SPOP and CHD1 alterations in prostate cancer: Relationship with PTEN loss, tumor grade, perineural infiltration, and PSA recurrence

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Abstract

Background: In the non-ETS fusion of prostate cancer (PCa) pathway, SPOP mutations emerge as a distinct oncogenic driver subclass. Both SPOP downregulation and mutation can lead to SPOP target stabilization promoting dysregulation of key regulatory pathways. CHD1 gene is commonly deleted in PCa. CHD1 loss significantly co-occurs with SPOP mutations, resulting in a PCa subclass with increased AR transcriptional activity and with a specific epigenetic pattern.

Methods: In this study, SPOP alterations at mutational and protein levels and CHD1 copy number alterations have been analyzed and correlated with ERG and PTEN protein expression and with the clinical pathological features of the patients.

Results: SPOP protein loss has been detected in 42.9% of the cases, and it has been strongly associated with PTEN protein loss (p < .001). CHD1 gene loss has been detected in 24.5% and SPOP mutations in 5.9% of the cases. Loss of CHD1 has been strongly associated with SPOP mutations (p = .003) and has shown a trend to be associated with ERG wt cancers (p = .08).

The loss of SPOP protein (p = .01) and the combination of PTEN and SPOP protein loss (p = .002) were both statistically more common in grade group 5 cancers, with a prevalence of 60% and 37.5%, respectively. Furthermore, SPOP loss/PTEN loss and SPOP wt/PTEN loss phenotypes were strongly associated with extraprostatic perineural infiltration (p = .007). Strong CHD1 loss was associated with a shorter time to PSA recurrence in the univariate (p = .04), and showed a trend to be associated with the PSA recurrence risk in the multivariate analysis (p = .058).


1 | INTRODUCTION

Data from large-scale genomic studies agree that most primary prostate cancers (PCa) can be ascribed to one of two broad categories: one related to ERG and other ETS fusions, and the other related to somatic mutations.1-5 ERG fusion is the most prevalent oncogenic driver subclass, and PTEN loss is a concomitant event that cooperates in progression.1,6 In the non-ETS fusion PCa pathway, SPOP mutations represent another oncogenic driver subclass that shows a characteristic pattern of associated alterations such as CHD1 loss, SPINK messenger RNA (mRNA) overexpression and an increased AR activity.1-4,14

Several studies have determined that SPOP acts as a tumor suppressor gene in many tumor types, including PCa. Both downregulation or mutation of SPOP can lead to the stabilization of SPOP targets, thus promoting the malfunction of key regulatory pathways.4,15-21 In PCa, the percentage of SPOP mutations ranges from 6% to 15%.1,2,4,14,22-27 SPOP protein has a role as a tumor suppressor that promotes the destabilization of various substrates, including ERG.18,19,28-31 It has been reported that SPOP mutations are mutually exclusive with ERG fusions and that therefore they could represent an alternative pathogenic event that could increase ERG protein levels in the absence of ERG fusion. In addition, SPOP mutations seem to be mutually exclusive not only with ERG fusion but also with all the ETS-fusion subclass.1

CHD1 gene is the second most frequently deleted or mutated gene in PCa, occurring in a range about 10%–25% cases.1,4,12-37 ETS fusions and homozygous loss of CHD134,35 have also been reported to be mutually exclusive. On the other hand, CHD1 loss significantly co-occurs with SPOP mutations,40 resulting in a PCa subclass with increased AR transcriptional activity.1 CHD1 has been identified as a key tumor suppressor in the prostate that can restrict AR binding and function, limiting in this way cancer progression.32 In addition, CHD1 loss seems to be associated with an increase in genomic instability and chromosomal rearrangements, and it sensitizes prostatic epithelial cells to DNA damaging treatments. Thus, CHD1 status could be used to stratify PCa for effective treatments.1,37,41

