Genetic predisposition to early recurrence in clinically localized prostate cancer.

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Abstract:	Abstract Objectives • To evaluate the genetic susceptibility to early biochemical recurrence (EBCR) after radical prostatectomy (RP), as a prognostic factor for early systemic dissemination. • To build a preoperative nomogram to predict EBCR combining genetic and clinicopathological factors. Patients and Methods • We evaluated 670 patients from six University Hospitals, subjected to RP for clinically localized prostate cancer (PCa), and followed-up for at least five years or until biochemical recurrence (BCR). • EBCR was defined as PSA>0.4ng/mL within one year of RP; preoperative variables studied were: age, prostate specific antigen (PSA), clinical stage,

	biopsy Gleason, and the genotype of 83 PCa-related single nucleotide polymorphisms (SNPs). • Univariate allele association tests and multivariate logistic regression were used to generate predictive models for EBCR, with clinicopathological factors and adding SNPs. • We internally validated the models by bootstrapping and we compared their accuracy using the area under the curve (AUC), net reclassification improvement (NRI), integrated discrimination improvement (IDI), calibration plots, and Vickers' decision curves. Results • Four common SNPs at KLK3, KLK2, SULT1A1 and BGLAP genes were independently associated with EBCR. • A significant increase in AUC was observed when SNPs were added to the model: AUC (I.C.95%) 0.728 (0.674-0.784) vs. 0.763 (0.708-0.817). • NRI showed a significant increase in probability for events of 60.7% and decrease for non-events of 63.5%. • IDI and decision curves confirmed the superiority of the new model. Conclusion • Four SNPs associated with EBCR significantly improved the accuracy of clinicopathological factors. • We present a nomogram for preoperative prediction of EBCR after RP.

Title Page:

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Abstract

Objectives

- To evaluate the genetic susceptibility to early biochemical recurrence (EBCR) after radical prostatectomy (RP), as a prognostic factor for early systemic dissemination.
- To build a preoperative nomogram to predict EBCR combining genetic and clinicopathological factors.

Patients and Methods

- We evaluated 670 patients from six University Hospitals, subjected to RP for clinically localized prostate cancer (PCa), and followed-up for at least five years or until biochemical recurrence (BCR).
- EBCR was defined as PSA>0.4ng/mL within one year of RP; preoperative variables studied were: age, prostate specific antigen (PSA), clinical stage, biopsy Gleason, and the genotype of 83 PCa-related single nucleotide polymorphisms (SNPs).
- Univariate allele association tests and multivariate logistic regression were used to generate predictive models for EBCR, with clinicopathological factors and adding SNPs.
- We internally validated the models by bootstrapping and we compared their accuracy using the area under the curve (AUC), net reclassification improvement (NRI), integrated discrimination improvement (IDI), calibration plots, and Vickers' decision curves.

Results

- Four common SNPs at KLK3, KLK2, SULT1A1 and BGLAP genes were independently associated with EBCR.
- A significant increase in AUC was observed when SNPs were added to the model: AUC (I.C.95%) 0.728 (0.674-0.784) vs. 0.763 (0.708-0.817).
- NRI showed a significant increase in probability for events of 60.7% and decrease for non-events of 63.5%.
- IDI and decision curves confirmed the superiority of the new model.

Conclusion

- Four SNPs associated with EBCR significantly improved the accuracy of clinicopathological factors.

- We present a nomogram for preoperative prediction of EBCR after RP.

Introduction:

Nowadays, up to 80% of prostate cancer (PCa) patients are diagnosed of clinically localized disease[1]. Radical prostatectomy (RP) is the most common curative treatment option for those patients. However, after RP approximately 35% of patients will experience rising prostate specific antigen (PSA) levels, referred to as biochemical recurrence (BCR)[2]. Rising PSA levels will be due to either a local recurrence as a consequence of non-radical local surgery, or to systemic recurrence because of tumor dissemination before surgery.

An early biochemical recurrence (EBCR) after RP, especially within one year, has prognostic relevance, as it suggests that systemic disease was already present before surgery[3,4,5].

It is surprising that clinically or even pathologically localized PCa could trigger a systemic failure. Thus, how can we explain an early predisposition to systemic failure from supposedly localized stages? Can we expect a genetic predisposition to early systemic dissemination? Could we predict this predisposition preoperatively?

Knowing the genetic predisposition of a patient to early systemic dissemination, even though having a clinically localized PCa, would lead us to consider radiotherapy with hormonal adjuvant treatment instead of RP, or to suggest the inclusion of such patients in early adjuvant protocols or clinical trials, despite being pathologically localized PCa.

Recently, several germline genetic polymorphisms have been associated with the risk of developing PCa[6], its aggressiveness[7] and the risk of BCR[8]. We hypothesized that certain of those polymorphisms, could also promote early systemic dissemination.

