Impact of Genotyping on Outcome of Prostatic Biopsies: A Multicenter Prospective Study

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Single nucleotide polymorphisms (SNPs) have been associated with prostate cancer (PCa) risk and tumor aggressiveness in retrospective studies. To assess the value of genotyping in a clinical setting, we evaluated the correlation between three genotypes (rs1447295 and rs6983267(8q24) and rs4054823(17p12)) and prostatic biopsy outcome prospectively in a French population of Caucasian men. Five hundred ninety-eight patients with prostatic-specific antigen (PSA) >4 ng/mL or abnormal digital rectal examination (DRE) participated in this prospective, multicenter study. Age, familial history of PCa, body mass index (BMI), data of DRE, International Prostate Symptom Score (I-PSS) score, PSA value and prostatic volume were collected prospectively before prostatic biopsy. Correlation between genotypes and biopsy outcome (positive or negative) and Gleason score (≤6 or >6) were studied by univariate and multivariable analysis. rs1447295 and rs6983267 risk variants were found to be associated with the presence of PCa in univariate analysis. rs6983267 genotype remained significantly linked to a positive biopsy (odds ratio (OR) = 1.66, 95% confidence interval (CI): 1.06–2.59, P = 0.026) in multivariable analysis, but rs1447295 genotype did not (OR = 1.47, 95% CI: 0.89–2.43, P = 0.13). When biopsy outcome was stratified according to Gleason score, risk variants of rs1447295 were associated with aggressive disease (Gleason score ≥7) in univariate and multivariable analysis (OR = 2.05 95% CI: 1.10–3.79, P = 0.023). rs6983267 GG genotype was not related to aggressiveness. The results did not reach significance concerning rs4054823 for any analysis. This inaugural prospective evaluation thus confirmed potential usefulness of genotyping PCa assessment. Ongoing clinical evaluation of larger panels of SNPs will detail the actual impact of genetic markers on clinical practice.
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INTRODUCTION

Prostate cancer (PCa) is a high-incident disease with an estimated 217,730 new cases diagnosed in the United States in 2010. Ninety-two percent of tumors are expected to be diagnosed at local or regional stage (1). Localized PCa is an heterogeneous disease, and the range of treatment options is wide (2). Hence intense efforts have been made to individualize new initial prognostic factors and integrate them into prediction tools to guide therapeutic decisions (3). New biomarkers also are introduced to overcome limitations of a PSA blood test (4).

Currently used predictive and prognostic models in localized prostate cancer do not integrate genetic susceptibility (5). However, genetic variants such as single nucleotide polymorphisms (SNPs) may provide useful information at the time of diagnosis or during initial disease evaluation. Indeed, genetic susceptibility, initially associated with a higher risk of PCa, also has been shown to be correlated with disease aggressiveness.

SNPs located at 8q24 (rs1447295 and rs6983267) present a well-documented association with PCa risk (6,7), highly evolutive disease (8–12) and clinical outcome (13–16). More recently, rs4054823 risk variant, located on 17p12, has been shown to be the first-reported SNP predisposing to aggressive but not indolent PCa (17). However, these results are based on retrospective studies, and the utility of genotyping remains to be assessed in clinical practice. Notably, the impact of genotyping on biopsy results has scarcely been evaluated (18). To assess effectiveness of genotyping in a clinical setting, we prospectively genotyped French Caucasian men who underwent prostatic biopsy for three SNPs (rs1447295, rs6983267 and rs4054823) related to PCa aggressiveness.

MATERIALS AND METHODS

Study Design

Consecutive patients were recruited between 2006 and 2010 in four tertiary reference centers among men who were referred to a urologist because of PSA value over 4.0 ng/mL and/or abnormality at digital rectal examination (DRE). All patients included in the study are Caucasian. Patients with a personal history of PCa or those having PSA >50 ng/mL were excluded. All patients gave written informed consent at the time of inclusion. The protocol had been approved by our internal review board. This report is a preliminary analysis of a large prospective multicenter study about genotyping and PCa.

For every patient, the following data were collected at the inclusion visit: age, treatment by 5-α reductase inhibitors, personal history of cancer, familial history of PCa (classified positive if at least one first-degree relative had PCa, and negative otherwise), height, weight, body mass index (BMI) and data of DRE (classified as normal or abnormal). All patients completed a dedicated questionnaire including International Prostate Symptom Score (I-PSS) and Androgen Deficiency of Aging Male (ADAM). Baseline PSA value, free/total PSA ratio and prostatic volume (measured by ultrasonography) were assessed in a prospective manner.