SPOP mutations are considered an early event.1,4,15,30,42 CHD1 deletion would be a later, subclonal event.2,4 Paradoxically, the percentage of SPOP mutations is much lower, thus suggesting that perhaps gene or protein expression alterations could also have a role in this setting. This possibility has received little attention in the literature.17,43,44 Recently, our group has reported that ERG rearrangement with its concomitant overexpression is associated with low levels of SPOP mRNA, and that this downregulation of SPOP expression is much more common than the mutations of this gene. In addition, this finding was associated with a shorter time to PSA recurrence.23 In any event, the prognostic role of SPOP mutations remains uncertain as different papers have reported conflicting results.14,17,23,42,45-48

The aim of the present study has been to further investigate the relationship between SPOP, CHD1, PTEN, and ERG alterations in a more integrated approach, in an attempt to understand the meaning of the crossovers between ERG and SPOP carcinogenic pathways, and the potential role of all these genes as prognostic factors. For this purpose, we have investigated SPOP alterations at mutational and protein levels and CHD1 copy number alterations in a representative and well characterized cohort of localized PCa. We have also analyzed their relationship with ERG and PTEN protein expression and with the clinical-pathological features of the patients.

2 | MATERIALS AND METHODS

2.1 | Patients and tumor samples

Two-hundred and twenty PCa obtained from formalin-fixed paraffin-embedded (FFPE) radical prostatectomy specimens, and 30 additional FFPE tumors, were selected retrospectively from the files of the Parc de Salut MAR Biobank (MARBiobanc). Prostatectomy specimens are wholly sectioned, embedded and examined to determine the number of tumor foci and their pathological features. We consider two tumor foci as different when they are separated in the same slide and/or they do not overlap with other tumor foci in the consecutive proximal and/or distal sections of the prostate.

Grade Group (GG) WHO-ISUP 2016 classification, tumor stage, preoperative PSA levels, perineural infiltration, and tumor topographic distribution (unifocal vs. multifocal) information are shown in Table 1. Preoperative PSA, and extraprostatic perineural infiltration was not available in one and four cases, respectively.

2.2 | Immunohistochemistry for SPOP, PTEN, and ERG

Immunohistochemical (IHC) study for SPOP, PTEN, and ERG proteins was performed in 581 tissue microarray (TMA) cores derived from the 220 cases. Twenty-two cases were noninformative because there was no available information for all the three immunostainings and so they were
excluded. Therefore, the final number of analyzed cases was 198. As previously reported,49 from 1 to 3 cores of the different Gleason Score (GS) cancer areas were selected in each case from the haematoxylin-eosin (H&E)-stained sections of donor blocks (N. J. and J. Ll.), and a total of 9 TMAs were constructed using a manual tissue arrayer (Chemicon ATA 100). In 54 cases, only one core was available for the analysis. SPOP protein expression was also analyzed by IHC in 30 FFPE tumors in which SPOP mRNA expression levels had been previously reported.23 IHC analysis of SPOP was carried out using the anti-rabbit polyclonal SPOP antibody (Abcam). IHC analysis of ERG and PTEN was carried out as previously reported.49 For ERG, two patterns of nuclear expression were considered: negative (no detectable staining) or positive (any detectable staining), and endothelial cells were used as an internal control. For the assessment of PTEN and SPOP loss, a semi-quantitative scoring system was implemented, considering loss of expression as complete (0), partial (1) or absent (wt expression) (2). Internal references for intensity of expression were adjacent normal tissue for PTEN. For SPOP, smooth muscle tissue was used as internal positive control and stromal tissue as internal negative control. In addition, in some macro tumor tissue sections, additional slides were immunostained replacing the primary antibody with buffer solution as additional control to test the specificity of the immunostaining.