In our study, we analyze the association of common single nucleotide polymorphisms (SNPs) with the risk of EBCR within one year after RP, as a surrogate for systemic failure in clinically localized PCa.

In addition, we attempt to develop new preoperative nomograms to predict EBCR, combining standard clinicopathological parameters and SNPs. Finally, we compare the predictive accuracy of models with and without SNPs.

Patients and Methods:

After exclusion of 33 patients due to missing data, a total of 670 patients were evaluated. All patients gave written informed consent. The study was approved by the Clinical Research Ethical Committee of University Hospital Vall d'Hebron (Barcelona), and it was in accordance with the Helsinki Declaration and the European Medicines Agency recommendations.

Study inclusion criteria were: a) clinically localized PCa subjected to RP, b) without adjuvant treatment, c) followed until BCR or for at least five years after RP, and d) Caucasian origin. From January 2002 to May 2009, 703 PCa patients were enrolled, from six institutions.

All patients were genotyped using a microarray with allele-specific probes for 83 SNPs, which have been selected by their **association with PCa risk and/or aggressiveness** according to published literature (Supplementary Table 1). **As the study was focused on germline variants, there is no concern about the time point of sample collection**. Briefly, DNA from blood or saliva was used for amplifying target genes in 6 multiplex-PCRs. PCR products were fluorescently labeled and hybridized (Ventana Medical Systems, Tucson, USA). The microarrays were scanned (Innopsys S.A., Carbonne, France) and genotypes were determined using MG1.0 software[9,10].

Age, preoperative PSA, clinical stage, biopsy Gleason score, and the SNPs, were analyzed as candidate predictors. EBCR was defined as a PSA>0.4ng/mL[11] within one year of surgery. PSA was evaluated at 1.5-3 months after RP, and then, every 3-6 months depending on the previous value.

A preliminary variable selection was done based on univariate association with EBCR for clinicopathological variables, and on allele association tests for SNPs, using chi-square and Mann-Whitney test. Subsequently, stepwise logistic regression was used to determine the optimal predictive model.

For multivariate prediction models, PSA was modeled as its natural logarithm, clinical stage and Gleason score were grouped into 3 categories, and a weighted risk score (WRS)[12] was built using selected SNPs. For this purpose, we defined a new variable, SNP= $\sum_{k=1}^{i} w_k g_k$ where one variable is considered per SNP, gk=0; 1; 2, depending on the number of risk alleles carried at the SNP k, and the weights were estimated using a

logistic regression model. For backward selection procedure, the cut-off p-value was set at 0.1, and the stopping rule was based in Akaike's information criterion. Two predictive models were built, one based in clinicopathological variables, and the other adding the genetic score.

Discrimination accuracy of the two models was compared using the area under the curve (AUC)[13], along with the net reclassification improvement (NRI) and the integrated discrimination improvement (IDI)[14]. Calibration was assessed graphically, and clinical utility was studied using Vickers' decision curves[15]. All analyses were performed using R programming language v.2.11.1 with the rms, Hmisc and pROC libraries added and HelixTree software.

Results:

Among 670 patients, 13.3% had a PSA>0.4ng/mL within one year of RP (Table 1). Our cohort included clinically localized PCa patients (T1-T2) and half of them were T1c. More than 66% had a preoperative PSA<10ng/mL and a 76% had a Gleason score <7. Understaging and/or positive surgical margins were found in around 30% (Table 1).

Gleason score, clinical stage, preoperative PSA, and four SNPs located at KLK3 (-5429T/G, rs2569733), KLK2 (Arg250Trp, rs198977), SULT1A1 (Arg213His, rs9282861) and BGLAP (-198T/C, rs1800247) genes showed independent association with EBCR (Tables 2-3).

We generated two predictive models: a baseline model using clinicopathological variables (Figure 1) and another one using clinicopathological variables and the genetic score, constructed from the four SNPs independently associated with EBCR (Figure 2). Both models showed good discrimination. The AUC of the baseline model was 0.728 (I.C.95%: 0.674–0.784), and the AUC of the model with the genetic score was 0.763 (I.C.95%: 0.708–0.817) (Table 4). The latter showed a significant increase in discrimination ability compared to the baseline model (AUC difference, 0.034, p=0.025)[13](Figure 3).

We performed an internal validation using 10,000 bootstrap samples with similar proportion of EBCR than the original database, following the procedure described by Harrell et al.[16]. Bias-corrected AUCs for the two models were 0.714 and 0.748, respectively.

The improvement of the model with the genetic score was analyzed through the NRI category-free[17] and the IDI. The analysis showed a NRI increase of 60.7% for events and a decrease of 63.5% for non-events ($p=2.14*10^{-5}$), and an IDI superiority of the model with the genetic score ($p=5.63*10^{-5}$).

