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Germline polymorphisms associated with impaired survival outcomes and somatic tumor alterations in advanced prostate cancer

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Abstract

Introduction: Germline variants in androgen metabolism genes may influence clinical response to androgen deprivation therapy (ADT) in advanced prostate cancer. We sought to investigate the prognostic significance of germline variants in androgen metabolism genes with respect to overall survival (OS) after ADT, and to associate germline variants with tumor genomic features.

Methods: Germline and somatic whole-genome sequencing (WGS) data were evaluated in a cohort of 101 men with metastatic castration-resistant prostate cancer (mCRPC). Survival analyses were performed to identify polymorphisms associated with impaired OS after primary ADT.

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Germline variants found to be prognostic of OS were associated with tumor somatic DNA-sequence alterations based on WGS performed on paired metastasis biopsies from the same 101 patients. Gene set enrichment analysis was performed based on tumor RNA-sequencing data to identify genomic pathways differentially expressed in patients with germline variants.

Results: A comprehensive literature review identified 17 candidate polymorphisms in nine androgen metabolism genes that have been previously shown to have an association with response to ADT in prostate cancer. Of these, the variant rs1856888 allele located 13kb upstream of *HSD3B1* was found to be significantly associated with impaired OS ($P=0.029$). Variant rs1856888 was commonly co-inherited with the well-characterized *HSD3B1*(1245A>C) polymorphism, and there was a trend towards shorter median OS in patients with *HSD3B1*(1245A>C) compared to homozygous wild-type patients ($P=0.052$). While *HSD3B1* germline variants were not associated with common somatic tumor DNA alterations, they were associated with increased tumor expression of cell proliferation and cell cycle genes.

Conclusions: This study presents a comprehensive assessment of germline variants in androgen metabolism genes and highlights *HSD3B1* polymorphisms as prognostic of OS after ADT and associated with an aggressive gene expression tumor profile in mCRPC.

INTRODUCTION

Genetic variants have been implicated in both the development and progression of prostate cancer¹⁻³. Of particular clinical interest is the relationship between germline polymorphisms in androgen metabolism genes and response to androgen deprivation therapy (ADT)⁴. ADT is often used as an effective therapy for metastatic castration-sensitive prostate cancer (mCSPC). However, while mCSPCs generally demonstrate a favorable initial response to ADT, most tumors eventually progress to a castration-resistant state. A major mechanism by which tumors develop ADT resistance is synthesis of androgens from extragonadal adrenal precursor steroids⁵. In particular, overexpression of the *SRD5A1* gene product leads to increased conversion of the adrenal steroid androstenedione to 5 α -androstenedione, which is then converted to dihydrotestosterone (DHT). Increased DHT synthesis can then drive the progression of metastatic castration-resistant prostate cancer (mCRPC)⁵.

Agents such as abiraterone acetate that inhibit the androgen biosynthesis pathway have thus been of great interest and have demonstrated clinical promise. Abiraterone inhibits the enzyme cytochrome P450 17 alpha-hydroxylase (CYP17), which drives the synthesis of androgens in both the testes and adrenal glands⁶. Abiraterone acetate has been shown to significantly improve survival outcomes in mCRPC and mCSPC^{7,8}. However, despite the overall clinical benefit of abiraterone acetate, response to treatment is highly variable among patients. Motivated by the intuition that innate patient-to-patient variation in androgen biosynthesis may explain this variability in response to treatment, a recent study demonstrated that the commonly observed *HSD3B1*(1245A>C) germline polymorphism alters the metabolism of abiraterone acetate⁹. In addition, clinical outcomes studies have demonstrated significant associations between specific germline polymorphisms in androgen-related genes such as *CYP19A1*, *HSD3B1*, and *HSD17B4* and survival outcomes from time of initiating primary ADT¹⁰⁻¹³. Altogether, these studies highlight the importance

of germline variants in androgen biosynthesis genes as potential predictors of ADT response.

To our knowledge, no study has comprehensively assessed all of these germline variants in a cohort of mCRPC patients to see if they are predictive of ADT response or overall survival. Additionally, little is known about the association between prostate cancer-related germline variants and molecular tumor characteristics, as none of the published studies to date have coupled germline sequencing results with genomic profiling of the patients' clinical tumor samples. We performed the first comprehensive analysis of germline polymorphisms integrated with solid tumor RNA-sequencing and DNA-sequencing results to investigate germline predictors of ADT response in metastatic castration-resistant prostate cancer (mCRPC).