2.3 | SPOP mutational analysis by PCR and Sanger direct sequencing

Representative tumor areas were selected from the FFPE prostatectomy specimens for manual microdissection and DNA extraction in a subset of 102 cases out of the total of 198 cases analyzed. Dissected areas contained at least 70% and usually around 90% tumor cells. The respective H&E slides served as templates. DNA was extracted using the DNeasy Tissue Kit (Qiagen GmbH) from two to three consecutive 10-μm sections.

Mutational analysis of exons 5 and 6 of SPOP (hotspot regions) and surrounding intronic regions was performed from DNA. Primer sequences, annealing temperature, PCR product size, exons, and hotspot codons analyzed are shown in Table S1. PCR products were migrated in 2% E-gel® precast agarose electrophoresis system (Invitrogen, Thermo Fisher Scientific), and imaged with the Gene Flash transilluminator (Syngene Bioimaging). PCR products were purified and sequenced with the Big Dye Terminator Kit v.3.1 in the ABI 3730xl DNA Analyzer (Applied Biosystems, Thermo Fisher Scientific) by the UPF-Genomics Core Facility (DCEXS-UPF).

2.4 | CHD1 gene loss by TaqMan® Copy Number Assay (quantitative PCR [qPCR])

CHD1 gene loss was assessed with the ABI PRISM 7500 Sequence Detection System, using the TaqMan® Copy Number Assay (qPCR) probe and primer mix (Applied Biosystems, Life Technologies Corporation). The assay identification number for CHD1 was Hs01976012_cn, and RNase P gene was used as internal control to normalize levels of DNA. The samples were run in triplicate and the mean value was calculated for each case. CHD1 copy number loss was considered for 2ΔΔCt <0.76. Strong CHD1 loss was considered for 2ΔΔCt <0.66.
2.5 | Statistical and survival analysis

Categorical variables are presented as frequencies and percentages, and quantitative variables as median and range. Pearson Chi-Square test or Fisher’s Exact test were used to assess the relationship between two categorical variables, while the Student t test was used to study the quantitative variables. Statistical analysis was performed using the STATA statistical package version 15.1 (STATA Corp.). A p value less than .05 was considered to be statistically significant.

The relationship with time to PSA recurrence was analyzed using Kaplan–Meier (Log-Rank) test in 195 patients (3 cases in this series were lost for follow-up).

In addition, a multivariate Cox proportional hazard regression analysis was used to assess the association between the categorical variables that showed a statistical association in the Log-Rank analysis, with their corresponding hazard ratio (HR), 95% confidence intervals, and p values, after adjusting for other prognostic variables (GG, tumor stage, focality, age, and preoperative PSA). A p < .05 was taken as statistically significant.

Patients were followed at regular intervals of 3 months for 1 year and every 6 months for the subsequent years, and a PSA test was performed before every follow-up visit. None of the patients received pre- or postoperative radiation or hormone therapy. Patients were censored when an increase in serum PSA >0.2 ng/ml was detected at the time of their last clinical follow-up appointment (i.e., two consecutive increases), Patients’ follow-up ranged from 5 to 234 months, with a mean value of 87.1 months and a median of 93 months. For the Log-Rank test the time of follow-up was adjusted to 120 months. A p value <.05 was considered as statistically significant. Statistical analysis was performed using the STATA statistical package version 15.1 (STATA Corp.).

3 | RESULTS

3.1 | Immunostaining analysis of SPOP and relationship with ERG and PTEN status

IHC expression of SPOP, PTEN, and ERG was assessed in the 198 informative cases. In 157, more than one core was available for the analysis, and the one with the highest grade was selected to analyze the relationship between SPOP, PTEN, and ERG expression and the clinical-pathological parameters. In all cases, the core with the highest grade was representative of the global GS of the patient.

Expression of SPOP protein was predominantly found in the cytoplasm and only two cases showed expression additionally in the nucleus. Loss of SPOP protein expression was detected in 85 of the 198 cases (42.9%). A total of 22 (11.1%) cases showed complete, and 63 (31.8%) showed partial loss of expression. PTEN loss was detected in 72 of 198 cases (36.4%), with complete loss in 24 (12.1%) and partial loss in 48 (24.3%). ERG overexpression was found in 96 tumor samples (48.5%).