Both models showed good calibration (Figure 4 and Figure 5). Although calibration is not a good metric for model comparison[18], we found a better calibration in the high probability range for the model with the genetic score. Finally, decision curves analysis showed a superior clinical benefit of the model with the genetic score, particularly for intermediate risk patients for whom classic predictive models are least accurate (Figure 6).

Discussion:

We report the identification of four common SNPs located in KLK2, KLK3, SULT1A1 and BGLAP genes, independently associated with the risk of EBCR. In parallel, we have developed a model to predict EBCR within one year of RP, based on classic preoperative clinicopathological variables. The model showed a high discrimination capacity and a correct calibration, and confirmed the predictive ability of clinical variables included in previously published nomograms[19,20,21]. In addition, the incorporation of a genetic score, based on those four SNPs, resulted in a significant improvement of the model in terms of both discrimination and calibration. The gain in accuracy was more noticeable in intermediate and high risk patients. The decision curve analysis showed a greater net benefit at the same cut-off point for the clinical-genetic model, in agreement with the results of NRI and IDI.

The detection of systemic recurrences after RP is not feasible with objective techniques such as bone scan or computed tomography, until advanced stages and long time after RP (i.e. 8 years)[22]. In order to maximize the probability of having patients with systemic recurrences, we defined patients with EBCR as patients with detectable PSA in their first control after RP, or with rising PSA during the first year after RP[3,4,5]. Therefore, we used EBCR as a surrogate for high probability of systemic recurrence. Lymph node/seminal vesicles involvement or high prostatectomy Gleason score could be also considered as risk factors of systemic recurrence[23], but they are not known preoperatively.

EBCR is associated with metastases[22] and PCa specific mortality (PCSM)[24,25]. For this reason, accurate EBCR risk assessment is critical. With this aim, a postoperative nomogram to predict EBCR within two years of RP was reported a few years ago[29]. However, the model was based on postoperative variables and is not intended for preoperative use. In contrast, we have developed a model based on preoperative variables and germline genetic variants to predict the risk of EBCR within one year of RP. Patients at high risk of EBCR might be eligible for radiotherapy with concomitant hormonal therapy or subjected to RP in early adjuvant systemic treatment clinical trials, despite conflicting results[26,27]. Hence, our predictions could assist clinicians in disallowing RP alone and/or considering multimodal approaches or early adjuvant therapies.

Patients with pathological features associated with local recurrence (e.g. pT3a/pTxR1) could have been excluded in order to best evaluate the associations with systemic recurrence. However, pathological features of those patients were not known preoperatively, so their exclusion would have prevented from using the nomogram as a preoperative tool. Thus, all patients were included irrespective of their pathological features as in most published preoperative nomograms[28]. Instead, our study focused on EBCR within 1st year to minimize the chances of including local recurrences.

Our observed rate of EBCR (13.3%) within one year of surgery is close to the 13.1% and 8.9% within two years, reported by Walz et al.[29]. The BCR rate within one year in those cohorts is unknown. Of note, despite using a more sensitive definition of BCR (0.1-0.2 vs. 0.4ng/mL), Walz el al. reported lower BCR rate than that observed in our study. Several reasons might explain that: a) an artefactually high proportion of recurrence due to retrospective recruitment of our patients; b) a lower rate of non-palpable T1c tumors in our cohort than in the others (49.6% vs. 62.8% and 66.7%), which suggests a poorer prognosis of our patients; c) patients having EBCR with PSA >0.1-0.2ng/ml within two years are likely to reach 0.4ng/ml in a short period of time, suggesting similarity between these series; d) differences in ethnic composition between cohorts may involve different inherited genetic or environmental risk factors influencing the discrepancy. The latter hypothesis emphasizes the interest of **studying** the genetic contribution in PCa prognosis.

To date, D'Amico risk classification[19], the UCSF-CAPRA score[20], and the Stephenson nomogram[21] can be cited among the main preoperative models for prediction of BCR[30]. Those nomograms incorporate the same clinical variables than our model, except the latter two which include the number of positive cores (non-available in our cohort) and the UCSF-CAPRA which includes the age (non-significant in our analysis). **Of note, we observed a 76% of cases with biopsy Gleason score less than 7. Traditionally, high risk patients are more frequently derived to radiotherapy with hormone therapy rather than to radical prostatectomy in our health care setting. This may have resulted in a slight enrichment in not so aggressive disease in our cohort. However, despite being higher, our 76% is very close to the percentage of Gleason score less than 7 reported in other cohorts from widely validated preoperative nomograms (e.g. 72%, 68%, 70% and 74%, for D'Amico, Sthephenson, Walz, and UCSF-CAPRA, respectively). Thus, we consider that this issue would not jeopardize the applicability of our nomogram**. Another model