MATERIALS AND METHODS

Literature review of germline polymorphisms associated with ADT in prostate cancer

SciMiner¹⁴ was used to perform a comprehensive review of genes in which polymorphisms were associated with ADT and prostate cancer. Specifically, the following Pubmed search query was input into SciMiner: “polymorphism” AND “prostate cancer” AND “androgen deprivation therapy.” The output list of genes was then manually reviewed, and all androgen synthesis genes and their corresponding polymorphisms were included in a final filtered list of candidate polymorphisms.

Data processing and variable definitions

A cohort of 101 men with mCRPC was used to investigate the prognostic significance of all candidate polymorphisms. Biopsy tissue and clinical data were obtained as part of a prospective IRB-approved trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02432001) identifier: [NCT02432001](https://clinicaltrials.gov/ct2/show/study/NCT02432001)), and informed consent was obtained from all study participants. Germline and somatic DNA-sequencing and RNA-sequencing data were processed as previously reported^{15,16}. For each SNP identified from literature review, each patient was classified as “wild-type” (homozygous wild-type) or “variant” (either heterozygous or homozygous variant). Somatic DNA alterations were assessed for genes commonly altered in mCRPC as previously described^{15,16}. For loss-of-function alterations in *RBI*, *PTEN*, *TP53*, *CDK12*, *BRCA2*, and *CHD1*, samples were considered altered if two or more DNA alterations (mutations, copy number loss, inactivating structural variants) were present, i.e., presumed bi-allelic loss. ETS fusions included fusions involving *ERG*, *ETV1*, *ETV4*, and *ETV5* as the 3' partner. For *SPOP*, *MYC*, and *AR*, the following known driver alterations were considered: *SPOP* mutations, *MYC* amplifications, and *AR* amplifications (including *AR* upstream enhancer amplifications). All DNA-sequencing and RNA-sequencing data are available in dbGaP (accession number: phs001648).

Statistical methods

Retrospective, single-cohort survival analyses were performed to examine the prognostic significance of each candidate germline variant. The primary endpoint was overall survival (OS) from time of initiating primary ADT. Kaplan-Meier curves were plotted, and patients

with wild-type and variant SNPs were compared using a log-rank test. Multivariable analysis was conducted by including genomic variants significantly associated with impaired OS on univariate analysis as well as the following well-characterized clinicopathologic prognostic factors: ECOG performance status, Gleason score, PSA laboratory value, metastatic disease at time of initiating primary ADT, and treatment with enzalutamide or abiraterone acetate. Association of germline variants with common DNA alterations was performed using a Fisher's exact test. RNA-seq differential expression analyses were performed by first performing a Wilcoxon signed-rank test comparing the wild-type and variant patients. The $-\log_{10}$ of the P -values (positive score if expression was higher in the variant samples, negative score if higher in the wild-type samples) was used to rank genes in order from most overexpressed to least in the tumors of patients harboring a polymorphism. Gene set enrichment analysis (GSEA) was performed using this ranked list of genes and the previously published GSEA Preranked tool¹⁷ to identify differentially expressed gene sets. Gene sets were considered significantly differentially expressed if they had FDR q -value < 0.25 . All statistical comparisons were performed at a two-sided significance level of 0.05 unless otherwise stated. R version 3.5.0 was used to perform all statistical analyses, and code is available upon request.

RESULTS

Comprehensive literature review of all previously reported germline polymorphisms associated with ADT response identified 17 polymorphisms in the following nine genes: *HSD17B4*¹⁰, *HSD17B2*¹⁸, *SRD5A2*^{19,20}, *SLCO2B1*^{21–23}, *SLCO1B3*^{23,24}, *CYP19A1*^{10,25,26}, *CYP17A1*^{18,27}, *AKR1C3*^{28,29}, and *HSD3B1*^{11–13,30,31}. Assessing the prognostic significance of these polymorphisms in our cohort, we found that the only gene with a polymorphism significantly associated with OS was *HSD3B1*.