Examples of SPOP immunostaining are shown in Figure 1. Figure 1A (GG3 cancer) and 1B (GG5 cancer) show total loss of expression, and Figure 1C (GG2 cancer) and 1D (GG1 cancer) show SPOP wt expression.

In an attempt to assess the concordance between SPOP mRNA and protein expression levels, SPOP immunostaining was performed as well in a selected series of 30 FFPE PCs in which we had previously analyzed the mRNA expression levels by qPCR. In this series, 13 (42%) and 14 (45.2%) cases showed mRNA and protein expression loss, respectively. There was a strong association between both of them (Chi-Square test, p = .004). Thus, 76.9% (10 out of 13) of the cases with SPOP mRNA expression loss also showed SPOP protein loss, and 76.5% (13 out of 17) were wt for both SPOP mRNA and protein expression.

Loss of SPOP expression (either complete or partial) was strongly associated with PTEN loss (either complete or partial as well) (Chi-Square test, p < .001). Thus, SPOP loss was detected in 58.3% (42 out of 72) of the cases with PTEN loss and in 34.1% (43 out of 126) with PTEN wt expression (Figure 2A). However, SPOP loss was not associated with ERG overexpression (Chi-Square test, p = .95), for it was very similar in cases with ERG overexpression (41 out of 96; 42.7%), and in cases with ERG wt (44 out of 102; 43.1%). On the other hand, as expected, PTEN loss was strongly associated with ERG overexpression (Chi-Square test, p = .002).

As PTEN loss showed a strong statistical association with both SPOP loss and ERG overexpression (ERG+), we also analyzed the relationship of SPOP status with the combination of PTEN and ERG status (Figure 2B). From all the possible combinations of the three genes, our results indicate that SPOP loss is more strongly associated with PTEN loss that is not related to ERG overexpression (74.1%), compared to the other combinations (Chi-square test, p = .001). Percentage of wt SPOP was significantly higher in PTENwt/ERGwt cancers (68%) and in PTENwt/ERG+ (62.8%) cancers.

3.2 | CHD1 gene loss in PCs. Relationship with SPOP mutations and SPOP, ERG, and PTEN immunostaining

SPOP mutations were detected in 6 out of 102 (5.9%) cancers. All mutations were located in the MATH domain: G75R (n = 1), F102L (n = 1), F104V (n = 1), S119N (n = 1), and F133L (n = 2). Only two of these cases did not show loss of SPOP protein expression and both of them harbored the missense change F133L. Conversely, five of these six SPOP mutated cases had ERG wt protein expression. Finally, four of the six SPOP mutated cancers showed PTEN protein loss.

CHD1 copy number loss was considered for 2(-ΔCt) <0.76, and was detected in 25 out of 102 cancers (24.5%). CHD1 copy number loss was strongly associated with SPOP mutations (Fisher’s Exact test, p = .003), because 5 of the 6 (83.3%) SPOP mutated cancers showed also CHD1 loss, but only 20 of the 96 (20.8%) SPOP wt cancers did.

Percentage of CHD1 loss was substantially higher in ERG wt cancers (32%; 16 out of 50) than in ERG overexpressing cancers (17.3%; 9 out of 52), with a trend to approach statistical significance (Chi-square test, p = .08). On the other hand, CHD1 loss was not associated with IHC expression of SPOP (Chi-square test, p = .89), as it was found in 23.9% (11 out of 46) cancers with SPOP protein loss and in 25% (14 out of 56)
wt cancers, respectively. CHD1 loss was detected in a similar percentage of cases with PTEN loss (25%; 11 out of 44) and cases with wt PTEN (24.1%; 14 out of 58) (Chi-square test, $p = .92$).

