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Kallikrein-Related Peptidase 3 (*KLK3/PSA*) Single Nucleotide Polymorphisms and Ovarian Cancer Survival

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There is substantial evidence suggesting a role for hormone-regulated kallikrein-related peptidases (KLKs) in carcinogenesis and tumour metastasis. KLKs are considered to have potential as prognostic biomarkers for hormone dependent cancers, particularly ovarian cancer. The purpose of this study was to evaluate the association between *Kallikrein-related peptidase 3* (*KLK3*) gene single nucleotide polymorphisms (SNPs) located in hormone response elements and ovarian cancer survival. DNA samples were analyzed from 304 Australian women diagnosed with epithelial ovarian cancer. The *KLK3* rs266882 and rs11084033 SNPs were genotyped by the Sequenom iPLEX Mass Array platform. Hazard ratios (HR) and 95% confidence intervals (CI) were estimated using Cox regression models. An association was observed with ovarian cancer survival for homozygote carriers of the rare allele of rs11084033 (adjusted HR 2.12, 95% CI 1.08–4.15). This finding is consistent with bioinformatic analysis predicting the rs11084033 rare allele to be responsible for the loss of a confirmed androgen response element, and with published expression data suggesting that aggressive ovarian cancers show decreased *KLK3* tumor expression. The rs11084033 has potential prognostic significance in ovarian cancer. However, this finding requires replication, and further investigation regarding the functional significance of rs11084033 and correlated SNPs.

■ **Keywords:** *kallikrein-related peptidase 3 SNP,* ovarian cancer, survival, prognosis, hormone dependant cancers

Ovarian cancer is sixth most common cause of cancer death among women worldwide, and the leading cause of death due to gynecological malignancies (Parkin et al., 2005). Ovarian cancer is often associated with vague symptoms and consequently approximately 75% of women are diagnosed with advanced stage disease (FIGO stage III or IV; Fisch & Bruera, 2003). The prognosis of women with advanced stage disease is greatly reduced with a 56-year survival rate of 30% compared with a 90% 5-year survival rate among women diagnosed with early stage disease (FIGO stage I; Fisch & Bruera, 2003).

The kallikrein-related peptidases (KLKs) are a family of serine proteases that have been identified as potential diagnostic and prognostic cancer biomarkers because of their altered expression in hormone-related cancer. The most well-known and well-researched member of this family is KLK3, more commonly known as prostate specific antigen (PSA), which is the current diagnostic marker for prostate cancer (Borgono et al., 2004; Clements et al., 2004; Obiezu & Diamandis, 2005). With regard to a role in ovarian cancer, the KLKs are generally upregulated in cancer versus normal tissue (Borgono et al., 2004; Obiezu

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& Diamandis, 2005). However, increased expression of some KLKs (KLK4, KLK5, KLK6, KLK7 and KLK10) is reported to be associated with *poor* survival after ovarian cancer, whereas increased expression of other KLKs (KLK8. KLK9, KLK13 and KLK14) is reported to be associated with *improved* survival (Borgono et al., 2004; Obiezu & Diamandis, 2005). Only limited studies have been performed investigating *KLK3* expression in ovarian cancer: one microarray study has identified *KLK3* to be overexpressed in ovarian cancer compared with the adjacent normal tissue (Adib et al., 2004) and another identified *KLK3* expression to be decreased in tumors of serous invasive ovarian cancer compared with those of low malignant potential (Gilks et al., 2005). This would suggest that *decreased KLK3* expression may be a marker of *poor* prognosis for ovarian cancer.

Single nucleotide polymorphisms (SNPs) in the *KLK* genes that influence gene expression levels have the potential to be useful prognostic or predictive markers. A number of *KLK3* SNPs, have been reported to be associated with increased prostate cancer risk (Borgono et al., 2004; Lai et al., 2007). A well-characterized polymorphism (rs266882, -158G>A) is found within androgen response element I (AREI), which has been shown to alter androgen driven PSA expression by decreasing the response element's ability to bind the androgen receptor (Lai et al., 2007). Studies investigating the association of this SNP with prostate cancer have been conflicting (Jesser et al., 2008). While our study reported an increased risk of prostate cancer associated with the A-allele (Lai et al., 2007), a meta-analysis of all rs266882 association studies performed to date by Jesser et al., (2008) was not able to confirm these findings. However, issues that could affect these findings, such as selection of controls by PSA screening were not discussed by the authors. The SNP rs11084033 (A-4233C) is located within androgen response element V (AREV) of *KLK3* (Huang et al., 1999), and was originally identified by direct sequencing of prostate cancer samples from our laboratory (Prof Clements). Androgens have been implicated in the etiology of ovarian cancer (Wang & Chang, 2004), and have also been linked to other ovarian disease such as polycystic ovarian syndrome (Nisenblat & Norman, 2009). These two SNPs, selected because of their location within androgen response elements of the *KLK3* gene, were assessed for their association with survival among an Australian ovarian cancer patient cohort.