Two polymorphisms at the *HSD3B1* locus were identified *a priori* from review of the literature: rs1047303, i.e., *HSD3B1*(1245A>C), and rs1856888, located 13kb upstream of the *HSD3B1* promoter. At the rs1047303 locus, 58% (59/101) of patients were homozygous wild-type, 35% (35/101) were heterozygotes, and 7% (7/101) were homozygous variant. At the rs1856888 locus, 53% (54/101) of patients were homozygous wild-type, 36% (36/101) were heterozygotes, and 11% (11/101) were homozygous variant (Table 1). The variant rs1047303, i.e., *HSD3B1*(1245A>C), and variant rs1856888 alleles were commonly co-inherited, as all patients with *HSD3B1*(1245A>C) also demonstrated variant rs1856888 and only five patients with variant rs1856888 had homozygous wild-type rs1047303. The variant rs1856888 polymorphism was significantly associated with shorter OS (median OS [95% CI]: 5.0 [3.3–8.4] vs. 6.9 [5.2–9.8] years, $P=0.029$; Figure 1A). The *HSD3B1*(1245A>C) polymorphism was borderline significantly associated with OS differences between the wild-type and variant groups (median OS [95% CI]: 5.0 [3.1–8.4] vs. 6.5 [5.2–9.8] years, $P=0.052$; Figure 1B). Examining additional clinical endpoints, we found that variant rs1856888 was associated with borderline-significant shorter time to progression (TTP) from ADT initialization to mCRPC (median TTP [95% CI]: 2.0 [1.1–3.8] vs. 3.1 [1.7–7.3] years, $P=0.077$; Figure S1A) and shorter OS from time of mCRPC diagnosis (median OS [95% CI]: 2.5 [1.6–3.4] vs. 3.9 [3.4–4.9] years, $P=0.036$; Figure S1C). Variant rs1047303 was associated with borderline-significant shorter TTP from ADT initialization to mCRPC

(median TTP [95% CI]: 2.3 [1.1 – 3.8] vs. 3.1 [1.7–7.3] years, $P=0.084$; Figure S1B) and a trend toward shorter OS from time of mCRPC diagnosis (median OS [95% CI]: 2.5 [1.6–4.1] vs. 3.5 [2.9–4.2] years, $P=0.13$; Figure S1D).

We next sought to assess the association of these *HSD3B1* germline variants with molecular changes in the mCRPC tumors themselves by utilizing WGS and RNA-seq on metastatic tumor biopsies obtained from these patients. Given the frequent joint inheritance of the *HSD3B1*(1245A>C) and rs1856888 variant polymorphisms and prior studies suggesting both may be important in ADT response^{10,31}, we grouped the two together for the purposes of assessing associations with somatic tumor alterations. We identified the most common somatic DNA alterations in mCRPC¹⁵ and found that the *HSD3B1* germline variant was not significantly associated with any of these (Figure 2, Table S1). To assess whether the *HSD3B1* germline variant was prognostic of OS independently of bi-allelic *RB1* loss in the tumor, which has been recently highlighted as the key somatic DNA alteration associated with poor prognosis in mCRPC^{16,32}, we performed a multivariable survival analysis including both variant rs1856888 and bi-allelic *RB1* loss along with well-characterized, adverse clinicopathologic features. The variant *HSD3B1* allele was associated with shorter OS after ADT independently of bi-allelic *RB1* loss and other clinicopathologic features (HR:1.85[1.01–3.37], $P=0.045$; Table 2).

While the *HSD3B1* germline variant was not associated with any of the common somatic DNA alterations in mCRPC, we wanted to examine if there were differences in the tumor gene expression patterns between the *HSD3B1* variant and wild type patients. We performed differential expression analysis comparing the *HSD3B1* variant and wild type groups and used GSEA to perform pathway analysis. Strikingly, 10 out of the top 10 pathways differentially overexpressed in the tumors of patients with variant *HSD3B1* were related to proliferation and cell cycle regulation (Figure 3) suggesting an interplay between the germline genomics and the tumor gene expression.

DISCUSSION

We performed a comprehensive assessment of all androgen synthesis germline variants previously suggested to be associated with ADT response and found that one germline *HSD3B1* variant (rs1856888) was significantly associated with impaired survival outcomes and that the *HSD3B1*(1245A>C) polymorphism was borderline significantly associated. We also leveraged somatic variant data generated from whole genome sequencing and RNA-seq experiments to perform the first assessment of *HSD3B1* germline variants in relation to somatic tumor DNA-seq and RNA-seq alterations.