### 3.3 Relationship of loss of SPOP and PTEN expression, SPOP mutations, and CHD1 loss with clinical-pathological variables

We investigated the relationship among these alterations and the main clinical-pathological variables: GG, tumor stage, preoperative PSA, perineural infiltration and tumor uni- or multifocality (Table 2).

Loss of SPOP expression was statistically associated with the grade group classification. It was less frequent in GG1 cancers (22.2%; 8 of 36), than in GG2 (40.8%; 29 of 71), GG3 (52.2%; 12 of 23) and GG4 (42.8%; 12 of 28) cancers, and reaching a 60% (24 of 40) in the GG5 group (Chi-square test, $p = .01$) (Figure 3A).

To assess if loss of SPOP expression occurs only in high grade Gleason patterns or it is already present in Gleason pattern 3, we compared SPOP expression between patterns 3 and 4 in a subset of 35 GG2 PCa. From these, 15 out of 35 (42.8%) GG2 PCa showed SPOP loss, and 20 (57.2%) did not. In 34 cases (97.1%), SPOP expression was the same in both patterns, whereas in one case there was loss of SPOP expression only in Gleason pattern 4.
As stated above, loss of expression of SPOP and PTEN were strongly associated, and they also showed a strong statistical association with GGs. Thus, PTEN plus SPOP loss was present in only 5.6% of GG1 cancers (2 of 36), but in 37.5% of GG5 (15 of 40) cancers. Isolated PTEN loss was present in only 5.6% of GG1 cancers as well (2 of 36). The frequency of SPOP loss was higher in GG3 PCA (8 of 28; 28.6%), but did not show a clear trend to be associated with any GG. Finally, SPOP/PTEN wt phenotype was very frequent in GG1 PCA (26 of 36; 72.2%) and moderately frequent in GG2 cancers (31 of 71; 43.8%) (Chi-square test, \( p = .002 \)) (Figure 3B).

CHD1 loss was less commonly found in GG1 cancers, but it was not statistically associated with the GG classification (Fisher’s Exact test, \( p = .44 \)). Among the 102 cancers in which this analysis was available, CHD1 loss was detected in 10% of GG1 (2 of 20), 25% of GG2 (10 of 40), 27.3% of GG3 (3 of 11), 33.3% of GG4 (4 of 12) and in 31.6% of GG5 (6 of 19) cancers.

As stated above, we found SPOP mutations in 6 out of 102 PCA (5.9%). None of them belonged to GG1, 2 cases (5%) were GG2 PCA, there was 1 case in GG3 (9.1%) and GG4 (8.3%), and 2 mutated cancers (10.5%) belonged to GG5. Again, these results were not statistically significant.

Stage, preoperative PSA, extraprostatic perineural infiltration and tumor focality information are shown in Table 2. Neither SPOP loss of expression, combined SPOP plus PTEN loss, SPOP mutations nor CHD1 loss showed association with tumor stage or preoperative PSA levels.

Regarding tumor focality, there was no association between either uni- or multifocality and SPOP mutations (Fisher’s Exact test, \( p = 1 \)) or CHD1 loss (Chi-square test, \( p = .27 \)). Conversely, IHC loss of SPOP protein expression was found in 27 of 51 (52.9%) unifocal cases, and in 52 of 132 (39.4%) multifocal cancers (Chi-square test, \( p = .09 \)). Finally, the combination SPOP loss/PTEN wt was present in 17 of 51 (33.3%) unifocal cancers, but in 22 of 132 (16.7%) cases (Chi-square test, \( p = .08 \)) (Table 2).

We investigated the relationship of these gene alterations with extraprostatic perineural infiltration as well. We did not find any association between perineural infiltration and loss of IHC expression of SPOP, SPOP mutations or CHD1 loss. However, the protein combinations of SPOP loss/PTEN loss and SPOP wt/PTEN loss were statistically associated with cancers with extraprostatic perineural infiltration (Chi-square test, \( p = .007 \)) (Table 2).

