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Association of Genetic Variation of the Six Gene Prognostic Model for Castration-Resistant Prostate Cancer with Survival

Wanling Xie¹, Konrad H. Stopsack⁴, Sarah J Drouin², Henry Fu², Mark M. Pomerantz², Lorelei A. Mucci³, Gwo-Shu Mary Lee^{2,*}, and Philip W Kantoff^{4,*}

¹Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute and Harvard Medical School, 450 Brookline Ave., Boston, MA 02215.

²Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, 450 Brookline Ave., Boston, MA 02215.

³Harvard T. H Chan Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02215,

⁴Department of Medicine, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065.

Abstract

Background: We previously identified a blood RNA transcript-based model consisting of six immune or inflammatory response genes (*ABL2*, *SEMA4D*, *ITGAL*, *CIQA*, *TIMP1* and *CDKN1A*) that was prognostic for survival in cohorts of men with castration-resistant prostate cancer (CRPC). We investigated whether inherited variation in these six genes was associated with overall survival (OS) in men with CRPC.

Methods: The test cohort comprised 600 patients diagnosed with CRPC between 1996 and 2011 at Dana-Farber Cancer Institute. Genotyping of 66 tagging single nucleotide polymorphisms (SNPs) spanning the six genes was performed on blood derived DNAs. For the top four SNPs ($p < 0.05$), validation was conducted in an independent cohort of 223 men diagnosed with CRPC between 2000 and 2014. Multivariable Cox regression adjusting for known prognostic factors estimated hazard ratios (HR) and 95% confidence intervals (CI) of the association of genetic variants with OS.

Results: Two thirds of patients in both cohorts had metastases at CRPC diagnosis. Median OS from CRPC diagnosis was 3.6 (95%CI 3.3–4.0) years in the test cohort and 4.6 (95%CI 3.8–5.2) years in the validation cohort. 59 SNPs in Hardy-Weinberg equilibrium were analyzed. The major alleles of rs1318056 and rs1490311 in *ABL2*, and the minor alleles of rs2073917 and rs3764322 in *ITGAL* were associated with increased risk of death in the test cohort (adjusted-HRs 1.27–1.39; adjusted- $p < 0.05$; false discovery rate < 0.35). In the validation cohort, a similar association with OS was observed for rs1318056 in *ABL2* (adjusted-HR 1.44; 95%CI 0.89–2.34) and rs2073917 in *ITGAL* (adjusted-HR 1.41; 95%CI 0.82–2.42). The associations did not reach statistical significance most likely due to the small sample size of the validation cohort (adjusted- $p = 0.142$).

* **Correspondence:** Philip W. Kantoff, Phone: 212-639-5851; Fax: 929-321-5023; kantoff@mskcc.org. Gwo-Shu Mary Lee, Phone: 617-632-5088; gwo-shu_lee@dfci.harvard.edu.

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and 0.209, respectively). Additional eQTL analysis indicated that minor alleles of rs1318056 and rs1490311 in *ABL2* are associated with a lower *ABL2* expression in blood.

Conclusions: These findings corroborate our initial work on the RNA expression of genes involved in immunity and inflammation from blood and clinical outcome and suggest that germline polymorphisms in *ABL2* and *ITGAL* may be associated with the risk of death in men with CRPC. Further studies are needed to validate these findings and to explore their functional mechanisms.

Keywords

Prostate Cancer; Castration-Resistant Prostate Cancer; SNP

Introduction

Androgens and androgen receptor (AR) play essential roles in the development and progression of prostate cancer (1–7). Androgen deprivation therapy (ADT) has been the mainstay of treatment for men with advanced hormone-sensitive prostate cancer. Unfortunately, prostate cancer eventually becomes resistant to ADT and progresses to castration-resistant prostate cancer (CRPC). While CRPC is usually fatal (8–12), the survival of patients with CRPC can be highly variable (13). Existing nomograms and prognostic tools do not account for the great biologic and clinical heterogeneity of CRPC and improvement in prognostication is needed to optimize patient stratification and treatment (13–19).

