Received: 8 February 2022 | Accepted: 1 June 2022

DOI: 10.1002/pros.24407

ORIGINAL ARTICLE

The Prostate WILEY

Gene variation impact on prostate cancer progression: Lymphocyte modulator, activation, and cell adhesion gene variant contribution

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Funding information

Ministerio de Educación, Cultura y Deporte, Grant/Award Number: FPU15/02897; Agència de Gestió d'Ajuts Universitaris i de Recerca, Grant/Award Number: 2017/SGR/1582; FP7 People: Marie-Curie Actions, Grant/Award Number: FP7/2007/2013; 229673; Ministerio de Ciencia e Innovación,

Abstract

Background: The view of prostate cancer (PCa) progression as a result of the interaction of epithelial cancer cells with the host's immune system is supported by the presence of tumor infiltrating lymphocytes (TILs). TILs fate and interaction with the tumor microenvironment is mediated by accessory molecules such as CD5 and CD6, two signal-transducing coreceptors involved in fine-tuning of T cell responses. While the nature of the CD5 ligand is still controversial, CD6 binds CD166/ALCAM, a cell adhesion molecule involved in progression and dissemination of epithelial cancers, including PCa. The purpose of the present study was to determine the role of *CD5*, *CD6*, and *CD166/ALCAM* gene variants in PCa.

Methods: Functionally relevant *CD5* (rs2241002 and rs2229177), *CD6* (rs17824933, rs11230563, and rs12360861) and *CD166/ALCAM* (rs6437585, rs579565, rs1044243, and rs35271455) single nucleotide polymorphisms (SNPs) were genotyped in germline DNA samples from 376 PCa patients. Their association with PCa prognostic factors, namely biochemical recurrence (BCR) and International Society of Urological Pathology (ISUP) grade was analyzed by generalized linear models and survival analyses.

Result: Proportional hazards regression showed that the minor *CD6* rs12360861^{AA} and *CD166/ALCAM* rs579565^{AA} genotypes were associated with earlier BCR, with hazard ratios of 2.65 (95% CI: 1.39–5.05, $p = 0.003$) and 1.86, (95% CI: 1.02–3.39, $p = 0.043$), respectively. Individually, none of the analyzed SNPs was significantly associated with ISUP grade, but haplotype analyses revealed association of the *CD5* rs2241002^{C}-rs2229177^{T} haplotype with ISUP grade ≥2, with odds ratio of 1.52 (95% CI: 1.05–2.21, $p = 0.026$).

Grant/Award Number: PID2019-106658RB-I00; Ministerio de Economía y Competitividad, Grant/Award Number: SAF2016-80535-R

Conclusion: The results show the impact on PCa aggressiveness and recurrence brought about by gene variants involved in modulation of lymphocyte activation (*CD5*, *CD6*) and immune-epithelial cell adhesion (*CD166/ALCAM*) in PCa aggressiveness and recurrence, thus supporting a role for host immune response in PCa pathophysiology.

KEYWORDS
CD166/ALCAM, CD5, CD6, polymorphism, prostate cancer, SNP

1 | INTRODUCTION

Prostate cancer (PCa) is the second most commonly diagnosed cancer and the sixth leading cause of cancer death among men worldwide.[1] Newly diagnosed prostate tumors are often localized PCa, confined to the prostate.[1] A combination of environmental and lifestyle risk factors in addition to genetic variants influences the development and prognosis of PCa in terms of recurrence and metastasis. Treatment options include active surveillance, radical prostatectomy, radiotherapy, focal therapy, androgen deprivation and, more recently, immunotherapy in case of metastatic disease.[2,3] Positive surgical margins, high International Society of Urological Pathology (ISUP) grade, and short interval to biochemical recurrence (BCR) are the main adverse clinical prognostic factors.[4]

PCa is generally an indolent disease over long time spans that enable antitumor immune responses.[2] However, PCa is often described as a "cold" tumor with minimal inflammatory or immunosuppressive microenvironment, where tumor infiltrating lymphocytes (TILs) may even contribute to PCa progression.[5] Such TILs include $CD4^+$ T cells skewed toward T regulatory (Treg) and T helper 17 (T_H17) phenotypes, and $CD8^+$ T cells with tolerogenic phenotype.[6,7] Recent immunophenotypical analyses of PCa microenvironment have revealed a subset of "immune-activated" patients with favorable outcomes who may benefit from novel immune checkpoint inhibitors.[3]

