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Polymorphisms in *MMP9* and *SIPA1* are associated with increased risk of nodal metastases in early stage cervical cancer

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Abstract

Objective—Heritable polymorphisms modulate metastatic efficiency in cancer. Single nucleotide polymorphisms (SNPs) in *MMP9* (rs17576) and *SIPA1* (rs746429, rs931127) have been associated with nodal metastases in multiple cancers. We investigated the association of these SNPs with nodal metastases in early stage cervical cancer.

Methods—Consecutive patients with stage IB cervical cancer who underwent a pelvic lymph node (LN) dissection were included. Cases (≥ 1 positive LN, n=101) were compared with controls (negative LN pathology, n=273). Genotyping was performed on genomic DNA in the 3 SNPs using a Taqman assay, and correlated with clinical variables.

Results—The G allele at *SIPA1* rs931127 was associated with an increased risk of nodal disease (OR 1.9, p=0.03), and approached significance at *SIPA1* rs746429 (OR 2.2, p=0.09) and *MMP9* rs17576 (OR 1.5, p=0.08). In patients with stage Ib1 lesions (n=304), the G allele at both *SIPA1* SNPs were associated with LN metastases (rs746429 OR 10.1, p=0.01; rs931127 OR 2.4, p=0.01). In patients with no lymphovascular space invasion, *SIPA1* SNPs were again associated with LN metastases, and all patients with nodal disease had at least one G allele at *SIPA1* rs746429.

Conclusions—In this case control study, SNPs in *SIPA1* varied statistically in cervical cancer patients with and without nodal metastases, and in *MMP9* after controlling for stage and lymphovascular space invasion. Further work is needed to characterize inherited polymorphisms that provide a permissive background for the metastatic cascade.

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Article Precise: SNPs in *MMP9* (rs17576) and *SIPA1* (rs746429, rs931127) differ between cervical cancer patients with and without nodal metastases and after controlling for clinicopathologic factors.

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INTRODUCTION

Despite advances in screening and prevention, cervical cancer remains a significant source of morbidity and mortality worldwide, accounting for a remarkable number of life years lost. [1–3] Cervical cancer remains the only gynecologic malignancy that is clinically staged; however clinical assessment is accurate in only 75% of cases, mostly due to the inability of clinical staging to predict patients with nodal metastases.[4] Patients with disease that is outside the pelvic and para-aortic fields targeted by radiation therapy are likely to die of cancer, and even pelvic nodal metastases within the radiation field significantly decrease five year survival from 82–90% to 38–61% in patients with Stage I disease.[5]

Increasing evidence suggests that genetic factors may contribute to the likelihood of metastases, providing a permissive background environment in which further somatic mutations or epigenetic events may lead to the full metastatic cascade potential. [6–9] Family studies in bladder and renal cell cancer have shown that good and poor survival aggregates in families, suggesting a genetic contribution to cancer related prognosis.[10] Approximately 5% of patients with widespread cancer present with either a small well differentiated lesion, or no identifiable primary tumor even at autopsy.[6] These patients may demonstrate a high metastatic potential genotype, upon which few secondary events are necessary to obtain metastatic spread. Hunter *et al.* recently bred mice transfected with a highly metastatic polyoma mammary tumor to a variety of inbred strains, and found significant variation in the tumor metastatic capacity despite each animal having the same transgene, supporting the influence of germline polymorphisms on the development of metastatic disease.[7]

A variety of genes have been implicated in metastatic disease in multiple cancer types, and strong evidence supports *SIPA1* and *MMP9* as key metastases modifier genes, therefore chosen for investigation in this study. Signal induced proliferation associated gene 1 (*SIPA1*) is a GTPase activating protein involved in mitogen induced cell cycle regulation and is important in disease progression. Haplotype mapping and linkage studies between high and low metastatic phenotypes have shown *SIPA1* to be a possible genetic susceptibility modifier locus in a mouse model, and polymorphisms in *SIPA1* in a mouse model had a major effect on metastatic potential of aggressive mammary tumor cell line.[6][11] In breast cancer patients, two SNPs in *SIPA1* were associated with an increased risk of nodal involvement: the 313G>A polymorphism (rs931127) in the promoter and exonic polymorphism 2760G>A (rs76329).[6, 12] Matrix metalloproteinases (MMP) are a family of proteolytic enzyme implicated in tumor invasion, angiogenesis, and metastasis, and *MMP9* specifically has been described in pancreatic tumor cell angiogenesis and growth, and correlated with advanced stage at presentation in neuroblastoma.[13,14] Multiple studies have examined functional SNPs in *MMP9*. Specifically, rs17576 in the enzymatic binding motif has strong evidence to support its role in the occurrence and metastatic spread in lung cancer [15], cutaneous malignant melanoma transit [16], and metastases but not invasion in gastric cancer.[17] Additionally, multiple gene expression studies support the importance of *MMP9* in metastatic disease.[18] [19][20][21]