We previously tested the hypothesis that expression of genes in whole blood derived cells could yield prognostic information in men with CRPC. We developed a whole blood RNA transcript-based prognostic model in CRPC, which predicted the overall survival (OS) of men with CRPC (24). Of 168 inflammation and prostate cancer-related genes tested, decreased expression of *ABL2*, *SEMA4D* and *ITGAL* and increased expression of *C1QA*, *TIMP1* and *CDKN1A* was significantly associated with prolonged OS in two independent cohorts. The 6-gene model was able to distinguish two prognostic groups that had a 2.2 year difference in OS. *ABL2*, *ITGAL*, and *SEMA4D* are involved in T-cell motility, antigen surveillance, and T-helper cell activity (25–29). The downregulation of these genes indicates a downregulation of cellular and humoral immunity. *C1QA*, *CDKN1A* and *TIMP1* showed increased expression in enriched monocytes, presumably indicating a process driving monocyte differentiation towards the production of tissue macrophages and away from dendritic cells (30–35). Taken together, these observations suggested that alterations of the immune system towards macrophage differentiation, with a decrease in cellular and humoral immunity, might have a significant impact on OS. To further explore this finding, we studied the association of inherited variation in these six genes on prognosis in two independent CRPC cohorts. We hypothesized that genetic variation might modify their biologic effect and in turn the association with OS.

PATIENTS AND METHODS

Patient population

The test and validation CRPC cohorts were identified from our established institutional Clinical Research Information System (CRIS) database of patients at Dana-Farber Cancer Institute (36). The test cohort was comprised of 600 patients diagnosed with CRPC between 1996 and 2011, with or without the presence of radiographic metastases. The validation set is a more recent CRPC cohort consisting of 223 men from the same institution, diagnosed with CRPC between 2000 and 2014. All patients had experienced biochemical progression after the treatment with primary ADT (orchiectomy or luteinizing hormone-releasing hormone [LHRH], with or without an anti-androgen) and had a blood sample available. All patients gave consent to an IRB-approved protocol that collects clinical and genomic data. The database lock date was June, 2012 for the test cohort and May, 2017 for the validation cohort.

SNP selection and Genotyping

Tagging SNPs for *ABL2*, *CIQA*, *CDKN1A*, *ITGAL*, *SEMA4D* and *TIMP1* genes were selected based on the HapMap phase II data in a Caucasian population (CEU). The tagging SNPs were selected by pairwise algorithm implemented in the Haploview 4.1 program (<http://www.broad.mit.edu/mpg/haploview>) to capture unmeasured variants with $r^2 > 0.8$, which capture all common variation ($>5\%$) among the CEU population of all gene loci. In total, 66 SNPs were genotyped in the test CRPC cohort from the six genes. Seven SNPs were removed from the analysis because they were not in Hardy Weinberg equilibrium (Supplemental Table S1) including all four SNPs selected for *TIMP1*. Thus, the final analysis contained 59 SNPs from 5 genes: 15 in *ABL2*, 3 in *CIQA*, 4 in *CDKN1A*, 15 in *ITGA*, and 22 in *SEMA4D*. Genomic DNA was prepared from whole blood using a QIAamp DNA Blood mini kit (Qiagen Inc, Valencia, CA, USA). Genotyping was done using Sequenom iPLEX matrix-assisted laser desorption/ionization–time of flight mass spectrometry technology at the core facility of Boston Children Hospital.

Statistical Methods

The primary outcome was OS, defined as the period from diagnosis of CRPC to patient death from any cause, with censoring on the day of last follow-up. The distribution of OS according to genotypes was estimated using the Kaplan-Meier method. Log-rank tests were performed to test the association between individual SNPs and OS.

In the test cohort, each SNP was treated as a categorical variable with a common homozygote, a rare homozygote, and a heterozygote allele. Rare homozygotes were combined with heterozygotes if the proportion of rare homozygotes was below 0.02. We also combined homozygotes and heterozygotes if they showed a similar association with the outcome (OS), which significantly differed from the other homozygous genotype (for two SNPs). As we were testing 59 different SNPs, false discovery rates (FDR) were reported to account for multiple comparisons (37). For a subset of SNPs selected on the basis of log-rank test results ($p < 0.05$), we performed multivariable Cox regression models adjusted for known clinical prognostic factors including biopsy Gleason score, time to progression on

primary ADT, metastatic status, PSA and age at diagnosis of CRPC. The top SNPs ($p < 0.05$) were selected for further validation if there was statistical significant association in the multivariable models. Similar analyses were conducted in the validation cohort to confirm findings for the selected SNPs.

To validate that the SNPs functioned as expression Quantitative Trait Loci (eQTL), we used the Genotype-Tissue Expression (GTEx) resource. The data used for the analyses described in this manuscript, including beta distribution-adjusted empirical p-values from FastQTL, were obtained from <https://www.gtexportal.org/> on 05/30/2018.

Statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). All P values are two sided.