TIL function modulates T-cell activation and fate through stable adhesive contacts with cancer cells via accessory immunomodulatory molecules such as CD5 and CD6. These are highly homologous signal-transducing lymphocyte surface receptors expressed by all T cell types from early stages of their development and by the B1a cell subset, with lower expression in other immune cell types (e.g., macrophages, dendritic cells, or natural killer cells).[8,9] Both CD5 and CD6 fine-tune the intracellular activation and differentiation signals delivered by the T cell receptor complex (TCR), to which they physically associate.[10–12] This is supported by CD5 and CD6 signalosome's integration of both inhibitory and activator effectors of TCR-mediated signals.[13,14] The relevance of CD5 and CD6 immunomodulatory properties in cancer immune surveillance is sustained by in vitro and in vivo experimental evidence,[15–18] as well as by clinical transcriptomic studies.[19]

Adhesive contacts between cancer cells and microenvironment components (extracellular matrix, immune, and nonimmune cells) are important for local tumor growth and metastasis,[20,21] making adhesion molecule regulation relevant in cancer biology.[22] One such molecule is CD166/ALCAM (for activated leukocyte cell adhesion molecule), a *bona fide* ligand of CD6.[23,24] CD166/ALCAM is a member of the immunoglobulin superfamily with a broad tissue distribution including normal epithelia, endothelia, neurons, hematopoietic and mesenchymal progenitors, bone marrow stromal cells, and an extensive list of malignancies including PCa.[25] Their homotypic (ALCAM-ALCAM) and heterotypic (ALCAM-CD6) interactions are relevant in several physiological processes (e.g., leukocyte transmigration, T-cell activation, hematopoiesis, osteogenesis, neurite outgrowth, angiogenesis, and embryonic implantation)[25] as well as in cancer growth and metastasis.[20]

Genome-wide (GWAS) and candidate gene-driven association studies support involvement of several *CD5* (rs2241002 and rs2229177), *CD6* (rs17824933, rs11230563, and rs12360861), and *CD166/ALCAM* (rs6437585, rs579565, and rs1044243) gene variants as susceptibility and/or disease modifier markers in different autoimmune disorders (i.e., rheumatoid arthritis, lupus nephritis, multiple sclerosis, psoriasis, Behçet's disease, and inflammatory bowel disease).[26–33] Cancer and autoimmunity are considered two opposite sides of the same coin, but there is still scarce information on the influence of *CD5*, *CD6*, and/or *CD166/ALCAM* gene variation in cancer, except for *CD5* in melanoma and chronic lymphocytic leukemia[34,35] and *CD166/ALCAM* in breast cancer.[36,37]

Given the involvement of CD5, CD6, and CD166/ALCAM in TIL function and T-epithelial cell interplay, and the association of some of their gene variants with inflammatory diseases and cancer, we have explored their impact on PCa. Our results show association of certain *CD5*, *CD6*, and *CD166/ALCAM* gene variants with PCa prognosis (BCR and ISUP grade), and support a role for the host's immune response in PCa pathophysiology.

2 | MATERIALS AND METHODS

2.1 | Subjects and samples

We conducted a retrospective study with 376 PCa patients attending the Urology Department of the Hospital Clínic de Barcelona, Spain, between 2008 and 2016. All patients had localized prostate

adenocarcinoma and were treated by means of radical prostatectomy, radiotherapy, or cryotherapy. Exclusion criteria included the presence of other active neoplasm at the moment of diagnosis. Tumor dissemination was controlled postoperatively by prostate-specific antigen (PSA) measurement. The study was approved by the local Hospital Ethics Committee, in accordance with the Declaration of Helsinki and the Regulation 536/2014 of the European Parliament and of the Council, and written informed consent was obtained from all participants before inclusion (ref. HCB2013/8753).

Clinical data, including age, ISUP grade, stage, treatment (radical prostatectomy, cryotherapy, radiotherapy), BCR, metastases, and PCa-related death, was retrospectively collected from medical records. BCR was defined as increase of PSA >0.2 ng/ml after radical prostatectomy or increase of PSA >2 ng/ml over nadir after radiotherapy or cryotherapy.