The ability to identify patients preoperatively who are at high risk of metastatic disease could allow the physician to tailor initial therapy and to individualize surveillance protocols. Additionally, it may identify molecular markers that may be targets for cancer therapy or chemoprevention. [22–26] The purpose of this case control study was to evaluate differences in allele frequency in one SNP in *MMP9* (rs17576) and two SNPs in *SIPA1* (rs746429 and rs931127) between patients with and without nodal disease at diagnosis of clinical stage Ib cervical cancer.

MATERIALS AND METHODS

Patient Population

Consecutive patients with stage IB cervical cancer were identified through an ongoing tissue collection and clinical database in place at Washington University School of Medicine and the Siteman Cancer Center. Only patients who had a complete pelvic and paraortic nodal dissection were included in this analysis. For the purpose of this study, cases were defined as patients with nodal disease present at the time of radical hysterectomy or lymph node dissection (n=101), and controls were defined as patients with negative nodes on hysterectomy specimen (n=273). The Washington University Human Research Protection Office approved this study.

All patients (both cases and controls) meeting the above criteria with genomic DNA samples available were included in the analysis, which resulted in approximately two controls being defined per case. Clinical information had already been extracted from patient medical records, self completed questionnaire, and pathology reports, and entered into the database. Variables assessed included age, race, stage, lymph node status (positive or negative), histology, years from diagnosis to last follow up, vital status at last follow up, HPV typing (if available), and presence or absence of lymph vascular space invasion. The primary outcome of this study was the incidence of single nucleotide polymorphism in cases versus controls. Additional analyses were performed to investigate associations with the above variables that may also influence the presence of metastatic disease and patient outcomes.

Genotyping

DNA was extracted from blood or buccal samples for SNP analysis, as well as from snap frozen tissue or formalin fixed, paraffin-embedded tissue for HPV analysis by overnight digestion with proteinase K, purified with phenol/chloroform and then quantified on the NanoDrop/ND-1000 spectrophotometer as described by Zhang et al.[27] Individual SNPs were evaluated using a 384-well format with four 96 well trays containing 3µL/well of sample DNA (0.5 ng/µL) on a 7900HT Real-Time PCR System (Applied Biosystems, Foster City, CA). Commercially available primers were used from ABI in *MMP9* (rs17576, 279A>G) and *SIPA1* (rs931127, 313G>A and rs746429, 2760G>A) to assess allele frequency amongst cases and controls. Cases and controls were randomly alloquotted in equal proportions on 96 well preparation tray, with two negative controls and one positive control for the primer also included. Overall genotyping success rates was between 97 and 98% and similar between cases and controls, with 6 (2%) samples failed for rs17576 (2 cases, 4 controls), 10 (2.6%) for rs931127 (6 cases, 4 controls), and 13 (3.4%) for rs746429 (6 cases, 7 controls).

Statistical Analysis

Overall frequency was compared between cases and controls, with additional subgroup analyses performed looking at specific variables described above. All genotyping data was blinded to clinical and pathologic data. Single SNP association tests was performed using regression models with appropriate covariates.[28] The genomic distribution of each SNP was correlated with clinical variables. Frequencies were calculated for the number of samples successfully genotyped or each SNP. Data was analyzed using the chi-square and Fischer's Exact Test, with 95% confidence intervals calculated where appropriate.

RESULTS

Patient characteristics of the 101 patients with nodal disease present (cases) and 273 patients with negative lymph node sampling (controls) are shown in Table 1. Mean age at diagnosis was similar for cases (42.6) and controls (40.0) (p=0.052). Cases and controls were also similar with respect to race. Squamous histology was more common than adenocarcinoma among

cases compared to controls (81% versus 65.3%, $p<0.05$). The groups differed slightly with respect to stage, with 71% of cases and 84% of controls having stage IB1 tumors. ($p<0.01$) Specific treatment regimens received are also shown in Table 1. The majority (79%) of controls received radical hysterectomy primarily, with 17% of patients receiving additional treatment based on tumor characteristics despite negative lymph node pathology. Of patients with negative lymph nodes, 80% received radiation with or without chemotherapy, and 70% of these also underwent radical hysterectomy with their initial treatment.