incorporating immunofluorescent biomarkers has been reported[31]. None of those models predicts BCR within one year of RP which makes difficult their comparison with our model. Nevertheless, the c-index reported for their external validations (D'Amico[19,32] 65.5-70.4%; UCSF-CAPRA[20,32] 68-81%; Stephenson[21,32] 75.2-79%; Donovan 73%[31]), and that obtained in our clinicopathological model (original 72.8%; bootstrap-corrected 71.4%) confirms a highly similar discrimination ability. Interestingly, the addition of genetic variables to the clinicopathological model resulted in a significant improvement in discrimination ability (original 76.3%; bootstrap-corrected 74.8%).

The improvement achieved by including the SNPs is modest, albeit consistently significant across all tests evaluated. The consistent improvement observed demonstrates how genetic factors can enhance the accuracy of PCa prognostic models.

We have previously reported the usefulness of common SNPs for postoperative 5-year BCR predictions[10]. In the present study, we have identified four SNPs independently associated with the preoperative risk of EBCR within one year. **These findings complements our previous results on postoperative long-term BCR predictions[10]**. Two of those SNPs, located at KLK2 and SULT1A1 genes, were also identified in the postoperative study whereas other two, on KLK3 and BGLAP genes, were not. A potential reason for that is that certain germline SNPs may contribute to specific histological phenotypes which once expressed, are reflected in the pathological variables preventing the causal SNPs from remaining in the models. Although that may be more obvious in the postoperative model, SNPs could also have an impact in preoperative variables. **The SNPs on KLK2, KLK3 and BGLAP genes, have been previosuly associated with aggressive disease by different authors, which supports our results. In contrast, the SNP on SULT1A1 has been associated with PCa risk but, to our knowledge, not with PCa aggressiveness. Thus, validation of the latter in external cohorts would help confirm our results**.

Kallikrein-related peptidase 3 gene (KLK3) encodes PSA, a prostate-specific and androgen-induced protease. Several SNPs throughout the kallikrein gene region on chromosome 19q13.33 have been consistently associated with PCa risk, aggressiveness and PCSM[10,33,34]. One of those SNPs (-5429T/G, rs2569733), which belongs to a major linkage block in the upstream enhancer region of KLK3, has been significantly associated **with increased PSA levels and PCA risk. Conversely, we**

found that carriers of the G allele had a decreased risk of BCR. Some authors have reported that this association might be due to a PSA bias[35]. Individuals with the PSA allele might be biopsied earlier due to increased PSA levels, and thus, have lower Gleason score and less aggressive PCa. Therefore, the SNP may not be etiologically implicated in PCa[35]. However, this hypothesis only partially explains the observed associations[7]. Indeed, in our study we could not find an association between SNP rs2569733 in KLK3 gene and histologic grade or clinical stage. Moreover, we found that the SNP was independently associated with the risk of EBCR, and the SNP remained significant in the multivariate model which included PSA, Gleason score and stage. In agreement with our results, Gallagher et al. reported that the association of SNPs in KLK3 gene with PCSM remained significant in a model which also included PSA and stage[7], which strengthens the hypothesis that this locus may play a biological role in PCa aggressiveness.

Another human Kallikrein is hK2 protein (kallikrein-related peptidase 2) which is codified by the KLK2 gene. We have analyzed a non-synonymous polymorphism at codon 250 of the KLK2 gene (Arg/Trp, rs198977). This functional SNP maps at 19q13.4 chromosome, close to one of the most well-established susceptibility loci for PCa[6,36]. We found that the T allele was also associated with increased risk of EBCR.

Sulfotransferase 1A1 (SULT1A1) activates dietary carcinogens and metabolizes protective agents[37]. The SNP rs9282861 at SULT1A1 gene (Arg213His, SULT1A1*1/SULT1A1*2) leads to decreased enzyme activity and thermostability[38]. Decreased SULT1A1 levels and enzymatic activity have been associated with decreased PCa risk[39]. Consistently, we found that carriers of SULT1A1*2 allele had a decreased risk of EBCR suggesting a protection against PCa progression.

Overexpression of osteocalcin (bone gamma-carboxyglutamic acid-containing protein, BGLAP) gene has been reported in metastatic bone tumors, including PCa[40]. One SNP in the promoter region of BGLAP gene (-198T/C, HindIII, rs1800247) has been associated with PCa risk[39]. Our study showed that patients carrying this variant were at increased risk of EBCR.