Materials and Methods

Study Participants

This study included 304 women diagnosed with primary invasive epithelial ovarian cancer between 1985 and 1997. Over half of the women (n = 199, 65%) had participated in a large population-based case-control study of the etiology of ovarian cancer (Purdie et al., 1995). The remaining women (n = 105, 35%) were ascertained as incident cases from the Royal Brisbane Hospital (RBH), Queensland, Australia (Spurdle et al., 2001). The characteristics of the cases have been reported elsewhere (Nagle et al., 2007). The study was approved by the Institutional Ethics Committees of the University of Queensland, The Queensland Institute of Medical Research and all participating hospitals where the women were originally diagnosed and treated.

Data Collection

Clinical and pathologic information including disease stage (using the International Federation of Gynecologists and Obstetricians [FIGO] criteria), tumor histologic subtype and grade and treatment was abstracted retrospectively from the women's medical records and pathology reports or, for a subset of incident RBH cases, from the RBH Gynecology Oncology database. Full details have been reported previously (Nagle et al., 2003). The women were followed for mortality using personal identifiers that were linked to the Australian National Death Index (NDI), state cancer registry records and the RBH Gynecology Oncology database (Nagle et al., 2006).

DNA Extraction and Genotyping

DNA was extracted as described in previous studies (Marsh et al., 2007; Nagle et al., 2007) and genotyping of the *KLK3* SNPs rs266882 and rs11084033 were completed using the Sequenom iPLEX MassArray platform (San Diego, CA, USA) according to manufacturer instructions. To determine the effect of rs11084033 on AREV and rs266882 on AREI, *in silico* analysis was performed using the SigScan program (http://www-bimas.cit.nih.gov/molbio/signal/) with default parameters (Prestridge, 1991). Two hundred and ninety-nine women and 304 women were successfully genotyped for rs266882 and rs11084033 respectively.

Statistical Methods

Survival time was calculated from date of diagnosis to date of death (from ovarian cancer) or censored at September 1, 2004, or death from another cause. The Kaplan-Meier technique was used to plot crude survival curves and estimate crude overall survival probabilities, and adjusted hazard ratios (HR) and 95% confidence intervals were obtained from Cox regression models. Hazard ratios and 95% CIs were adjusted for age (< 40, 40–49, 50–59, 60–69, 70+), FIGO stage (I, II, III, IV), histologic grade (1, 2 3–4) and subtype (serous, mucinuous, endometrioid, clear cell and other). Haplotypes were inferred for each individual using software PHASE (Stephens et al., 2001). Haplotypes with a frequency of less than 5% were pooled, and per-haplotype HR was estimated relative to the most common haplotype. All statistical analyses were performed using the Statistical Packages for Social Sciences for Windows, version 13.0 (SPSS Inc., Chicago, IL).

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Results

Characteristics of Study Participants

Among the 304 women with ovarian cancer, 182 (60%) died from the disease during the follow-up period, giving a 5-year survival proportion of 48%. Selected clinical and pathologic characteristics of the women are shown in Table 1. A little over three-quarters of the women were older than ≥ 50 years at diagnosis (77%), and many presented with late stage disease (72%) and high-grade tumors (54%). Serous was the most common histological subtype of ovarian tumor (64%).

TABLE 1

Descriptive Characteristics of the Women Included in the Study

	N[1] (%)
Age group (years)	
< 40	21 (7)
40–49	48 (16)
50–59	79 (26)
60–69	92 (31)
70+	61 (20)
FIGO stage	
I	49 (16)
II	34 (11)
III	83 (63)
IV	26 (9)
Histologic subtype	
Serous	189 (64)
Mucinuous	21 (7)
Endometrioid	32 (11)
Clear cell	21 (7)
Other	32 (11)
Histologic grade	
Well differentiated	37 (13)
Moderately differentiated	92 (33)
Poorly/undifferentiated	148 (54)