RESULTS

Patient Characteristics

The demographic, disease and past treatment characteristics of the test cohort ($N=600$) and validation cohort ($N=223$) are presented in Table 1. The two cohorts had similar disease characteristics at diagnosis and at the time of development of CRPC. The majority of patients (over 90%) were Caucasian. In both cohorts, approximately 70% of patients had received a local therapy (radical prostatectomy or radiation therapy); very few (<5%) had received chemotherapy during their treatment with ADT. At the time of CRPC diagnosis, median age was 68 years (interquartile range (IQR): 60–74) in the test cohort and 65 years (IQR: 58–72) in the validation cohort. More than 60% of patients in both cohorts had metastases at CRPC diagnosis. In the test cohort, there were 430 (72%) deaths and median OS from diagnosis of CRPC was 3.6 (95% CI: 3.3, 4.0) years. In the validation cohort, there were 129 (58%) deaths and median OS was 4.6 (3.8–5.2) years. Median follow-up in alive patients was 3.6 years in the test cohort and 3.9 years in the validation cohort.

Association of genetic variations with OS in the CRPC test cohort

In total, 59 SNPs covering 5 gene loci (*ABL2*, *CIQA*, *CDKN1A*, *ITGAL* and *SEMA4D*) were analyzed in the test cohort (Supplemental Table S2). Two SNPs in *ABL2* (rs1318056 and rs1490311) and two in *ITGAL* (rs2073917 and rs3764322) were associated with OS, with unadjusted P-values < 0.05 and FDR < 0.35 (Table 2, Figure 1, Supplemental Table S2). The genotyping success rate was $> 98\%$ for each of the four SNPs. *ABL2*-rs1318056 and *ABL2*-rs1490311 were analyzed under a dominant genetic model where the rare homozygote (frequency $\approx 2\%$) was combined with the heterozygote. Linkage disequilibrium (LD) as measured by r^2 was 0.54 between rs1318056 and rs1490311, indicating some degree of linkage between the two loci. *ITGAL*-rs2073917 was analyzed in a recessive model and *ITGAL*-rs3764322 in a dominant genetic model, as we observed their association with OS following that model (supplemental Table S2). The linkage between *ITGAL*-rs2073917 and *ITGAL*-rs3764322 loci was low ($r^2 = 0.01$). The detailed genotype distributions of the 4 SNPs are summarized in Supplemental Table S3.

In multivariable models adjusted for clinical prognostic factors, the carriers of major homozygote of rs1318056-CC and rs1490311-GG had worse OS compared to patients

carrying rs1318056-CG/GG and rs1490311-AA/AG genotype, respectively (adjusted hazard ratio [aHR] for rs1318056, 1.33; 95% CI, 1.02–1.73; $p=0.037$; a HR for rs1490311, 1.27; 95% CI, 1.02–1.58; $p=0.030$). For *ITGAL*-rs2073917, the minor homozygote AA was associated with poorer OS compared to AG/GG genotypes (aHR, 1.39; 95% CI, 1.01–1.90, $p=0.043$, FDR=0.27). For *ITGAL*-rs3764322, patients with one or two copies of G allele had a significantly shorter OS (aHR for AG/GG versus AA, 1.30; 95% CI, 1.06–1.58, $p=0.01$, FDR=0.20). Results were consistent if we restricted the analysis in the Caucasian population (data not shown).

Association of genetic variations with OS in the validation cohort

Four polymorphisms in *ABL2* (rs1318056 and rs1490311) and in *ITGAL* (rs2073917 and rs3764322) were genotyped in an independent validation cohort. We observed a similar trend and impact on OS in the validation cohort for *ABL2*-rs1318056 (aHR, 1.44; 95% CI, 0.89–2.34) and *ITGAL*-rs2073917 (aHR, 1.41, 95% CI 0.82–2.42). However, the associations did not reach statistical significance most likely due to the small sample size of the validation cohort ($p=0.142$ and 0.209 respectively, Table 2, Figure 1). The other two SNPs (*ABL2*-rs1490311 and *ITGAL*-rs3764322) did not show an association with OS similar to the test cohort. Results from the combined analyses including both cohorts ($N=823$) are displayed in Table 2.