### 3.4 | Relationship of SPOP, CHD1, and PTEN alterations and PSA recurrence

PSA recurrence was detected in 46 (23.9%) out of the 192 cases in which there was information on SPOP and PTEN protein analysis and follow-up data. In addition, PSA recurrence was also detected in 23 (23.2%) of the subset of 99 cases with information on SPOP mutations and CHD1 copy number loss and follow-up data. Kaplan–Meier analysis was performed for SPOP protein expression loss versus SPOP wt levels (Log-rank test, \( p = .53 \)), the different combinations of SPOP and/or PTEN protein loss (Log-rank test, \( p = .31 \)), SPOP mutations versus SPOP wt (Log-rank test, \( p = .77 \)) and CHD1 loss (<0.76) versus CHD1 wt (Log-rank test, \( p = .39 \)). In the case of CHD1 loss, we included an additional cut-off \( (\Delta C T) <0.66 \) to assess the specific impact of marked copy number loss for the Log-rank test, and we found that this approach showed a statistical association with a shorter time to PSA recurrence (Log-rank test, \( p = .04 \)) (Figure 4).

Moreover, also a multivariate Cox proportional hazard regression analysis was used to assess the association between strong CHD1 loss and PSA-recurrence risk after adjusting for other prognostic variables (Table 3), showing 2.45 higher risk of PSA-recurrence, with a trend to be statistically associated (HR = 2.45, \( p = .058 \)).

### 4 | DISCUSSION

Data from different studies have revealed that SPOP protein has a role as a tumor suppressor and that negatively regulates the stability of various substrates, including ERG and AR. SPOP seems to play a key role in tumorigenesis of different human neoplasms, and downregulation or mutation of this gene could promote stabilization of SPOP targets thus favoring the development of cancer. The mutational landscape of SPOP in PCa has been extensively studied. However, the prognostic role of SPOP status...
remains currently uncertain.\textsuperscript{14,17,23,41,45–48} CHD1 has also been identified as a key tumor suppressor in PCa. Deletion or mutation of this gene represents another frequent alteration in this cancer, but the impact of these alterations in PCa is also unclear.\textsuperscript{1,4,32–39,51} In this study we have analyzed the role of SPOP and CHD1 in the pathogenesis of PCa, and their potential use as prognostic factors. To the best of our knowledge, this is the first paper that addresses the relationship of SPOP with ERG and PTEN protein expression.

Few studies have dealt with the role of SPOP expression alterations in PCa.\textsuperscript{17,23,43,44} Our results, in keeping with some of the previous papers, indicate that a remarkable percentage of PCa (43%) show loss of SPOP protein expression. A similar percentage was obtained by Kim et al.\textsuperscript{43} that reported loss of SPOP protein in 37% of tumors.

We previously reported SPOP expression loss at RNA level in about 25% of cancers in a different PCa cohort.\textsuperscript{23} This alteration was associated with ERG overexpression and TMPRSS2-ERG rearrangement. Our present results show that loss of SPOP protein is much more common than loss of SPOP mRNA. In the present study we have assessed the relationship between SPOP mRNA and protein expression levels, and we found a strong association between both features. On the other hand, we detected only 6 cases (=6%) with SPOP mutations, representing an uncommon aberrant event in our series of PCa. It is interesting to note that only two of them were not associated with decreased SPOP protein, and these two cases were the only ones in this small group that had the same missense change F133L. However, the finding that loss of SPOP expression is much more common than SPOP mutations suggests that decrease in this protein must be related to other mechanisms.