2.2 | SNP genotyping

One 10 ml EDTA tube of peripheral blood was collected before treatment. Genomic DNA was extracted from peripheral blood by salting out.[38] DNA samples (20 ng) were subjected to real-time polymerase chain reaction (RT-PCR) in a LightCycler® 480 Instrument (Roche) using the TaqMan Genotyping Master Mix and the TaqMan probes for rs2241002, rs2229177, rs17824933, rs11230563, rs12360861, and rs6437585, following manufacturer's instructions (Thermo Fisher Scientific). *CD166/ALCAM* SNPs rs579565, rs1044243, and rs35271455, which lie in a SNP hotspot spanning 7 bp, were PCR amplified for subsequent sequence-based typing (PCR-SBT) using the Hs00666884_CE assay (Thermo Fisher Scientific).

2.3 | Statistical analyses

Statistical analysis was performed with R 3.6.0 (R Foundation for Statistical Computing), with the packages "SNPassoc", "survival", "survminer", and "haplo.stats" available at the Comprehensive R Archive Network (CRAN) repository. Association of individual SNPs (predictor variable) with ISUP grade (binary response variable, codified as ISUP = 1 vs. ISUP ≥ 2) was assessed by logistic regression, using the "association" function included in the "SNPassoc" package. For each analysis, four models were generated (codominant, dominant, recessive, and log-additive), and the model with the lowest Akaike information criterion (AIC) was chosen. For haplotypic analyses, putative haplotypes were inferred with the expectation-maximization (EM) algorithm and association with clinical parameters was assessed with generalized linear models, both using functions implemented in the "haplo.stats" package. BCR-free survival curves were estimated by the Kaplan-Meier method with the "survival" package. Hazard ratios for each genotype and their respective confidence intervals were calculated and hazard ratio differences between genotypes were assessed with Cox proportional hazard regression, all using the "survminer" package.

3 | RESULTS

The clinicopathological characteristics of the study cohort are listed in Table 1. Median follow-up time was 62.00 months (interquartile range 31.00–77.00 months), during which 119 patients (31.6%) developed BCR. Median time to BCR was 25.00 months (interquartile range: 12.00–48.00). Five patients (1.3%) developed metastases, and six (1.6%) died due to cancer-related causes.

The Table 2 lists allele frequencies of the *CD5* (rs2241002 and rs2229177), *CD6* (rs17824933, rs11230563, and rs12360861), and

TABLE 1 Characteristics of the prostate cancer patient cohort (n = 376)

Age, years. Mean (SD)	68.7 (7.3)
PSA at diagnosis (ng/ml). Mean (SD)	8.4 (4.6)
ISUP grade, *n* (%)	
1	126 (33.5)
2	149 (39.6)
3	67 (17.8)
4	17 (4.5)
5	13 (3.5)
Unknown/missing	4 (1.1)
Treatment	
Radical prostatectomy	287
Radiotherapy	9
Cryotherapy	76
Pathology after radical prostatectomy	
pT2	237 (82%)
pT3	47 (16%)
Positive margins	70 (24%)

TABLE 2 Summary of single nucleotide polymorphisms (SNPs) genotyping

Gene	SNP	Alleles (major/minor)	Major allele frequency	Hardy-Weinberg equilibrium (*p*-values)
CD5	rs2229177	T/C	51.2	0.302
	rs2241002	C/T	77.8	0.765
CD6	rs17824933	C/G	75.9	0.572
	rs11230563	C/T	57.1	0.916
	rs12360861	G/A	76.9	0.383
CD166/ALCAM	rs6437585	C/T	93.7	0.647
	rs579565	G/A	71.7	0.129
	rs1044243	C/T	89.3	0.399
	rs35271455	T/C	99.6	1.000