All patients underwent ultrasound-guided 18-gauge needle core prostatic biopsies. A minimum of six tissue samples were obtained at the time of biopsies (median 10). Pathologic samples were studied by a reference pathologist in each center. All biopsies were initial biopsies.

Study Endpoints

The primary endpoint of the study was the histologic presence of PCa, based on prostatic biopsies. Secondary endpoint was the global Gleason score evaluation. All data were stored in a centralized database.

Genotyping Analysis and SNPs

The three SNPs tested were assessed in all patients. Genotyping was performed with TaqMan assays for all samples as described previously (7). All the samples have been processed in a central lab. As mentioned previously, genotype groupings were based on a dominant model for rs14487295 (risk genotype = AA + AC) and a recessive model for rs6983267 (risk genotype = GG) and for rs4054823 (determined by the TT genotype) (7,11,17,19). All SNPs tested satisfied the Hardy-Weinberg equilibrium in our population.

Statistical Analysis

Cases were defined as patients with PCa on prostatic biopsies and controls were men with no evidence of cancer. Patients with Gleason score ≤6 were considered as "low Gleason score" and those with Gleason score >6 as "high Gleason score." Factors potentially influencing the presence of PCa (age, familial history of prostate cancer, presence of lower urinary tract symptoms [LUTS], abnormality of DRE, PSA value, prostate volume and BMI) were evaluated in the two groups as categorical variables and compared with the chi-square test. Allele frequencies for each SNP were calculated for cases and controls and the distributions were compared by logistic regression. Odds ratios (ORs) as a measure of relative risk, and their 95% confidence intervals (CIs) were estimated. To check for confounding factors, we conducted a multivariable analysis with a logistic regression model containing the three SNPs, age, BMI, PSA, prostate volume, DRE data and familial history of PCa. A complementary analysis was conducted by comparing the age at diagnosis for the different genotypes groups through a Kaplan-Meier analysis with the log-rank test.

As a secondary outcome, relations between risk alleles of each SNP and PCa aggressiveness were studied. OR compared with control cases associated with each SNP was evaluated after a stratification of cases by Gleason score. Associations also were tested in a multivariable

analysis, through a logit logistic regression model including the three SNPs, age, BMI, PSA, prostate volume, LUTS, DRE data and familial history of PCa as variables and cancer status as a multinomial outcome (no cancer, low Gleason score or high Gleason score).

RESULTS

Five hundred and ninety-eight consecutive patients have been included in the study and 589 patients were considered for statistical analysis because of missing data in 9 patients. Overall, 322 patients (54.6%) were found to have PCa. The mean ± SD (range) age at the time of biopsy was 64.6 ± 7.8 (40-93). The patients characteristics are presented in Table 1. Age at diagnosis, presence of low urinary tract symptoms, abnormalities of DRE and PSA value were found to be associated with presence of a PCa.

We first evaluated the distributions of the genotypes of the three SNPs in the cases and controls. The risk variants of rs1447295 (AA + AC genotypes) and the risk variant of rs6983267 (GG genotype) were associated significantly with the presence of PCa, but there was no association between PCa and the risk variant of rs4054823 (Table 2). Multivariate analysis shows that age, PSA and prostate volume are associated with a positive result at the prostatic biopsy ($P < 0.0001$, $P = 0.002$ and $P < 0.0001$ respectively). In this multivariable model, presence of the risk variant of rs6983267 remained linked significantly to a positive biopsy (OR = 1.66, 95% CI: 1.06–2.59, $P = 0.026$), but the risk variants of rs1447295 did not (OR = 1.47, 95% CI: 0.89–2.43, $P = 0.13$).

Cumulative incidence adjusted to age at diagnosis shows that cancer appeared in younger men in case of carriage of a risk variant of rs1447295 or rs6983267 (Figure 1). In the subgroup of 492 patients presenting no familial history of prostate cancer, risk variants of rs1447295 or rs6983267 also were associated significantly to a higher incidence of PCa in younger men.

When biopsy outcome was stratified according to Gleason score as a multi-

Table 1. Description of the study population.