The overall allele frequencies for the 3 SNPs included in the study as well as for subgroup analyses are shown in table 2 (*MMP9*, rs17576, 279A>G), Table 3 (*SIPA1*, rs746429, 2760G>A) and Table 4 (*SIPA1*, rs931127, 313G>A). A significant difference between cases and controls was present at the promoter SNP in *SIPA1*; at rs931127 having at least one G allele almost doubled the risk of LN metastases (OR 1.9, 95% CI 1.1–3.5). Overall allele frequency approached significance for the other two SNPs investigated. For *SIPA1* rs746429, the G allele was associated with an increased risk of nodal metastases (OR 2.2 (95% CI 0.9–6.5). For *MMP9* rs17576, patients with the GG genotype tended to have positive LN metastases at diagnosis (OR 2.0, 95% CI 0.9–4.6). No significant association was found in haplotype analysis of the two SNPs in *SIPA1* ($p=0.36$), likely due to a limited sample size.

In order to better understand possible relations between SNPs in these key three genes with nodal spread in cervical cancer, additional analyses were done investigating specific clinicopathologic features, including tumor size/stage, histology, and presence of lymph vascular space invasion. No consistent statistically significant associations were found between cases and controls amongst the different treatment regimens received (hysterectomy, chemotherapy, and radiation) or amongst the 181 patients with HPV 16 and 18 typing available. Results are summarized in Tables 2–4.

Tumor Size

To account for variations in tumor size amongst patients with stage IB cervical cancer, patients were separated into those with IB1 and IB2 lesions to investigate if size of the lesion and genetic polymorphism may interact and influence the development of metastatic disease. Both polymorphisms in *SIPA1* were significant in patients with smaller stage IB1 tumors. Having at least one G allele increased the risk of nodal metastases at both rs746429 (OR 10.1, 95%CI 1.6–417.9) and at rs931127 (OR 2.43, 95%CI 1.18–4.95). In patients with stage IB2 cervical cancer, having at least one G allele in *MMP9* rs17576 was marginally associated with an increased likelihood of nodal disease (OR 2.81, 95%CI 0.83–9.69). However, polymorphisms in *MMP9* were not significant in smaller lesions and polymorphisms in *SIPA1* were not significant in larger lesions.

Lymph vascular space invasion

Lymph vascular space invasion (LVSI) is an independent poor prognostic factor in cervical cancer patients and was therefore evaluated. Interestingly, both SNPs in *SIPA1* were significant in patients without, but not with LVSI. The GG genotype was associated with a significantly higher risk of nodal disease in both SNPs. At rs746429, no cases had the AA genotype compared to 12.2% of controls; odds ratios could not be calculated as AA was the reference allele. At rs931127 the GG genotype was associated with a significantly higher risk of nodal disease as well. (OR 12.4, 95% CI 1.4–567.5). Polymorphisms in *MMP9* were not associated with nodal disease in patients with or without LVSI.

Histology

To account for differences in histology between cases and controls, SNP frequency was evaluated amongst the different histologic types. Amongst the 99 patients with

adenocarcinoma, the G allele at rs17576 (*MMP9*) was associated with nodal disease (OR 8.2, 95% CI 1.7–78.4). Neither SNP in *SIPA1* was associated with nodal metastases in patients with adenocarcinoma (rs746429 $p=0.9175$, rs931127 $p=0.502$). However, no significant associations were found in any of the three polymorphisms and squamous histology.

Though not the primary outcome, no significant survival differences were found amongst cases and controls for any of the three SNPs investigated (Log rank test rs17576 $p=0.692$, rs746429 $p=0.906$, rs931127 $p=0.933$)

DISCUSSION

Compelling evidence supports a genetic link towards susceptibility to metastatic disease in cancer. In this case control study, we demonstrate that polymorphism in *MMP9* and *SIPA1*, genes implicated as potential metastases modifier genes, may play a role in the development of nodal metastases in cervical cancer patients. The G allele at rs931127 in *SIPA1* was associated with nodal disease in overall cases and controls (OR 1.9, 95% CI 1.1–3.5), and in the other two SNPs approached statistical significance (*SIPA1* rs746429 OR 2.2, 95% CI 0.9–6.5; *MMP9* rs17576 OR 1.5, 95% CI 0.9–2.6). Additional significant associations were found in *MMP9* in patients with adenocarcinoma, and in *SIPA1* in patients with stage IB1 cancers and in those without LVSI.