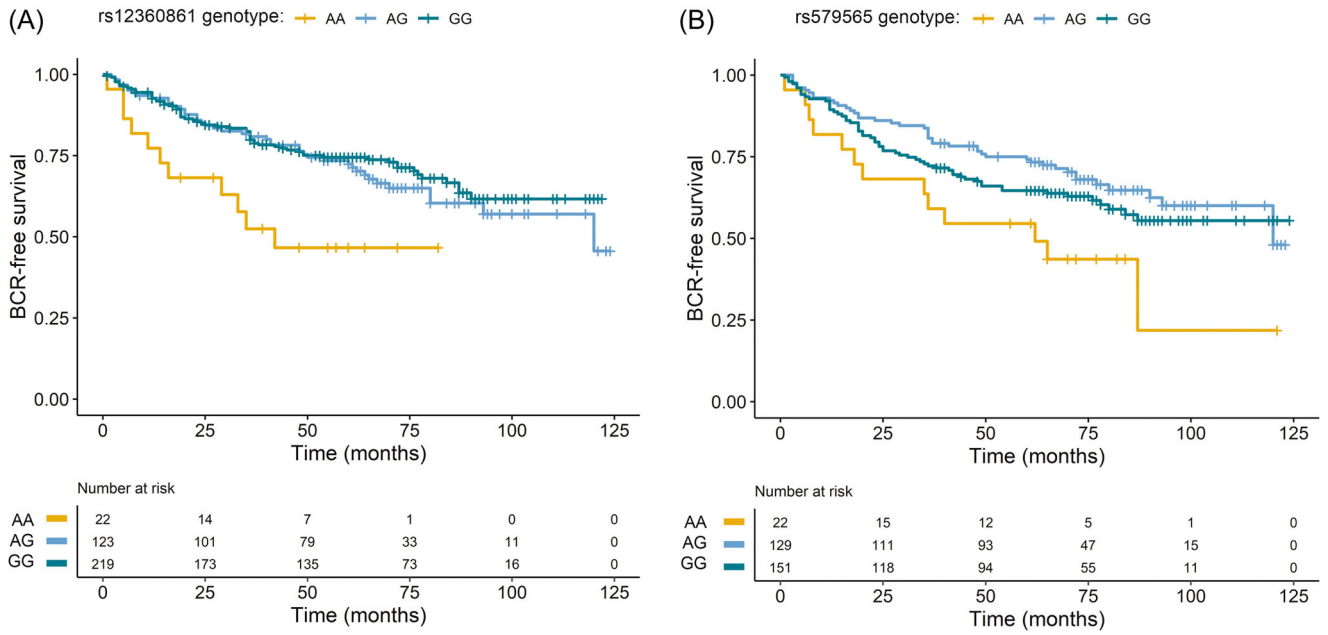

FIGURE 1 Biochemical recurrence (BCR)-free survival curves according to genotypes. (A) BCR-free survival curve of prostate cancer (PCa) patients according to *CD6* single nucleotide polymorphism (SNP) rs12360861 genotype. HR comparing homozygous rs12360861^{AA} with homozygous rs12360861^{GG} was 2.65, (95% CI: 1.39–5.05), $p = 0.003$. (B) BCR-free survival curve of PCa patients according to *CD166/ALCAM* SNP rs579565 genotype. HR comparing homozygous rs579565^{AA} with homozygous rs579565^{GG} was 1.86, (95% CI: 1.02–3.39), $p = 0.043$. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Logistic regression analysis of *CD5* haplotype association with ISUP grade

Haplotype rs2241002	rs2229177	% in cohort	ISUP = 1 (%)	ISUP ≥ 2 (%)	*p*-Value	OR (95% CI)
C	C	43.2	47.3	41.1		
C	T	34.7	28.3	38.0	0.026	1.52 (1.05, 2.21)
T	T	16.5	19.3	15.1	0.508	0.86 (0.55, 1.35)
T	C	5.6	5.1	5.9	0.508	1.34 (0.56, 3.17)

CD166/ALCAM (rs6437585, rs579565, rs1044243, and rs35271455) SNPs analyzed, which were all in Hardy-Weinberg equilibrium.

Cox regression analysis was used to test association between the selected *CD5*, *CD6* and *CD166/ALCAM* SNPs and BCR-free survival. As shown in Figure 1, association with shorter BCR-free survival was observed for the minor *CD6* rs12360861^{AA} genotype (HR = 2.65, 95% CI: 1.39–5.05, $p = 0.003$) and the minor *CD166/ALCAM* rs579565^{AA} genotype (HR = 1.86, CI: 1.02–3.39, $p = 0.043$).