Factor	Cancer (N = 322)	No cancer (N = 267)	P
Age group (y)			
≤65	140	165	< 0.0001
>65	182	102	
Familial history of prostate cancer[a]			
Absent	271	285	0.31
Present	51	42	
Lower urinary tract symptoms[b]			
≤7	117	67	0.004
>7	205	200	
Digital rectal examination			
Normal	218	207	0.011
Abnormal	104	60	
PSA (ng/mL)			
≤4	25	29	0.018
4.1–10.0	206	177	
10.1–20.0	64	54	
>20.0	27	7	
Prostate volume[c]			
<30 cc	73	49	0.013
30–60 cc	185	137	
>60 cc	64	81	
Body mass index (kg/m^2)			
≤25	133	105	0.58
25–30	155	139	
>30	34	23	

[a]Present in the case of one first degree relative having prostate cancer.
[b]Measured by International Prostate Symptom Score.
[c]Measured by transrectal ultrasound.

nomial variable, the risk variant genotypes of rs1447295 were found to be associated with aggressive disease (Gleason score ≥7) (Table 3). GG genotype of rs6983267 was significantly higher in both categories of PCa than in controls. The results did not reach statistical significance for rs4054823. Multivariable analysis (Table 4) showed that the pooled AA + AC genotypes of rs1447295 remained strongly associated with the risk of aggressive PCa. There was only a trend for
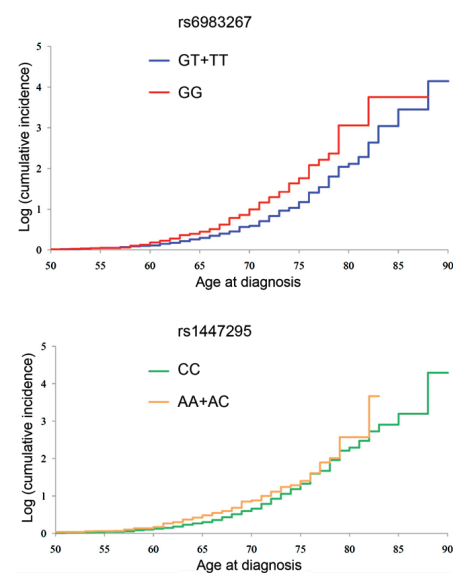
an association between rs6983267 genotypes and both groups of PCa cases.

DISCUSSION

Genome-wide association studies have highlighted that some SNPs are associated with PCa risk. However, few studies have been able to show a clear and replicated association between these genetic variants and prostate cancer aggressiveness (20). To our knowledge, only 8q24 SNPs (rs1447295 and rs6983267) have

Figure 1. Cumulative incidence of prostate cancer among the entire patient sample. Data are represented through a Kaplan-Meier analysis with estimation of the log of cumulative incidence (event = positive biopsy, time = age at diagnosis). Prostate cancer cases are diagnosed in younger men in the case of carriage of risk alleles at the 8q24 loci. Top panel: *P* = 0.001; bottom panel: *P* = 0.029.

been exposed as markers of aggressiveness, mainly through Gleason score, in multiple studies (8–12). In the present study, we also focused on a SNP located at chromosome17p12 that has been found associated specifically with high-risk disease in a robust case-case study (17). However, these previously published case-case or case-control studies are all retrospective, using patients taken from genetic analysis cohorts or screening programs, and often including people of different ethnic groups, and gathering variable clinical and biological information. We therefore decided to explore the role of genetic variability in a prospective manner, in a homogeneous ethnic group (men of European ancestry) to sort out if genotyping could provide information at the initial phase of diagnosis in a clinical setting. All the clinical and biological data recognized to be associated potentially with presence of a prostate cancer and biopsy Gleason score were evaluated.

Table 2. Associations between risk variants of each single nucleotide polymorphism and overall results of prostatic biopsy (cancer versus noncancer).

SNP	Alleles	Genotype Reference	Genotype Risk	Number with risk variants Cases	Number with risk variants Controls	OR (95% CI)	P
rs1447295	C,A	CC	CA or AA	80	48	1.51 (1.01–2.25)	0.045
rs6983267	G,T	TT or GT	GG	117	71	1.57 (1.11–2.24)	0.012
rs4054823	C,T	CC or CT	TT	110	86	0.92 (0.65–1.29)	0.617

Table 3. Association between risk variants of each single nucleotide polymorphism and biopsy outcome stratified by Gleason score.