In our series, we have found that the loss of SPOP and PTEN proteins are closely associated. On the other hand, the loss of SPOP protein did not bear any relationship with ERG protein expression. Previous studies have shown that both mutations of SPOP gene and rearrangements of ERG, would prevent degradation of ERG and eventually would result in an increase in ERG protein levels.\textsuperscript{1,3,4,18,28–30,52,53} On the other hand, Shoag et al.\textsuperscript{47} reported the lack of ERG protein overexpression in most of their SPOP-mutant PCa samples. Moreover, they suggested that ERG rearrangement and SPOP mutation could be divergent events leading to distinct biological classes of PCa. In keeping with this interpretation, we have also found that SPOP mutations are not associated with increased expression of ERG protein.

With regard to our finding of CHD1 copy number loss in 24.5% of PCa, previous reports indicate a frequent deletion or mutation of

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Distribution of SPOP and PTEN protein loss, SPOP mutations, and CHD1 loss in PCa according to clinical-pathological variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor stage</td>
<td>Tumoral focuses</td>
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<tr>
<td>pT2</td>
<td>pT3</td>
</tr>
<tr>
<td>SPOP loss</td>
<td>58 (40.6%)</td>
</tr>
<tr>
<td>SPOP wt</td>
<td>85 (58.4%)</td>
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<tr>
<td>p value</td>
<td>.277</td>
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<tr>
<td>SPOP/PTEN loss</td>
<td>26 (18.2%)</td>
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<tr>
<td>SPOP loss/PTEN wt</td>
<td>32 (22.4%)</td>
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<tr>
<td>SPOP wt/PTEN loss</td>
<td>20 (13.9%)</td>
</tr>
<tr>
<td>SPOP/PTEN wt</td>
<td>65 (45.5%)</td>
</tr>
<tr>
<td>p value</td>
<td>.228</td>
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<tr>
<td>SPOP mutation</td>
<td>5 (6.8%)</td>
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<tr>
<td>SPOP wt</td>
<td>68 (93.2%)</td>
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<tr>
<td>p value</td>
<td>.510</td>
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<tr>
<td>CHD1 loss</td>
<td>20 (27.4%)</td>
</tr>
<tr>
<td>CHD1 wt</td>
<td>53 (72.6%)</td>
</tr>
<tr>
<td>p value</td>
<td>.282</td>
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</table>
this gene in PCa, in a range of 10%–25%. In our series, loss of CHD1 was strongly associated with SPOP mutations but not with SPOP protein loss. A very high percentage of SPOP mutated cancers (≈83%) showed concomitant CHD1 loss. Our data also show a higher percentage of CHD1 loss in ERG wt than in ERG overexpressing cancers. Furthermore, other studies have shown a significantly higher prevalence of CHD1 deletions among ERG fusion-negative than among fusion-positive cancers.

With regard to the relationship with the clinical-pathological features of the cancers, SPOP protein loss and the combination of SPOP and PTEN loss were statistically associated with GG 5 of the ISUP/WHO 2016 classification. Conversely, the SPOP/PTEN wt phenotype was much more common in the low grade cancers (GG1, 72.2% and GG2, 43.8%). When comparing SPOP expression between Gleason patterns 3 and 4, our results suggest that loss of SPOP expression in GG2 PCa is present already in the Gleason pattern 3 component.

**TABLE 3** Multivariate Cox proportional hazard analysis for strong CHD1 copy number loss and PSA-recurrence risk

<table>
<thead>
<tr>
<th>COX regression for PSA recurrence</th>
<th>HR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD1 copy number</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Wt vs. strong loss (&lt;0.66)</td>
<td>2.456</td>
<td>(0.971–6.209)</td>
<td>.058</td>
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<tr>
<td>Grade group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2–3</td>
<td>0.873</td>
<td>(0.231–3.302)</td>
<td>.842</td>
</tr>
<tr>
<td>4–5</td>
<td>1.192</td>
<td>(0.271–5.234)</td>
<td>.815</td>
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<td>pT</td>
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<tr>
<td>pT2 vs. pT3–4</td>
<td>1.382</td>
<td>(0.547–3.487)</td>
<td>.493</td>
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<td>Tumor focality</td>
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<td>Unifocal vs. Multifocal</td>
<td>0.535</td>
<td>(0.214–1.337)</td>
<td>.181</td>
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<tr>
<td>Preoperative PSA</td>
<td>1.067</td>
<td>(0.942–1.209)</td>
<td>.305</td>
</tr>
<tr>
<td>Age</td>
<td>0.998</td>
<td>(0.931–1.071)</td>
<td>.966</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HR, hazard ratio.