No association was identified between any of the individual SNPs and the ISUP grade (Supporting Information: Table S1). However, some of the studied genetic variants are reported to show stronger association of haplotype-clinical feature association than individual SNPs.[27,34,35] We therefore performed haplotypic analyses, which revealed association between *CD5* and ISUP grade (Table 3). Particularly, the *CD5* rs2241002^{C}-rs2229177^{T} haplotype was associated with increased risk of ISUP grade ≥ 2 when compared with the most common haplotype rs2241002^{C}-rs2229177^{C}.

4 | DISCUSSION

PCa risk stratification based on tumor size, PSA level, and ISUP grade is conventionally used to predict the clinical outcome of patients. Clinical and biological markers remain insufficient to identify PCa patients at higher risk of progression at the time of treatment initiation (radical prostatectomy, cryotherapy, or radiotherapy). On this basis, individualized genetic and molecular prognostic factors will help to discriminate between high-risk patients who may benefit from adjuvant therapy or closer surveillance and low-risk patients for whom active surveillance may suffice.

The present candidate gene-driven approach has identified genetic variants involved in PCa aggressiveness and recurrence after radical prostatectomy. Our analysis includes functionally relevant SNPs from the functionally related *CD5*, *CD6*, and *CD166/ALCAM* gene products. *CD5* and *CD6* encode highly homologous lymphocyte co-receptors that modulate the activation and differentiation signals

transduced by the TCR complex.[10] There are reports on the impact of *CD5* and *CD6* variants on susceptibility and/or severity of autoimmune and malignant disorders, which are considered the two sides of the same coin.[26–35,39] Here, we found association of the minor *CD6* rs12360861^{A} allele with BCR. Clinical relevance of this SNP is illustrated by their role in immune-mediated diseases such as multiple sclerosis and psoriasis.[28–30,32] In the case of *CD5*, we found association of the rs2241002^{C}-2229177^{T} haplotype with a higher ISUP grade, reminiscent of the higher aggressiveness of melanoma in rs2241002^{C}-2229177^{T} carrier patients.[34] Such findings support the utility of *CD5* and *CD6* genotyping in risk stratification and discrimination of patients with clinically significant tumors from indolent ones.

The functional impact of *CD5* variation on T cell activation has been previously reported. The *CD5* rs2229177^{T} allele (Val471) is associated with stronger signaling via CD5,[40] which in turn results in stronger inhibition of TCR-mediated signals compared with the rs2229177^{C} allele (Ala471).[27] Thus, we hypothesize that attenuation of TCR signaling lowers the antitumor activity of T cells, concomitant with higher ISUP grades in rs2241002^{C}-2229177^{T} haplotype carriers. There is no available information on the functional consequences of the *CD6* rs12360861$^{G>A}$ SNP. We speculate that it behaves similarly to the *CD6* rs11230563$^{C>T}$ and rs17824933$^{C>G}$ SNPs, which have been associated with lower T cell expression of the CD6 receptor and deficient binding to CD166/ALCAM,[41–43] leading to poor endothelial/epithelial-T cell interplay.

The *CD166/ALCAM* gene encodes the best characterized CD6 ligand.[23,24] CD166/ALCAM provides heterotypic interactions (CD6-ALCAM) between T cells and epithelial/endothelial cells, but also homotypic interactions (ALCAM-ALCAM) involved in cell adhesion, migration, and progression of several malignancies such as melanoma, breast, colorectal and bladder cancers.[20] CD166/ALCAM is also necessary for engrafting hematopoietic stem cells into the hematopoietic niche.[44] Expression of CD166/ALCAM is known to impact tumor progression in PCa patients[45,46] and development of bone metastases in mouse models of PCa.[47] Moreover, PCa metastases occur mainly in the bone,[48] compromising patient survival.[49] Here we observed an association of the minor *CD166/ALCAM* rs579565^{A} allele with lower BCR-free survival. This is a clinically relevant SNP, as shown by its association to multiple sclerosis risk.[29] To the best of our knowledge, no information is available on the functional consequences of this synonymous *CD166/ALCAM* rs579565 SNP (e.g., introduction of cryptic splicing sites, changes in transcription efficiency, or linkage disequilibrium with other gene variants) that may account for its clinical significance. It is worth mentioning that other *CD166/ALCAM* SNPs such as rs6437585 and rs1044243 have been associated to breast and bladder cancer, supporting the role of genetic variation of *CD166/ALCAM* in cancer.[36,37,50]