Association of genetic variations with gene expression in GTEx

Since the six-gene prognostic model for CRPC was originally identified based on the transcriptional profiling of whole blood, we validated the association of the four positive SNPs with mRNA expression in blood and prostate tissue. In this eQTL analysis, we observed that the minor alleles of the two SNPs in *ABL2* (rs1318056, rs1490311) were associated decreased *ABL2* mRNA expression in whole blood (rs1318056, $p=0.035$; rs1490311, $p=0.015$; Figure 2). The two SNPs in *ITGAL* were not associated with *ITGAL* mRNA levels (rs2073917, $p=0.86$; rs3764322, $p=0.9$). Neither of the SNPs in *ABL2* or *ITGAL* was associated with mRNA expression in normal prostate tissue (all $p>0.44$).

DISCUSSION

We previously identified a whole blood RNA transcript-based model consisting of a six-gene signature of immune function or inflammatory response genes, which was prognostic for OS in cohorts of men with CRPC. In the current work, we tested the association of SNPs from the six genes with survival in a test and a validation cohort of patients with CRPC. The major homozygote of *ABL2*-rs1318056 and the minor homozygote of *ITGAL*-rs2073917 were significantly associated with decreased OS, independent of clinical factors. A similar trend of association with OS was also observed in the validation cohort, though the association was not statistically significant, likely due to the small sample size. The other two SNPs (*ABL2*-rs1490311 and *ITGAL*-rs3764322) showed association with OS in the test cohort but not in the validation cohort. A larger independent validation cohort is needed to re-validate the association of these SNPs with OS. Unfortunately, all SNPs in *TIMP1* failed genotyping and thus we were unable to examine these effects. Further eQTL analysis indicated that the minor alleles of rs1318056 and rs1490311, which are associated longer

OS, were associated with decreased *ABL2* mRNA expression in whole blood. This result corroborated our initial finding that decreased expression of *ABL2* is associated with lower risk of CRPC and better OS (24).

Several studies have shown that gene expression profiles in peripheral blood cells can help to distinguish controls from patients with various diseases (20–23). There is some evidence that white blood cell signatures can also be used for prognostication. The peripheral blood derived six-gene prognosis model was developed and validated based on the hypothesis that gene expression patterns in whole blood may be affected by exposure to neoplastic tissue at the primary site, or tumor tissue circulating in blood, thus potentially providing information on the characteristics of malignant cells in individual patients. The model was capable of predicting OS in patients with CRPC (24), with decreased expression of *ABL2*, *SEMA4D* and *ITGAL* and increased expression of *C1QA*, *TIMPI* and *CDKN1A* being associated with better OS. The current work further suggests that inherited variation in these genes could affect the expression level or the function of encoded proteins, and thus may be potentially associated with cancer prognosis. These blood-based genetic tests are a minimally invasive and may offer a means of improving the existing nomograms and disease classifiers.

ABL2 is a non-receptor tyrosine protein kinase of the Abelson family that plays an essential role in processes associated with cell growth and survival (38). *ABL2*-rs1318056 is a coding region SNP resulting in a missense mutation in which G to C converts AGT (Serine) to ACT (Threonine; G/C) at amino acid position 80. Such a mutation is generally considered a conservative mutation. However, if the serine resides in an interior protein active site, changing to Threonine may not be favored (39). The effect of *ABL2*-rs1318056 on protein function remains to be determined. rs1490311 is located at 3' UTR of *ABL2*. Our data suggested the potential role of rs1318056 and rs1490311 serve as eQTL. Both rs2073917 and rs3764322 are intronic SNPs in *ITGAL* and appeared negative in the eQTL analysis in GTEx. Little is known about the causal relationship between this six genes whole blood transcriptional signature and prognosis in patients with CRPC. The association of genetically inherited variations of some of these genes with the cancer aggressiveness provide yet another level of support for their prognostic significance in prostate cancer. Since these 6 genes signature are involved in the immune system and macrophage differentiation, accompanied by a decrease in both cell-mediated and humoral immunity, the identified SNPs in this study may be predictive of the way patients' immune systems deal with their cancer.

A limitation of this study includes the small size of validation cohort, which may have led to some findings not replicating. In addition, our prognostic models did not include some that have been shown to be prognostic in CRPC, such as lactate dehydrogenase (LDH), hemoglobin, and alkaline phosphatase (ALP). Given that we tested 59 SNPs, we reported of false discovery rates to account for multiple testing (37), and we leveraged an independent validation cohort. We note that the two SNPs in *ITGAL* were not associated with *ITGAL* mRNA expression. However, this testing in the GTEx resource may have lacked power and was performed in blood from individuals without CRPC. Furthermore, this was a hypothesis-generating study following our initial work on RNA expression of genes involved in immune function and inflammatory response. In conclusion, we identified polymorphisms

in genes that are involved in the immune system and macrophage differentiation may be associated with the OS in men with CRPC. Further studies are needed to validate these findings and to explore functionality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ADT	androgen deprivation therapy
CaP	Prostate cancer
TTP	Time to progression
OS	Overall Survival

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Figure 1.
Kaplan Meier Plots of overall survival from CRPC diagnosis in the Test and Validation cohorts.