Of all the germline variants interrogated in nine androgen metabolism genes, only variant *HSD3B1* was found to be associated with impaired OS. The differences in findings between the present and prior studies may be attributable to the relatively modest sample sizes in each study. Additionally, the ethnic composition of the present cohort and those examined in many prior studies was different, with many of the previous cohorts comprised predominantly of East Asian patients^{19–21,27} who are known to have different prevalence of germline variants and potentially different genomic risk factors than Caucasian patients^{13,21}.

Additional studies are needed to validate the clinical relevance of these germline variants across different ethnic groups.

HSD3BI(1245A>C) is the most well-characterized polymorphism with respect to its effects on androgen synthesis^{9,33,34} and response to ADT^{12,13,26,31} in prostate cancer. Furthermore, *HSD3BI*(1245A>C) is associated with shorter OS in men with low volume metastatic CSPC in a phase III trial of ADT vs. ADT and docetaxel³⁵. *HSD3BI*(1245A>C) was investigated in a recent Japanese study, which found that *HSD3BI*(1245A>C) was associated with impaired progression-free survival but no difference in OS in mCSPC, and improved response to abiraterone acetate in mCRPC²⁶. However, given the low number of patients with *HSD3BI*(1245A>C) in that prior study and known low prevalence of the *HSD3BI*(1245A>C) polymorphism in patients of East Asian descent¹³, it is unclear whether these findings were generalizable to cohorts of different ethnic composition. In our study of predominantly Caucasian patients, we observed that 42% of patients demonstrated the *HSD3BI*(1245A>C) variant, which was higher than the 14.1% observed in the Japanese mCRPC study and comparable to the 47.9% observed in a different predominantly Caucasian mCRPC cohort¹³. Of note, all cases of variant *HSD3BI*(1245A>C) were attributable to germline alterations, as there were no somatic mutations identified at this locus in our cohort. We found in our cohort that median OS was shorter in patients with variant *HSD3BI* than those homozygous wild-type, with a borderline-significant survival difference ($P=0.052$).

While most studies have focused on the *HSD3BI*(1245A>C) polymorphism, one prior study in metastatic CSPC highlighted rs1856888, a SNP 13kb upstream of *HSD3BI* as a prognostic factor associated with significantly shorter time to progression on primary ADT¹⁰. Our study found variant rs1856888 to be associated with poor prognosis with respect to OS as well. Additionally, our study is the first to report a high frequency of *HSD3BI*(1245A>C) and variant rs1856888 co-inheritance. Given the high frequency of co-inheritance, the relationship between the two variants and their individual contributions to the clinically-aggressive mCRPC phenotype are difficult to resolve. While the mechanistic significance of the missense-encoding *HSD3BI*(1245A>C) polymorphism and its effects on enzyme stabilization have been elucidated, the mechanistic consequences of rs1856888 are unknown. Additional studies are needed to assess the functional and clinical relevance of the rs1856888 variant independent of *HSD3BI*(1245A>C) status.

We found that inheritance of a *HSD3BI* germline variant was not associated with common somatic DNA-sequencing alterations in mCRPC. Somatic DNA alterations would not necessarily be expected to be associated with germline variants in androgen metabolism genes, since, unlike variant *HSD3BI*, none of the common somatic alterations examined (with the exception of *AR* amplification^{36,37}, *TP53* mutations, and *RBI* loss^{15,38}) are known to be enriched in mCRPC relative to mCSPC. As recent studies found that *RBI* loss was the key somatic alteration associated with impaired outcomes in mCRPC^{16,39}, we performed a multivariable analysis including both *RBI* loss and variant *HSD3BI* along with other clinicopathologic factors and found that both were independently prognostic of shorter OS after primary ADT. This further supports the independence between the germline *HSD3BI* variant and somatic *RBI* loss. Investigation in larger cohorts is needed to confirm these

findings and to examine the association between *HSD3B1* germline variants and lower-prevalence DNA-seq alterations. In our RNA expression-based gene set enrichment analysis of metastasis biopsies, we found that the *HSD3B1* germline variant was associated with increased expression of cell proliferation genes. These findings demonstrate that variant *HSD3B1* may be associated with more molecularly as well as clinically aggressive tumors.