Patients at high risk of EBCR are more likely to develop metastatic disease. Considering the costs and decreased quality of life derived from metastatic disease, the most cost-effective strategy for the management of high-risk

patients is the one that maximizes progression-free survival[41]. This highlights the need for improved risk classification methods. In a previous study, Zubek et al. demonstrated the cost-effectiveness of a tissue-based protein assay for the prediction of BCR[42]. Compared with such tests (i.e. tissue or serum- based patterns of protein or RNA expression), SNP-based tests have become cheaper and faster in recent years, and thus more suitable for clinical routine testing[43]. Therefore, considering the reduced cost of a SNP assay, and the increased clinical benefit derived from its use, the cost-effectiveness of the new test presented is warranted.

Despite the significant improvement achieved by the inclusion of those four SNPs, there is still scope for progress. Most of the candidate SNPs analyzed in our study had been originally associated with PCa risk rather than PCa progression. Thus, the potential phenotypic impact of those SNPs on the clinicopathological variables could have partially obscured their contribution over the predictive accuracy of said variables. In this respect, we hypothesize that the analysis of a panel of SNPs on genes specifically associated with the ability of PCa circulating cells to migrate, or to their tissue-specific tropism, such as nodes or bone, could further improve the prediction of EBCR. The finding of osteocalcin gene polymorphism associated with EBCR in our study, and the known involvement of this gene in bone metastasis, could support this hypothesis.

Several limitations may apply to our study. Firstly, we developed a multicentric retrospective study with its potential intrinsic limitations. For example, certain EBCR risk factors were not available in our cohort (e.g. number of positive cores) or were not equally recorded. **Indeed, a systematic review and re-grade of all cases by a single uro-pathologist may have helped increase the homogeneity of our data and the applicability of the results to contemporary cohorts. Nevertheless, a recent comparison of the predictive a accuracy of 2001 Partin Tables versus a new preoperative nomogram based on PSA, stage and Gleason, complying with the 2005 International Society of Urological Pathology consensus on Gleason grading, showed roughly similar performance in a series of 1,188 PCa patients[44], which suggests that this limitation may not substantially reduce the clinical utility of the new nomogram. Nonetheless, further validation of the nomogram in external, contemporary series would be desirable as it would strengthen our results**. Secondly, the effect of unfavourable alleles in ethnically different populations should be confirmed. Thirdly, it would be interesting to explore whether newly identified

PCa susceptibility loci[10] could further improve the prediction of EBCR. Finally, despite 1-year BCR risk being a clinically relevant endpoint, the impact of those SNPs on metastasis and PCSM may also be analysed in future studies.

Conclusions:

We have developed a new nomogram for preoperative prediction of BCR at 1 year using clinicopathological and genetic risk factors. The addition of genetic polymorphisms significantly improves the predictive power of classic nomograms, and opens the way for adding new biomarkers in future updates.

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Abbreviations:

AUC, area under the curve; **BCR**, biochemical recurrence; **BGLAP**, bone gamma-carboxyglutamic acid-containing protein; **EBCR**, early biochemical recurrence; IDI, integrated discrimination improvement; **KLK2**, Kallikrein-related peptidase 2; **KLK3**, Kallikrein-related peptidase 3; **NRI**, net reclassification improvement; **PCa**, prostate cancer; **PCSM**, prostate cancer specific mortality; **PSA**, prostate specific antigen; **RP**, radical prostatectomy; **SNPs**, single nucleotide polymorphisms; **SULT1A1**, Sulfotransferase 1A1; **WRS**, weighted risk score.

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Table 1: Descriptive statistics of the clinicopathological variables for 670 patients undergoing RP included in the study.

Variable	Total, *N* = 670
Preoperative PSA	
Mean, ng/mL	10.09
Median (IQR), ng/mL	7.94 (5.88)
<4, %	5.2
4-6.9, %	34.8
7-9.9, %	26.9
10-19.9, %	25.8
≥ 20, %	7.3
Age at diagnosis	
Mean, years	63.84
Median (IQR), years	64 (8)
<54, %	6.1
55-59, %	17.5
60-64, %	27.3
65-69, %	30.4
≥70, %	18.7
Biopsy Gleason sum, %	
2-6	76.1
7	19.7
8-10	4.2
Clinical stage, %	
T1c	49.6
T2a-T2b	35.4
T2c	15.1
Gleason sum at surgery, %	
2-6	56.1
7	32.8
8-10	11.0
Pathological stage, %	
T2a-T2b	21.5
T2c	50.4
T3-T4	28.1

Seminal ves. Involvement, %	
Negative	94.0
Positive	6.0
Lymph node involvement, %	
Negative	98.4
Positive	1.6
Surgical margins, %	
Negative	69.6
Positive	30.4
BCR within 1 year, %	
No	86.7
Yes	13.3

IQR = Interquartile range

Table 2: Univariate associations between baseline preoperative clinicopathological variables and EBCR within one year of RP. Results of chi-square and Mann-Whitney test