The size at which a tumor is more likely to metastasize may be different amongst individuals. In this study, we found an association between the G allele in both polymorphisms in *SIPA1* with a risk of metastatic disease among patients with smaller, stage IB1 tumors. The histologic finding of lymph vascular space invasion has been associated with aggressive behavior and poorer prognosis than those lesions without this finding. It is interesting that in our cohort, both polymorphisms in *SIPA1* were significant in patients without lymph vascular space invasion. Given that patients with LVSI are more likely to have nodal metastases, evaluating SNP frequency in patients without LVSI serves as a useful control to evaluate the impact of polymorphisms as a predisposing factor for cervical cancer metastases, prior to the histologic finding of LVSI. Perhaps SNPs in *SIPA1* lower the metastatic threshold for tumor to spread to lymph nodes prior to the histologic finding of LVSI on tumor section. The GG genotype at rs931127 was associated with a significantly higher risk of nodal metastases while at rs746429 all patients with nodal metastases had at least one G allele. It is possible that these polymorphisms may predispose patients towards earlier lymphatic spread by a different mechanism yet to be elucidated.

Strong evidence implicates *SIPA1* as a metastases modifier gene. *SIPA1* is a mitogen induced GTPase activating protein that may enhance or hinder cell cycle progression and plays a role in regulating cell adhesion.[29,30] Crawford *et. al.* found significant associations between SNPs in *SIPA1* to be associated with metastases in breast cancer patients. However, in their study, the AA genotype was associated with increased risk of nodal disease at both rs746429 and rs931127.[12] In our cohort, the opposite was true, with the GG genotype at both sites associated with increased risk of nodal metastases, with even one G allele significantly increasing the odds of nodal disease. The reason for this difference is not completely understood, though several potential explanations exist. Crawford's study included patients with breast cancer and this study focuses on cervical cancer, cancers at separate tissue organ sites with different characteristics, under different hormonal and cytokine influences, and the tumor biology may be inherently different.

The patient population in which genetic studies are done is one key factor, and it is possible that the patients include in Crawford's study exhibit a different genetic profile than those of our population. Associations of opposite alleles at the same locus even within the same disease

has been described and called “flip-flop” phenomenon. This phenomenon can be attributed to different factors, such as population differences that influence differences in linkage disequilibrium.[31,32] It is also possible that SNPs in *SIPA1* may serve as a proxy for a separate functional SNP yet to be identified. It is possible that the development of nodal metastases is a complex process, and single locus associations may be confounded by environmental factors as well as other loci that may be yet to be identified.[31] Cases and controls did not differ with respect to race, and the three SNPs selected for this study demonstrate similar frequencies between patients of different races.

Matrix metalloproteinases (MMP) are a family of proteolytic enzyme implicated tumor invasion, angiogenesis, and metastasis, and several members of the MMP gene family have been evaluated as modifiers of cancer risk. While polymorphisms in MMP2, 3, and 8 have been associated with risk of development and metastatic disease in esophageal, gastric, and breast cancer respectively, MMP9 was chosen as a focus of this study.[33–35][36] Matrix metalloproteinase 9 has been implicated in multiple types of cancer development, growth, angiogenesis, and metastases. The catalytic domain of *MMP9* allows it to bind to its substrate elastin; the 279G>A polymorphism (rs17576) in exon 6 results in substitution of a positively charged arginine with uncharged glutamine, affecting the binding capacity of the enzyme. [37,38] In 744 lung cancer patients compared to 747 controls, the 279GG genotype was associated with an increased risk of occurrence and metastases [15] and in 74 cases of gastric carcinoma, the 279GG genotype was also associated with increased risk of lymph node metastases.[17]. In our cohort, we found the GG genotype associated with nodal metastases in patients with stage IB2 tumors and in those with adenocarcinoma histology.