There are several limitations of the present study. First, we report on a multi-institutional cohort of relatively modest sample size, and no external validation cohort was available for this analysis. With this in mind, we chose to focus on a relatively succinct set of 17 germline variants previously reported to be associated with impaired clinical outcomes. Still yet, multiple survival comparisons were made, and the nominal *P*-values reported should be interpreted accordingly. Future work in independent cohorts is needed to validate the prognostic significance of *HSD3B1* polymorphisms. Another limitation of our study is that data on PSA dynamics (such as PSA doubling time) after ADT initiation were unavailable. Thus, we were unable to directly assess the prognostic significance of variant *HSD3B1* with respect to response to primary ADT. Further investigation of the clinical implications of variant *HSD3B1* is needed to potentially guide treatment decisions.

In summary, we applied whole-genome germline and somatic sequencing approaches to paired normal tissue and tumor biopsy samples to perform an integrated analysis of both inherited and acquired predictors of clinical outcomes. Future studies may shed further insights into the interplay between the germline *HSD3B1* variants in advanced prostate cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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CONFLICTS OF INTEREST

SGZ has patents filed unrelated to this work with Decipher Biosciences and Celgene. A patent for 3 β -hydroxysteroid dehydrogenase in steroid-dependent disease has been filed by Cleveland Clinic.

JJA has performed consulting or held an advisory role with Astellas Pharma, Bayer, Merck, and Janssen Biotech, Inc.

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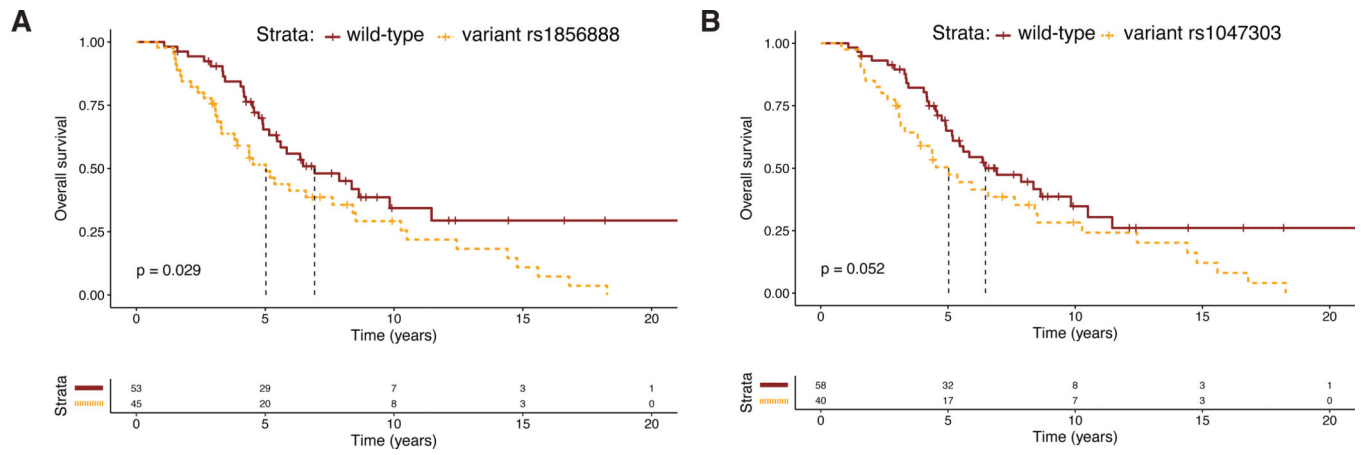


Figure 1:
Kaplan-Meier curves demonstrating overall survival differences between patients with 1–2 variant alleles and homozygous wild-type patients in the A) rs1856888 and B) rs1047303 (1245C) *HSD3B1* polymorphisms.

