correcting.

[a]regulates proliferation, survival, invasion, metastasis, angiogenesis [chromosome 7].

[b]MAPK scaffolding protein, reduced mRNA transcript levels in highly metastatic cells [chromosome 17].

[c]regulates cell differentiation [chromosome 9].

[d]also called cytokine receptor 2 regulates tumor microenvironment [chromosome 2].

Table 4.

Top Candidate SNPs Associated with Progression.

Gene Name	SNP	Region	Minor Allele	Hazard Ratio*#	95% Confidence Interval	*p*-value	Adjusted *p*-value$
Epidermal Growth Factor Receptor (EGFR)[a]	rs13222385	Intron	G	1.62	1.24 – 2.11	0.000	0.147
	rs845561	Intron	G	0.56	0.39 – 0.80	0.001	0.524
	rs884225	3' UTR	G	0.38	0.21 – 0.69	0.002	0.584
	rs884904	Downstream 500B	A	0.38	0.21 – 0.69	0.002	0.584
	rs845558	Intron	A	1.49	1.14 – 1.95	0.003	1
	rs7795743	Intron	G	0.66	0.49 – 0.87	0.004	1
Fibroblast Growth Factor 4 (FGF4)[b]	rs3168175	Intron	C	0.45	0.28 – 0.71	0.001	0.274
	rs3740640	Downstream 500B/intron	G	0.45	0.28 – 0.71	0.001	0.274
MutS Homolog 2 (MSH2) [c]	rs4638843	Intron	C	1.69	1.19 – 2.39	0.003	1
Nucleoside Diphosphate Kinase 1/2 (NME1/2)[d]	rs2159359	Intron	A	1.50	1.12 – 2.01	0.007	1

*Hazard ratio for progression with cox regression modelling using an unadjusted additive model for allelic dosage, evaluating 1 or 2 minor alleles verses no minor alleles (reference group).

#Events include women who recurred or experienced disease progression while alive and those who died due to disease progression.

$Adjusted for stage (IIIC vs IB or IC), grade (3 vs 1 or 2), age at diagnosis, institution and LVSI (present vs absent) and multiple test correcting.

[a]regulates proliferation, survival, invasion, metastasis, angiogenesis [chromosome 7].

[b]Oncogenic growth factor with broad mitogenic and cell survival activities [chromosome 11].

[c]Mismatch repair gene [chromosome 2].

[d]MAPK scaffolding protein, reduced mRNA transcript levels in highly metastatic cells [chromosome 17].