Our study is inherently limited by study design and relatively small sample size, which weakens our ability to solidify statistical associations, as demonstrated by some of the large confidence intervals of some of our odds ratios. This is seen in the homozygous high risk genotype groups. Due to a small sample size, we were unable to perform multivariate analysis of the associations found in this study. Additionally, important clinical characteristics including the presence of parametrial disease and the treatment modality received were not analyzed. We identified significant associations in tumors ≤ 4 cm (Stage IB1) and those without LVSI. However, multiple testing and subgroup analysis can lead to false positive associations and this should be validated in additional studies. Survival was not significant in this study likely due to the early stage of all subjects and variable treatments. The overall 5-year survival rate for node negative was 97% and node positive 80%.

Metastatic spread of cancer is a complex process in which multiple host genetic and environmental factors interplay to influence a tumor’s metastatic potential. This is an active and exciting area of ongoing research. In this case control study, a functional SNP in *MMP9* (rs17576) varied significantly in patients with adenocarcinoma histology. Polymorphisms in *SIPA1* (rs746429 and rs931127) varied significantly in Stage IB1 cervical cancer patients and those without LVSI, and between overall cases and controls at rs931127. Further work is warranted investigating the role of these and other polymorphisms in metastatic susceptibility to cervical cancer. In addition, fine mapping and sequencing will be needed to identify a functional variant for *SIPA1*.

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Table 1

Patient Characteristics

Patient Characteristics	Cases (n=101)		Controls (n=273)		P value
	n	%	n	%	
Ib					
Ib1	75	74.3	239	88.2	<.01
Ib2	26	25.7	32	11.8	
Race					
White	79	79.0	235	87.7	0.09
Black	20	20.0	30	11.2	
Asian	1	1.0	2	0.7	
Hispanic	0	0	1	0.4	
Cancer type					
Squamous cell carcinoma	81	81.0	177	65.3	<.05
Adenocarcinoma	16	16.0	86	31.7	
Neuroendocrine	3	3.0	8	3.0	
Treatment Details					
RH only	12	11.9	216	79.1	<0.001
RH + Radiation (+/-) chemo	57	56.4	46	16.8	
Radiation +/- chemo only	24	23.8	1	0.4	
Specific details unknown	8	7.9	10	3.7	

RH = Radical hysterectomy

* P-value based on fishers' test

Table 2

<i>MMP9</i> rs17576 279A>G	CONTROLS		CASES		P value	OR (95% CI)
	n	%	n	%		
Overall (n=364)						
AA	121	45.7	3	35.4		Reference
GA	119	44.9	9	49.5	0.17	1.4 (0.8–2.4)
GG	25	9.4	1		0.05	2.0 (0.9–4.6)
GA/GG	144	54.3	6	15.2	0.08	1.5 (0.9–2.6)
total	265	100	9	64.6		
IB1 (n=308)						
AA	102	43.6	2	35.1		Reference
GA	107	45.7	3	51.4	0.25	1.4 (0.8–2.6)
GG	25	10.7	1	13.5	0.30	1.6 (0.6–3.9)
GA/GG	132	56.4	4	64.9	0.20	1.4 (0.8–2.6)
total	234	100	7	100		
IB2 (n=56)						
AA	19	61.3	9	36.0		Reference
			1		0.25	

<i>MMP9</i> rs17576 279A>G	CONTROLS			CASES		
	n	%	n	%	P value	OR (95% CI)
GA	12	38.7	1	44.0		1.9 (0.5–7.0)
GG	0	0	5	20.0	0.01*	**
GA/GG	12	38.7	1		0.06	
total	31	100	6	64.0		2.8 (0.8–9.7)
No LVSI (n=160)						
AA	61	41.8	5	35.7		Reference
GA	70	47.9	8	57.1	0.58	1.4 (0.4–5.7)
GG	15	10.3	1	7.1	0.86	0.8 (0.02–8.1)
GA/GG	85	58.2	9	64.2	0.22	1.5 (0.7–3.3)
total	146	100	1	100		
Adenocarcinoma (n=99)						
AA	47	56.0	2	13.3		Reference
GA	27	32.1	1	73.3	0.006	9.6 (1.8–92.8)
GG	10	11.9	2	13.3	0.11	4.7 (0.3–69.7)
GA/GG	37	44.0	1		0.002	
total	84	100	3	86.6		8.3 (1.7–78.4)
total			1	100		
total	84	100	5	100		