Figure 2. Expression quantitative trait loci analysis using the GTEx resource of **A**, SNP rs1318056 in *ABL2* ($p = 0.035$) and **B**, SNP rs1490311 in *ABL2* ($p = 0.015$).

Table 1.

Patient and disease Characteristics of men with prostate cancer in the Dana-Farber Cancer Institute test and validation cohorts

	Test cohort (N=600)		Validation cohort (N=223)	
	N	%	N	%
Race				
White	569	94.8	204	91.5
Asian	2	0.3	2	0.9
Black	18	3	9	4
Hispanic	6	1	.	.
Other/Unknown	5	0.8	8	3.6
Biopsy Gleason Score				
6 or less	76	12.7	22	9.9
7	181	30.2	63	28.3
8 or more	252	42	115	51.6
Unknown	91	15.2	23	10.3
Type of local therapy				
RP +/-RT	212	35.3	83	37.2
RT only/other	190	31.7	70	31.4
None	198	33	70	31.4
Received antiandrogen during ADT	411	68.5	149	66.8
Received chemotherapy during ADT	20	3.3	13	5.8
Metastasis at CRPC diagnosis				
No	215	35.8	65	29.1
Yes	385	64.2	158	70.9
	Median	IQR	Median	IQR
Age at CRPC diagnosis, year	68	60–74	65	58–72
PSA at CRPC diagnosis, ng/mL	1.3	0.3–6.2	1.0	0.2–3.0

RP: prostatectomy, RT: radiation therapy, ADT: Androgen deprivation therapy, CRPC: castration resistant prostate cancer, IQR: interquartile range

Table 2.

Associations of genetic variations with overall survival after development of CRPC

		Test cohort (N=600 [*])				Validation cohort (N=223 [*])			
SNP	Allele	N(%)	No. of death	Median OS Year (95%CI)	Adjusted HR ^{**} (95%CI)	N(%)	No. of death	Median OS Year (95%CI)	Adjusted HR ^{**} (95%CI)
<i>ABL2</i>									
rs1318056	CG/GG	107(18)	68	4.0(3.0–5.4)	1(reference)	37(17)	21	4.7(3.1–6.7)	1(reference)
	CC	492(82)	361	3.5(3.3–3.9)	1.33(1.02–1.73)	178(83)	103	4.3(3.5–5.1)	1.44(0.89–2.34)
	p-value ^{***}			0.041	0.037			0.322	0.142
rs1490311	AA/AG	175(29)	116	3.6(3.3–5.2)	1(reference)	66(30)	39	4.4(3.5–5.6)	1(reference)
	GG	423(71)	313	3.5(3.2–4.0)	1.27(1.02–1.58)	152(70)	88	4.5(3.4–5.3)	1.09(0.74–1.60)
	p-value ^{***}			0.032	0.030			0.744	0.681
<i>ITGAL</i>									
rs2073917	AG/GG	538(90)	384	3.6(3.3–4.0)	1(reference)	189(88)	106	4.5(3.6–5.2)	1(reference)
	AA	62(10)	46	3.0(2.6–4.0)	1.39(1.01–1.90)	26(12)	18	4.4(2.2–5.8)	1.41(0.82–2.42)
	p-value ^{***}			0.020	0.043			0.287	0.209
rs3764322	AA	330(56)	225	3.8(3.4–4.3)	1(reference)	121(56)	68	4.4(3.5–5.2)	1(reference)
	AG/GG	259(44)	197	3.1(2.7–3.6)	1.30(1.06–1.58)	97(44)	59	4.9(3.4–5.6)	1.04(0.72–1.48)
	p-value ^{***}			0.005	0.010			0.943	0.846

^{*} Patients with unsuccessful genotyping for a particular SNP were excluded in that SNP analysis, therefore, the total N for a SNP may not add to the total of 600 and 223 in test and validation cohort.^{**} adjusted for biopsy Gleason score (7, >7 or unknown), time to progression on primary ADT, metastatic status (yes or no), PSA (<10, 10–20, 20, or unknown) and age at diagnosis of CRPC.^{***} log-rank test and Wald chi-square test from Cox regression.

HR, Hazard ratio; CI, Confidence Interval.