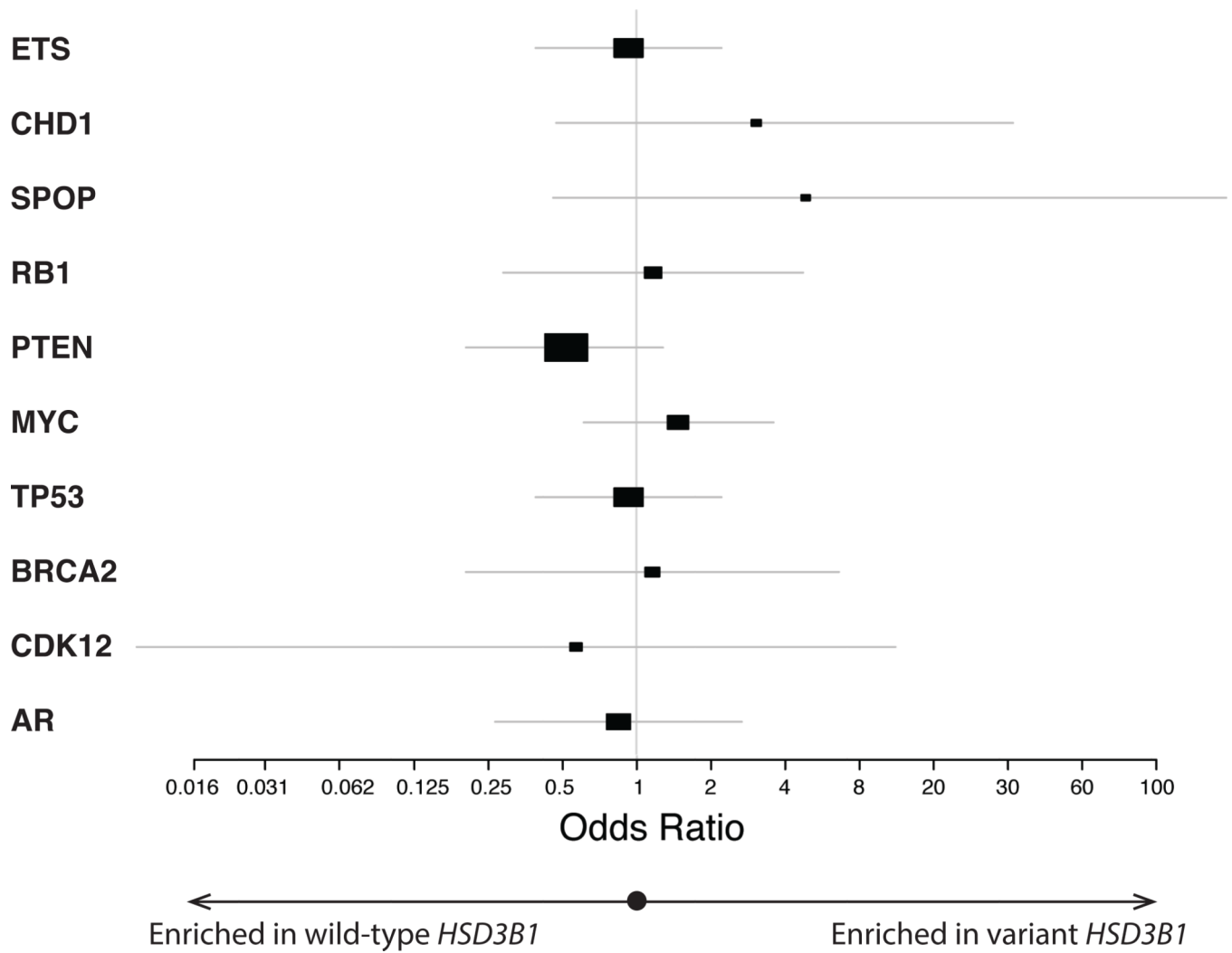


Figure 2:

Forest plot showing the association between variant germline *HSD3B1* and common somatic DNA alterations in mCRPC tumor biopsies. A odds ratio > 1 indicates positive association between variant *HSD3B1* and the somatic tumor alteration of interest. Details on the prevalence of each somatic alteration stratified by *HSD3B1* status as well as exact odds ratios including 95% confidence intervals can be found in Table S1.