CHD1 loss was less frequent in GG1 cancers and showed a trend to be higher from GG2 through GG4. All SPOP mutated cancers in GG3, GG4 and GG5 also harbored loss of CHD1.

We have previously shown that the prevalence of SPOP mRNA loss was the lowest in GG1 and the highest in GG5, although without statistical association. In keeping with these findings, García-Flores et al. reported that low levels of SPOP mRNA independently predicted a worse prognosis and Zhao et al. found, also at mRNA level, a significant association with the number of positive lymph nodes, GS, and pathologic stage.

It is noteworthy that in our study SPOP protein loss was present in a higher percentage of unifocal PCa, with a trend for statistical significance.

The combinations SPOP loss plus PTEN loss and SPOP wt plus PTEN loss were strongly associated with intra- and extraprostatic perineural infiltration. In fact, PTEN loss was associated with...
perineural infiltration in both SPOP wild type and SPOP loss cases. In keeping with this specific finding, there is an increasing body of information on the role of the nervous system in prostate carcinogenesis that deserves further investigations.1,5

Neither SPOP nor PTEN loss were statistically associated with shorter time to biochemical recurrence in our series. In agreement with previous reports, our results show that strong CHD1 gene loss is associated with a shorter time to PSA recurrence, and has a trend to be associated with a higher risk of PSA recurrence in the multivariate analysis.

Different evidences suggest that CHD1 status could have a prognostic role in PCa and thus be used to stratify human PCa for effective treatments.1,3,17 CHD1-loss or SPOP-mutant/CHD1-loss seems to be characterized by increased genomic instability and high levels of chromosomal rearrangements, suggesting a potential defect in DNA damage repair. In PCa, it has been reported that the frequency of CDH1 deletions increases with tumor grade,36 and that CHD1 deletions are associated with an increased risk of biochemical recurrence.1,4,36

The prognostic role of SPOP mutational status is controversial.1,3,14,17,23,44-48 Whereas some studies suggested an association between mutations and PCa progression, poor prognosis and pathologic parameters,1,17,18,48 other authors have reported that SPOP mutations are a favorable prognostic factor.47,48 Very recently, SPOP mutations have even been associated with improved survival outcome in men with de novo metastatic castration-sensitive PCa receiving standard androgen deprivation therapy.58 In our present series, SPOP mutations did not show any association with the investigated clinical-pathological parameters. Only one of the six patients with SPOP mutation showed PSA recurrence. Paradoxically, these results contrast with our previous study in an independent cohort of PCa, in which SPOP mutations were associated with a shorter time to PSA recurrence.23 A higher number of mutated cases must be available for comparison with wild type SPOP to draw any definitive conclusions.

5 | CONCLUSIONS

In conclusion, the present study has been focused on two genes that are usually associated to cancers not related to ETS fusions. In short, we have found that SPOP mutations are very uncommon, and therefore other alternative mechanisms, such as a decrease in SPOP protein expression, may be involved in the role of this gene in prostatic carcinogenesis. On the other hand, in our series an intense reduction in CHD1 copy number conveys a poor outcome for the patients. A potentially relevant finding for the clinical management of PCa patients is that the combination of PTEN and SPOP proteins loss is strongly associated with extraprostatic perineural extension. If the results of the study are confirmed by other authors they could be incorporated in the decision-making algorithms of PCa management.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ETHICS STATEMENT

CEIC Parc de Salut Mar approval status, project number 2015/3211/l.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION
Additional Supporting Information may be found online in the supporting information tab for this article.