The PCa immune microenvironment is heterogeneous, leading to different immunotherapy responses (e.g., immune checkpoint inhibitors). Recent immunogenomic work proposes a classification of PCa patients in three immunophenotypes based on gene enrichment signatures: nonimmune, immune-activated, and immune-suppressed.[3] The last two subtypes were dichotomized based on a stromal signature involving Wnt/TGF-β (for tumor growth factor), and C-ECM (for cancer-associated extracellular matrix) genes. The work states that PCa patients with an immune-activated gene signature could benefit from TGF-β inhibitors.[3] It would be interesting to know how *CD5*, *CD6*, and *CD166/ALCAM* expression and/or variation fit in those PCa immunophenotypes. Interestingly, CD166/ALCAM is a TGF-β-responsive marker and functional regulator of PCa metastasis[47] and gene expression studies in patients with resectable non-small cell lung cancer show that higher *CD5* and *CD6* intra-tumor expression associates to better overall and relapse-free survival.[19]

The strengths of this study include the large cohort of PCa patients, expert pathological review of cases and long-term follow-up of patients diagnosed with clinically localized diseases. Participating researchers were blinded to all clinical information, and genomic information was matched to clinical data only after all patient cases had been processed. We also acknowledge some study limitations. First, the use of BCR as a significant endpoint is questionable as only a proportion of patients with BCR show clinical progression.[51] However, the detection of BCR after radical prostatectomy is usually the indicator for the application of adjuvant therapies. So, an independent validation will be necessary to ascertain the role of *CD5*, *CD6*, and *CD166/ALCAM* gene variants as PCa prognostic and treatment response indicators.

5 | CONCLUSION

We report new genetic variants involved in the modulation of lymphocyte activation and immune-epithelial cell adhesion, which influence PCa aggressiveness and recurrence. The described associations position *CD5*, *CD6*, and *CD166/ALCAM* as putative prognosis markers and/or therapeutic targets. Our findings support that *CD5* variants attenuating T-cell activation associate with increased ISUP grade, and that *CD6* and *CD166/ALCAM* variants lowering T-epithelial cell adhesive contacts associate with shorter BCR-free survival. Further studies are encouraged to validate our findings and the mechanism underlying them.

AUTHOR CONTRIBUTIONS

Conceptualization: Francisco Lozano, and Lourdes Mengual. *Genetic studies*: Sergi Casadó-Llombart, Marta Consuegra-Fernández, Esther Carreras, Fernando Aranda, and Noelia Armiger. *Sample and clinical information collection*: Tarek Ajami, Antonio Alcaraz, and Lourdes Mengual. *Statistical analyses*: Sergi Casadó-Llombart, Marta Consuegra-Fernández, Esther Carreras, and Fernando Aranda. *Writing original draft*: Sergi Casadó-Llombart, Tarek Ajami, Lourdes Mengual, and Francisco Lozano. All authors read, critically revised and approved the final version of the manuscript.

ACKNOWLEDGMENTS

We thank Tessel E. Galesloot and Lambertus A. L. M. Kiemeney for their support in data analysis and critical comments, and Marcos Isamat for manuscript editing. This study was supported by Spanish Ministerio de Economía y Competitividad (MINECO; SAF2016-80535-R) and Ministerio de Ciencia e Innovación (MCIN/AEI/10.13039/501100011033; PID2019-106658RB-I00), co-financed by European Development Regional Fund "A way to achieve Europe" ERDF, and Agència de Gestió d'Ajuts Universitaris i de Recerca from Generalitat de Catalunya (AGAUR; 2017/SGR/1582). Sergi Casadó-Llombart and Esther Carreras are recipients of fellowships from the Spanish Ministerio de Educación, Cultura y Deporte (FPU15/02897), and European Community Seventh Framework Program (BIOTRACK, FP7/2007/2013; 229673), respectively. Funders had no access to clinical data and did not participate in study design, data analysis, or manuscript redaction.

CONFLICT OF INTEREST

Francisco Lozano is a founding partner at Sepsia Therapeutics SL. The other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated in the present study are available from the corresponding author upon reasonable request.

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How to cite this article: Casadó-Llombart S, Ajami T, Consuegra-Fernández M, et al. Gene variation impact on prostate cancer progression: lymphocyte modulator, activation, and cell adhesion gene variant contribution. *The Prostate*. 2022;82:1331-1337. doi:10.1002/pros.24407