Note: [1] Numbers may not sum to total because some data is missing

Survival and Bioinformatic Analysis

Both the rs266882 and rs11084033 genotyping distributions met Hardy-Weinberg equilibrium criteria. Results from Cox regression analysis are shown in Table 2. There was no association observed between the rs266882 *KLK3* SNP and ovarian cancer survival, before or after adjustment. The Kaplan-Meier survival curves (Figure 1) show a clear survival disadvantage for those women with the rs11084033 *AA* genotype, and in the adjusted analyses, patients with a homozygous variant genotype demonstrated poorer 5-year survival than those with a wild-type rs11084033 genotype (adjusted HR 2.12, 95% CI 1.08–4.15, $p = .04$, Table 2. When analyzed under a dominant model, A-allele carriers demonstrated poorer 5-year survival compared with non-carrier, although this was not significant (adjusted HR 1.26, 95% CI 0.91–1.74). The linkage

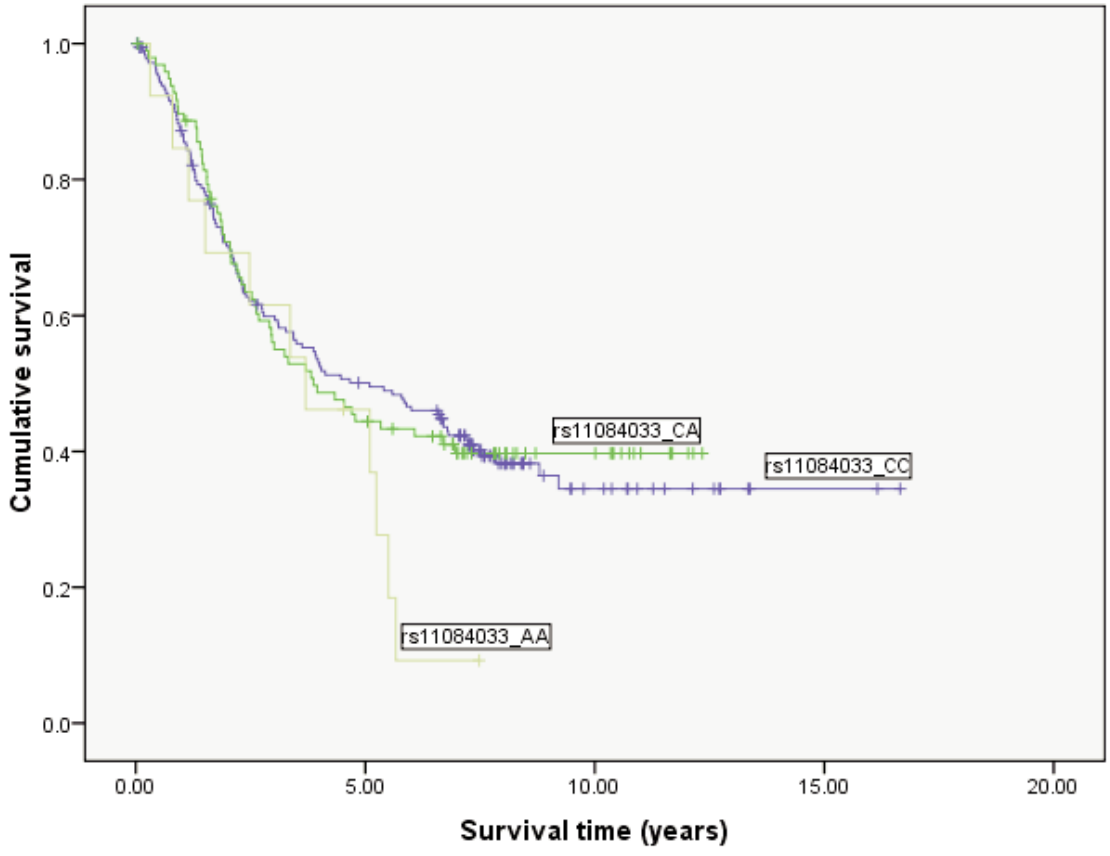

FIGURE 1

Kaplan-Meier survival curve for *KLK3* rs11084033.