Variable	Overall	No BCR	BCR	*p* value
	670 (100)	581 (86.7)	89 (13.3)	
Biopsy Gleason sum, n. (%)				<0.001
2-6	510 (76.1)	461 (79.4)	49 (55.0)	
7	132 (19.7)	103 (17.7)	29 (32.6)	
8-10	28 (4.2)	17 (2.9)	11 (12.4)	
Clinical stage, n. (%)				0.003
T1c	332 (49.6)	301 (51.8)	31 (34.8)	
T2a-T2b	237 (35.4)	201 (34.6)	36 (40.4)	
T2c	101 (15.0)	79 (13.6)	22 (24.8)	
PSA, ng/ml, Median (IQR)	7.9 (5.9)	7.5 (5.3)	11.4 (9.7)	<0.001

Table 3: Frequency distributions, odds ratios (OR) and univariate association *p* values for the presence of BCR by genotype, for SNPs on *KLK3* (rs2569733), *KLK2* (rs198977), *SULT1A1* (rs9282861), and *BGLAP* (rs1800247) genes. (T: Thymine; G: Guanine; C: Cytosine; A: Adenine).

	Genotype frequencies, n (%)			Frequency of risk genotype-carriers*, n (%), and ORs				
***KLK3*, -5429 T/G (rs2569733)**	**TT**	**TG**	**GG**	**TT***	**TG or GG**	**OR**	**95% CI**	***p* value**
No BCR	331 (57.0)	224 (38.6)	26 (4.5)	331 (57.0)	250 (43.0)	1.93	1.18 – 3.16	<0.01
BCR	64 (71.9)	23 (25.8)	2 (2.2)	64 (71.9)	25 (28.1)			
Total	395 (59.0)	247 (36.9)	28 (4.2)	395 (59.0)	275 (41.0)			
***KLK2*, Arg^{250}Trp (rs198977)**	**CC**	**CT**	**TT**	**CC**	**CT* or TT***	**OR**	**95% CI**	***p* value**
No BCR	259 (44.6)	253 (43.5)	69 (11.9)	259 (44.6)	322 (55.4)	1.69	1.06 – 2.69	0.025
BCR	28 (31.5)	45 (50.6)	16 (18.0)	28 (31.5)	61 (68.5)			
Total	287 (42.8)	298 (44.5)	85 (12.7)	287 (42.8)	383 (57.2)			
***SULT1A1*, Arg^{213}His (rs9282861)**	**GG**	**GA**	**AA**	**GG***	**GA or AA**	**OR**	**95% CI**	***p* value**
No BCR	277 (47.7)	254 (43.7)	50 (8.6)	277 (47.7)	304 (52.3)	1.78	1.12 – 2.81	0.014
BCR	55 (61.8)	27 (30.3)	7 (7.9)	55 (61.8)	34 (38.2)			
Total	332 (49.6)	281 (41.9)	57 (8.5)	332 (49.6)	338 (50.4)			
***BGLAP*, -198 T/C (rs1800247)**	**TT**	**TC**	**CC**	**TT**	**TC* or CC***	**OR**	**95% CI**	***p* value**
No BCR	354 (60.9)	190 (32.7)	37 (6.4)	354 (60.9)	227 (39.1)	2.00	1.27 – 3.14	<0.01
BCR	39 (43.8)	45 (50.6)	5 (5.6)	39 (43.8)	50 (56.2)			
Total	393 (58.7)	235 (35.1)	42 (6.3)	393 (58.7)	277 (41.3)			

Table 4: Multivariate models to predict the probability of EBCR within one year of RP (OR: Odds Ratio, 95% CI: 95% confidence interval) (T: Thymine; G: Guanine; C: Cytosine; A: Adenine)..

Variables included in the clinicopathological model	**OR**	**95% CI**	***P* value**
Preoperative PSA (log), ng/ml	1.85	1.42 – 2.41	<0.001
Biopsy Gleason sum			<0.001
7 *vs.* <7	2.37	1.40 – 4.01	0.001
>7 *vs.* <7	6.15	2.57 – 14.74	<0.001
Clinical stage			0,018
T2a-T2b *vs.* T1	1.9	1.11 – 3.23	0,019
T2c *vs.* T1	2.38	1.26 – 4.51	0,008

Variables included in the clinicopathological-genetic model	**OR**	**95% CI**	***p*value**
Preoperative PSA, ng/ml	1.83	1.40 – 2.40	<0.001
Biopsy Gleason sum			<0.001
7 *vs.* <7	2.40	1.40 – 4.12	0.001
>7 *vs.* <7	6.59	2.69 – 16.12	<0.001
Clinical stage			0.017
T2a-T2b *vs.* T1	1.88	1.09 – 3.25	0.023
T2c *vs.* T1	2.34	1.21 – 4.53	0.011
SNP genotyping	2.07	1.50 – 2.87	<0.001
KLK3 genotype (TT *vs.* TG or GG)	0.29	0.11 – 0.77	0.013
KLK2 genotype (CT or TT *vs.* CC)	2.04	1.02 – 4.10	0.044
SULT1A1 genotype (GG *vs.* GA or AA)	0.47	0.21 – 1.07	0.070
BGLAP genotype (TC or CC *vs.* TT)	2.48	1.15 – 5.33	0.020

Figure 1: Predictive clinicopathological nomogram of EBCR for clinically localized PCa, within one year of RP.