* Estimated using Fisher's Exact test

** Could not be estimated

Table 3

SIPAI rs746429 2760G>A	CONTROLS		CASES		P value	OR (95% CI)
	n	%	n	%		
Overall (n=358)						
AA	34	12.9	6	6.4		Reference
GA	118	44.7	41	43.6	0.15	2.0 (0.7–6.1)
GG	112	42.4	47	50.0	0.06	2.4 (0.9–7.4)
GA/GG	230	87.1	88	93.6	0.09	2.2 (0.9–6.5)
total	264	100	94	100		
IB1 (n=301)						
AA	30	12.9	1	1.5		Reference
GA	107	46.1	34	49.3	0.01	9.5 (1.5–400.1)
GG	95	41.0	34	49.3	0.01	10.7 (1.6–450.6)
GA/GG	202	87.1	68	98.6	0.01	10.1 (1.6–417.9)
total	232	100	69	100		
IB2 (n=57)						
AA	4	12.5	5	20.0		Reference
GA	11	34.4	7	28.0	0.41	0.5 (0.1–3.4)
GG	17	53.1	13	52.0	0.52	0.6 (0.1–3.5)
GA/GG	28	87.5	20	80.0	0.44	0.6 (0.1–3.1)
total	32	100	25	100		
No LYSI (n=160)						
AA	18	12.2	0	0.0		Reference
GA	59	40.1	2	15.4	1.0*	**
GG	70	47.6	11	84.6	0.21*	**
GA/GG	129	87.7	13	100	0.36*	**
total	147	100	13	100		

SIPA1 rs746429 2760G>A	CONTROLS		CASES		P value	OR (95% CI)
	n	%	n	%		
Adenocarcinoma (n=101)						
AA	9	10.5	1	6.7		Reference
GA	43	50.0	9	60.0	0.57	1.9 (0.2-91.6)
GG	34	39.5	5	33.3	0.81	1.3 (0.1-69.5)
GA/GG	77	89.5	14	93.3	0.65	1.6 (0.2-76.8)
total	86	100	15	100		

* Estimated using Fisher's Exact test
** Could not be estimated
* OR not calculable if zero value within cell

Table 4

SIPA1 rs931127 313G>A	CONTROLS		CASES		P value	OR (95% CI)
	n	%	n	%		
Overall (n=362)						
AA	89	33.3	2	21.1		Reference
			0			
GA	123	46.1	5	54.7	0.03	1.9 (1.0–3.6)
			2			
GG	55	20.6	2	24.2	0.07	1.9 (0.9–3.9)
			3			
GA/GG	178	66.7	7	78.9	0.03	1.9 (1.1–3.5)
			5			
total	267	100	9	100		
			5			
IB1 (n=305)						
AA	82	34.9	1	18.6		Reference
			3			
GA	106	45.1	4	58.6	0.01	2.4 (1.2–5.3)
			1			
GG	47	20.0	1	22.9	0.06	2.2 (0.9–5.3)
			6			
GA/GG	153	65.1	5	81.4	0.01	2.4 (1.2–5.0)
			7			
total	235	100	7	100		
			0			
IB2 (n=57)						
AA	7	21.9	7	28.0		Reference
			1			
	17	53.1		44.0	0.51	

SIPA1 rs931127 313G>A	CONTROLS		CASES		P value	OR (95% CI)
	n	%	n	%		
GA			1			0.7 (0.2–2.9)
GG	8	25.0	7	28.0	0.86	0.9 (0.2–4.7)
	25	78.1	1	72.0	0.59	
GA/GG			8			0.7 (0.2–2.9)
total	32	100	2	100		
	5					
No LYSI (n=160)						
AA	53	36.1	1	7.7		Reference
GA	64	43.5	5	38.5	0.17	4.1 (0.4–199.4)
GG	30	20.4	7	53.8	0.007	12.4 (1.4–567.5)
	94	63.9	1	92.3	0.04	6.8 (0.9–294.5)
GA/GG			2			
total	147	100	1	100		
			3			
Adenocarcinoma (n=100)						
AA	32	37.2	3	21.4		Reference
GA	41	47.7	8	57.1	0.30	2.1 (0.4–13.0)
GG	13	15.1	3	21.4	0.30	2.5 (0.3–20.5)
	54	62.8	1	78.5	0.25	2.2 (0.5–12.9)
GA/GG			1			
total	86	100	1	100		
			4			