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TABLE 2

Association Between *KLK3* Polymorphisms and Ovarian Cancer Survival

	Patients *n* (%)	Patients dead *n* (%)	5-year survival (%)	Adjusted[1] HR[2] (95% CI[3])
rs266882				
AA	95 (32)	56 (31)	53	1.0
GA	117 (39)	72 (40)	41	1.12 (0.77-1.62)
GG	87 (29)	52 (29)	50	1.10 (0.73-1.64)
rs11084033				
CC	190 (63)	108 (61)	50	1.0
CA	101 (33)	57 (32)	44	1.18 (0.84-1.66)
AA	13 (4)	11 (6)	46	2.12 (1.08-4.15)

Note: [1]Adjusted for age, FIGO stage, histologic grade and subtype
[2] HR — hazard ratio
[3] CI — confidence interval

disequilibrium observed between rs11084033 and rs266882 was low (r^2 = .15). Haplotype analysis was performed to assess if haplotypes may provide a better prediction of disease survival. No significant association of haplotypes with ovarian cancer was observed (data not shown). Bioinformatic analysis of rs266882 using SigScan showed that the AREI remained after introduction of the G allele (the effect of the SNP on AR binding is not assessed by this program). Analysis of rs11084033 predicted that AREV is abolished on introduction of the A-allele, (TTTCGAAGTT*TGTCCC*AGTATAA to TTTGCAAGTTTGTC*A*CAGTATAA).

Discussion

To our knowledge, this is the first study to examine the association of *KLK3* polymorphisms with ovarian cancer survival. KLK3 is the current diagnostic marker for prostate cancer and its expression has been associated with prognostic features of this disease (Borgono et al., 2004; Obiezu & Diamandis, 2005). Considering ovarian and prostate cancers are both hormone-related diseases, it seems conceivable that KLK3 may also be important in ovarian cancer biology, although there has been limited research on this topic. In this study, we have observed poorer 5-year survival among patients carrying the rs11084033 homozygote rare allele genotype after adjustment for age, grade and stage. This SNP falls within an androgen response element (AREV) in the *KLK3* enhancer region (Hunag et al., 1999). The functional effects of rs11084033 on this response element have not been tested in vitro, and the effect of this variant on *KLK3* expression level thus remains to be determined. However, bioinformatic analysis of rs11084033 predicted that the introduction of the rare variant results in the abolishment of AREV, which may consequently result in decreased expression of *KLK3*. Taken together, this suggests decreased expression of *KLK3*, caused by the loss of AREV by rs11084033, may explain the observed association with poorer ovarian cancer survival. Our findings are consistent with a microarray study reporting decreased expression of *KLK3* in serous invasive ovarian tumors compared with tumors of low malignant potential ovarian (Gilks et al., 2005). Although counter-intuitive to what is known with respect to PSA regulation in prostate cancer, the involvement of PSA in ovarian cancer is poorly understood and may not replicate that seen in prostate cancer. Moreover, there are several other KLKs that are upregulated in ovarian cancer versus normal tissue, but which are markers of improved prognosis, namely KLK8, KLK9 and KLK14 (Borgono et al., 2004; Obiezu & Diamandis, 2005). Although it is perhaps surprising that rs266882 was not found to be associated with ovarian cancer survival despite the reported functional significance of this SNP, this may also reflect some fundamental differences in regulation of PSA expression between prostate and ovarian cancer, namely differential tissue-specific co-regulator binding at the allelic change in the AREI as suggested for rs266882 in prostate cell lines (Lai et al., 2007), which may drive differential ovarian expression.

In conclusion, the rs11084033 SNP predicted in silico to abolish AREV of the *KLK3* gene was observed to be associated with poorer prognosis of ovarian cancer, and may be a potential prognostic marker for this disease. However, this finding requires replication, and further investigation regarding the functional significance of rs11084033 and correlated SNPs in ovarian cancer. Although the value of this SNP as a potential prognostic marker may be reduced by the low frequency of the AA genotype, the results nevertheless have implications for understanding the biology of ovarian cancer prognosis.

It should be noted that neither of the SNPs analyzed in this study are genotyped, nor are in strong linkage disequilibrium with SNPs genotyped by the Illumina Human 1M Duo Beadchip commonly used for genome-wide association studies. Therefore candidate gene studies will be required to replicate the findings of this study.

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References

Adib, T. R., Henderson, S., Perrett, C., Hewitt, D., Bourmpoulia, D., Ledermann, J., & Boshoff, C. (2004). Predicting biomarkers for ovarian cancer using gene-expression microarrays. *British Journal of Cancer, 90,* 686–692.

Borgono, C. A., Michael, I. P., & Diamandis, E. P. (2004). Human tissue kallikreins: Physiologic roles and applications in cancer. *Molecular Cancer Research, 2,* 257–280.