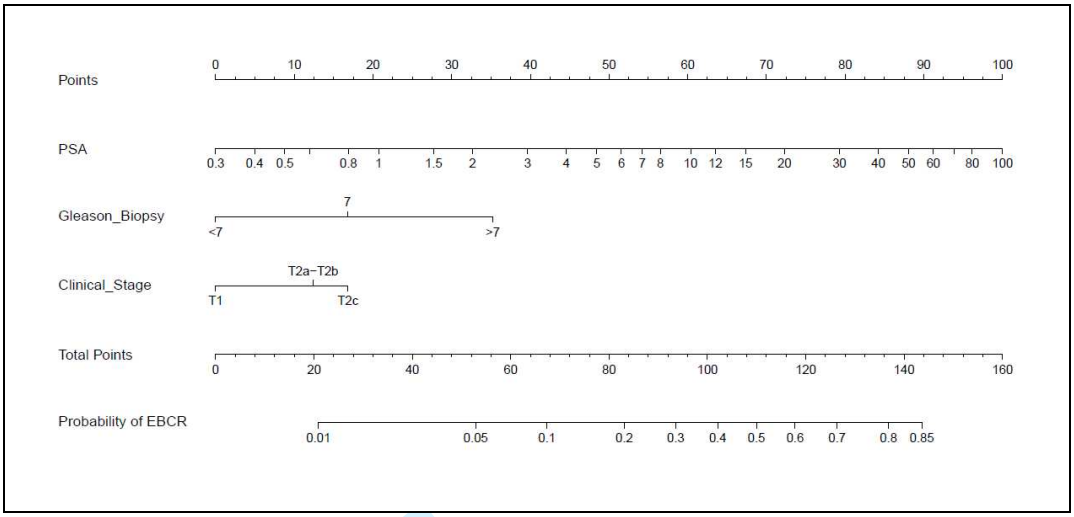

Figure 2: Predictive clinicopathological-genetic nomogram of EBCR for clinically localized PCa, within one year of RP. (SNP: Weighed risk score built from the SNP's information)**.**

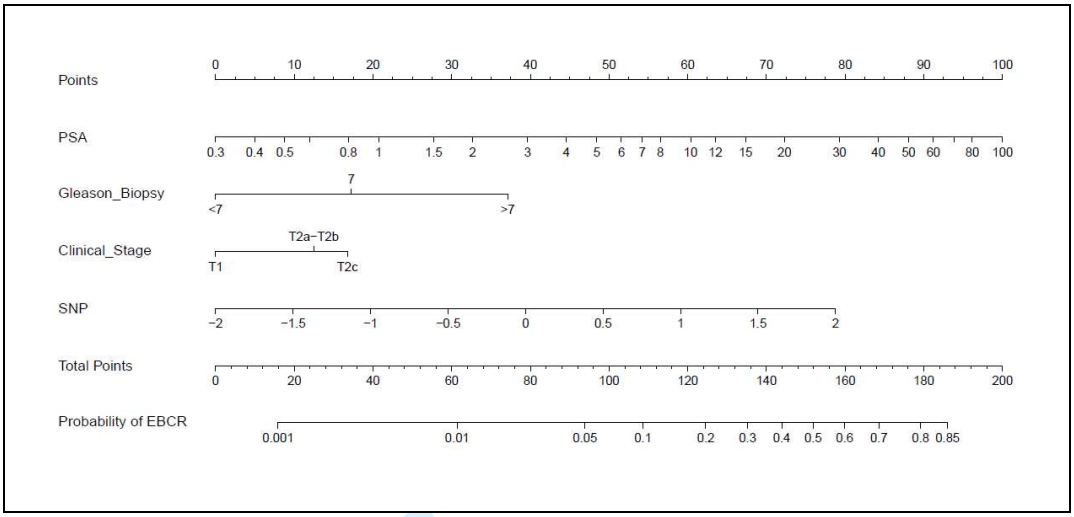

Figure 3: ROC curves of predictive models (dotted line: clinicopathological model; solid line: clinicopathological-genetic model).