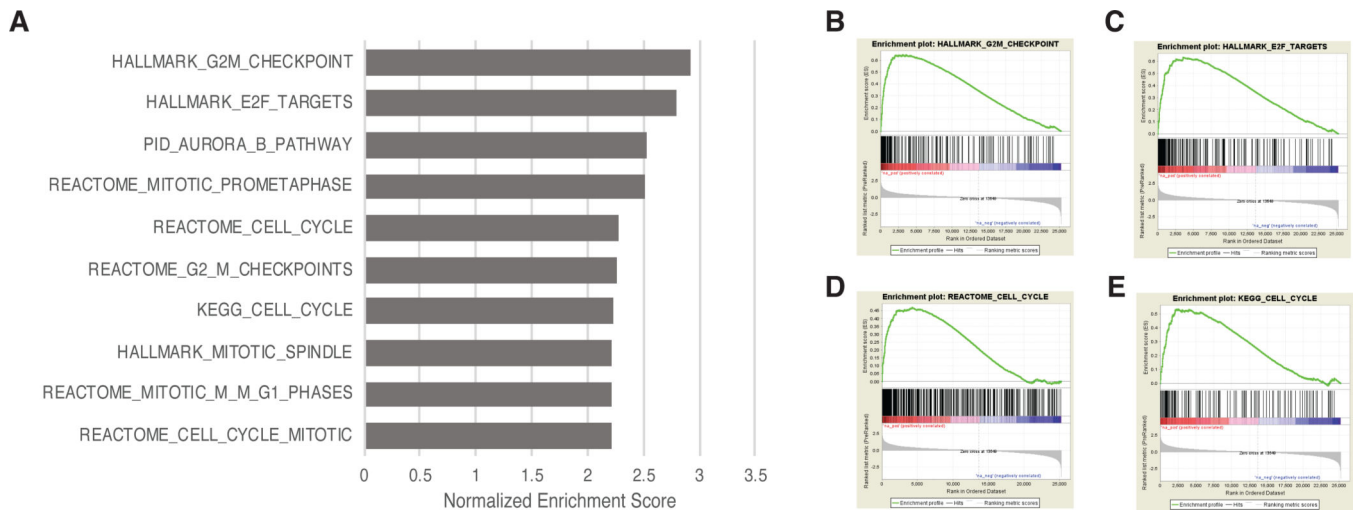


Figure 3:

A) Top 10 gene sets significantly overexpressed in the tumors of patients with variant *HSD3B1* based on Gene Set Enrichment Analysis (GSEA) performed on tumor RNA-seq data using the GSEA Preranked tool¹⁷. B-D) GSEA plots of four cell-cycle related pathways significantly overexpressed in tumors of patients with variant *HSD3B1*.

Table 1.

Patient demographics and clinicopathologic features

Characteristic	Pts. sequenced (N=101)
Age – years (SD)	71 (8.4)
Race	
Asian	4 (4.3)
Black or African American	5 (5.3)
White	85 (90.4)
Missing	7 (6.9)
Gleason score at diagnosis	
8+	52 (57.1)
< 8	39 (42.9)
Missing	10 (9.9)
ECOG performance status	
0	57 (56.4)
1	41 (40.6)
2+	3 (3)
Missing	0 (0)
Metastatic disease at time of ADT initiation	
Yes	51 (51.5)
No	48 (48.5)
Unknown	2 (2.0)
Metastatic sites at time of biopsy	
Liver	15 (14.9)
Visceral metastases (non-liver)	12 (11.9)
Bone +/- lymph node	64 (63.4)
Lymph node only	11 (10.9)
Missing	0 (0)
Prior treatment status (abiraterone / enzalutamide)	
Naïve / Naïve	38 (38)
Naïve / Treated	27 (27)
Treated / Naïve	15 (15)
Treated / Treated	20 (20)
Missing	1 (1)
rs1856888	
Homozygous wild-type	54 (53)
Heterozygous	36 (36)
Homozygous variant	11 (11)
rs1047303, i.e., <i>HSD3B1</i> (1245A>C)	
Homozygous wild-type	59 (58)
Heterozygous	35 (35)
Homozygous variant	7 (7)

Characteristic	Pts. sequenced (N=101)
Laboratory values – median (IQR)	
PSA, ng/mL	43 (15–148)
Alkaline phosphatase, U/L	91 (65–140)
LDH, IU/L	203 (166–291)
Hemoglobin, g/dL	13 (12–14)

Note: all clinicopathologic variables were measured at time of biopsy and are presented as “Number (%)” unless otherwise noted.

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Table 2.

Multivariable analysis including genomic and clinicopathologic features

	HR	LHR	UHR	P-val
Bi-allelic <i>RBI</i> loss	3.01	1.40	6.46	0.005*
Germline variant <i>HSD3B1</i>	1.85	1.01	3.37	0.045*
Metastases present at ADT initiation	3.21	1.72	5.98	0.0002*
ECOG 1	1.25	0.70	2.21	0.45
Gleason 8	0.95	0.55	1.64	0.85
Treatment with enzalutamide / abiraterone	1.00	0.54	1.85	0.995
log(PSA) at biopsy	1.54	1.01	2.35	0.046*

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