Clements, J. A., Willemsen, N. M., Myers, S. A., & Dong, Y. (2004). The tissue kallikrein family of serine proteases: functional roles in human disease and potential as clinical biomarkers. *Critical Reviews Clinical Laboratory Sciences, 41,* 265–312.

Fisch, M., & Bruera, E. (2003). Handbook of advanced cancer care. Cambridge, UK: Cambridge University Press.

Gilks, C. B., Vanderhyden, B. C., Zhu, S., van de Rijn, M., & Longacre, T. A. (2005). Distinction between serous tumors of low malignant potential and serous carcinomas based on global mRNA expression profiling. *Gynecologic Oncology, 96,* 684–694.

Huang, W., Shostak, Y., Tarr, P., Sawyers, C., & Carey, M. (1999). Cooperative assembly of androgen receptor into a nucleoprotein complex that regulates the prostate-specific antigen enhancer. *Journal of Biological Chemistry, 274,* 25756–25768.

Jesser, C., Mucci, L., Farmer, D., Moon, C., Li, H., Gaziano, J. M., Stampfer, M., Ma, J., & Kantoff, P. (2008). Effects of G/A polymorphism, rs266882, in the androgen response element 1 of the PSA gene on prostate cancer risk, survival and circulating PSA levels. *British Journal of Cancer, 99,* 1743–1747.

Lai, J., Kedda, M. A., Hinze, K., Smith, R. L., Yaxley, J., Spurdle, A. B., Morris, C. P., Harris, J., & Clements, J. A. (2007). PSA/KLK3 AREI promoter polymorphism alters androgen receptor binding and is associated with prostate cancer susceptibility. *Carcinogenesis, 28,* 1032–1039.

Marsh, A., Healey, S., Lewis, A., Spurdle, A. B., Kedda, M. A., Khanna, K. K., Mann, G. J., Pupo, G. M., Lakhani, S. R., & Chenevix-Trench, G. (2007). Mutation analysis of five candidate genes in familial breast cancer. *Breast Cancer Research and Treatment, 105,* 377–389.

Nagle, C. M., Chenevix-Trench, G., Spurdle, A. B., & Webb, P. M. (2007). The role of glutathione-S-transferase polymorphisms in ovarian cancer survival. *European Journal of Cancer, 43,* 283–290.

Nagle, C. M., Purdie, D. M., Webb, P. M., Green, A., Harvey, P. W., & Bain, C. J. (2003). Dietary influences on survival after ovarian cancer. *International Journal of Cancer, 106,* 264–269.

Nagle, C. M., Purdie, D. M., Webb, P. M., Green, A. C., & Bain, C. J. (2006). Searching for cancer deaths in Australia: National Death Index vs. cancer registries. *Asian Pacific Journal of Cancer Prevention,* 7, 41–45.

Nisenblat, V., & Norman, R. J. (2009). Androgens and polycystic ovary syndrome. *Current Opinion in Endocrinology, Diabetes and Obesity, 16,* 224–231.

Obiezu, C. V., & Diamandis, E. P. (2005). Human tissue kallikrein gene family: Applications in cancer. *Cancer Letters, 224,* 1–22.

Parkin, D. M., Bray, F., Ferlay, J., & Pisani, P. (2005). Global cancer statistics, 2002. *CA: A Cancer Journal for Clinicians, 55,* 74–108.

Prestridge, D. S. (1991). SIGNAL SCAN: A computer program that scans DNA sequences for eukaryotic transcriptional elements. *Computer Applications in the Biosciences, 7,* 203–206.

Purdie, D., Green, A., Bain, C., Siskind, V., Ward, B., Hacker, N., Quinn, M., Wright, G., Russell, P., & Susil, B. (1995). Reproductive and other factors and risk of epithelial ovarian cancer: An Australian case-control study. Survey of Women's Health Study Group. *International Journal of Cancer, 62,* 678–684.

Spurdle, A. B., Webb, P. M., Purdie, D. M., Chen, X., Green, A., & Chenevix-Trench, G. (2001). Polymorphisms at the glutathione S-transferase GSTM1, GSTT1 and GSTP1 loci: Risk of ovarian cancer by histological subtype. *Carcinogenesis, 22,* 67–72.

Stephens, M., Smith, N. J., & Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics, 68,* 978–989.

Wang, P. H., & Chang, C. (2004). Androgens and ovarian cancers. *European journal of Gynaecological Oncology, 25,* 157–163.

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