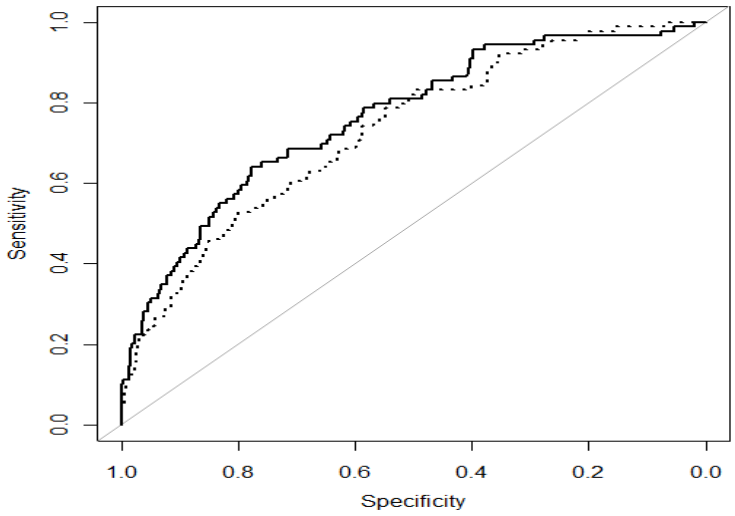

Figure 4: Calibration plot of clinicopathological model of EBCR within one year of RP.

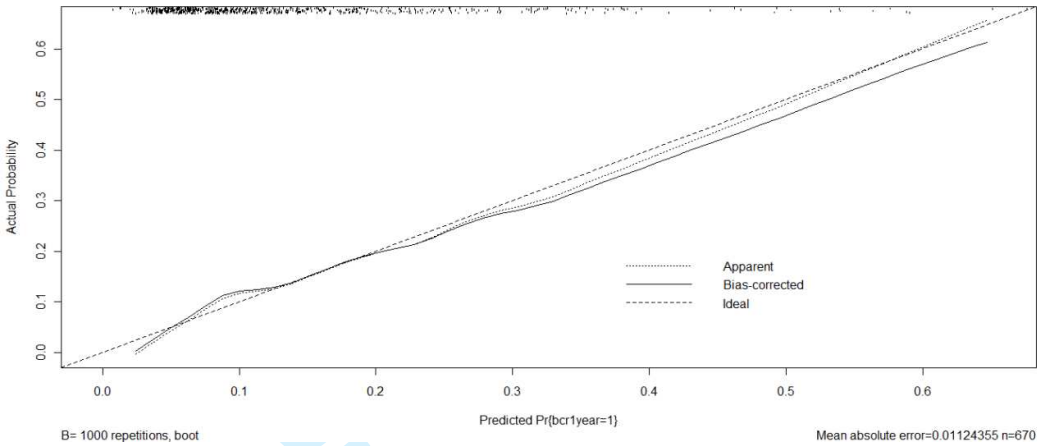

Figure 5: Calibration-plot of clinicopathological-genetic model of of EBCR within one year of RP.

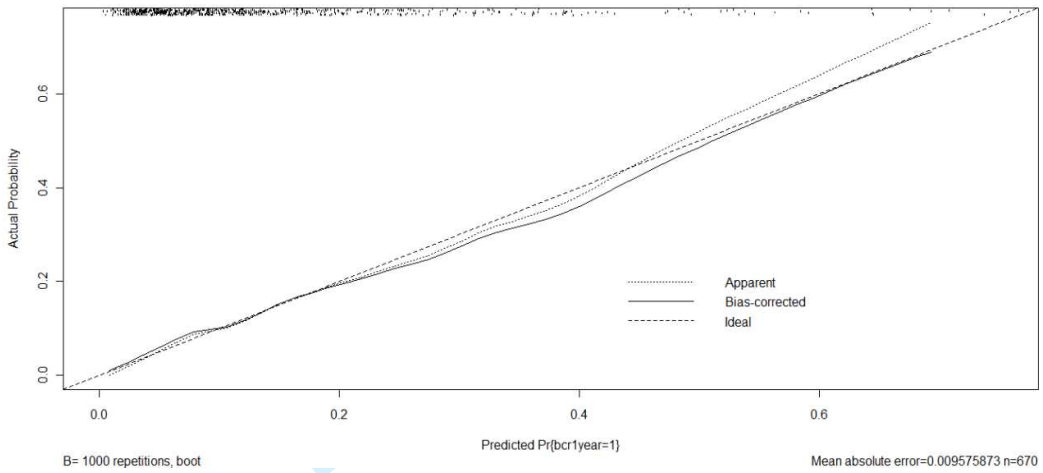

Figure 6: Decision curves-analysis of clinicopathological (Model 1) and clinicopathological-genetic (Model 2) models.