SNP	Gleason	Risk genotype	OR (95% CI)	*P*
rs1447295 (8q24)	≤6	CA + AA	1.17 (0.72–1.91)	0.512
	≥7	CA + AA	1.94 (1.213–3.09)	0.006
rs6983267 (8q24)	≤6	GG	1.61 (1.07–2.43)	0.024
	≥7	GG	1.53 (1.01–2.36)	0.049
rs4054823 (17p12)	≤6	TT	1.20 (0.80–1.79)	0.381
	≥7	TT	0.98 (0.64–1.50)	0.931

Our results showed that rs1447295 AA + AC genotypes were associated with an increased risk of PCa on biopsies and also with aggressive disease (biopsy Gleason score ≥7), in univariate and multivariable analyses. However, PSA, prostate volume and age were associated more strongly with Gleason score than SNPs in the logit regression model used. These data underline that clinical variables are of critical value and remain essential at initial evaluation.
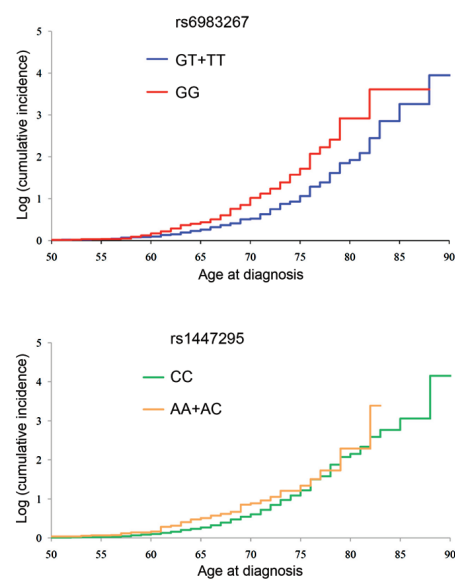
rs6983267 GG genotype was found to be associated with both low and high Gleason scores on biopsies. Thus, in-line with the literature, rs1447295 risk variants are linked in Caucasian men to an increased risk of aggressive PCa, whereas rs6983267 genotype is associated with PCa risk without any predictive value regarding aggressiveness. Opposing current literature, we were not able to confirm the association between rs4054823 genotype and aggressive, not indolent PCa exposed by Xu *et al*. (17). This may be related to a lack of power of the present study, but to our knowledge no other report has until now confirmed these findings. After adjusting on age, rs1447295 and rs6983267 were associated with an increased risk of PCa not only in the whole sample, but also in the subgroup of people with no familial history of PCa (Figure 2). In these cases, genotyping could therefore be useful to assess risk factors at the initial diagnosis phase, especially in younger men.

Our study thus suggests that SNPs located at 8q24, the most widely studied SNPs in the field of PCa (8), are of interest at the time of first prostate biopsy. Included in a predictive tool, its information could improve the model and potentially could influence the therapeutic decision. This issue has been investigated previously by the only report focused on correlation between SNPs and biopsy results. Nam *et al*. (18) have estimated the predictive values of the presence of genetic risk variants, when included in a nomogram in a retrospective study of about 3,000 patients. Unfortunately, even if the results reached statistical significance, they have shown that the improvement of prediction of biopsy results by genotyping was low, given the OR values of the SNPs tested. In the present report, we focused only on the main SNPs related to aggressiveness, and our goal was not to build a new extensive nomogram. But since our ongoing protocol deals with other available SNPs, it

Figure 2. Cumulative incidence of prostate cancer among patients with no familial history of prostate cancer. Data are represented through a Kaplan-Meier analysis with estimation of the log of cumulative incidence (event = positive biopsy, time = age at diagnosis). Prostate cancer cases are diagnosed in younger men in the case of carriage of risk alleles at the 8q24 loci. Top panel: *P* = 0.0001; bottom panel: *P* = 0.035.

would be interesting in the future to compare results obtained through a prospective work to those exposed previously (18).

Some limitations certainly apply to this study. First, given that this report is preliminary, the number of subjects here is relatively low, but is methodologically correct and adapted to the criteria of our multivariable analysis. However, we were not able to conduct a case-case study to compare the frequencies of the genotypes in the indolent and aggressive group. Second, our results do not integrate all the SNPs previously reported. This could impact the interpretation of our results since the influence of the SNPs has been shown to be multiplicative by previous studies (21). Results concerning a larger panel of SNPs evaluated prospectively in an ongoing protocol will be available in the coming years and provide data about these interac-

Table 4. Correlation between variant genotypes and prostate cancer risk stratified by Gleason score according to multivariate analysis adjusting for age, body mass index, PSA, prostate volume and familial history.

SNP	Gleason	Genotype	OR (95% CI)	*P*
rs1447295 (8q24)	≤6	CA + AA	1.18 (0.66–2.11)	0.568
	≥7	CA + AA	2.05 (1.10–3.79)	0.023
rs6983267 (8q24)	≤6	GG	1.63 (0.99–2.68)	0.053
	≥7	GG	1.75 (0.98–3.15)	0.058
rs4054823 (17p12)	≤6	TT	1.30 (0.80–2.11)	0.298
	≥7	TT	1.30 (0.73–2.33)	0.369

tions. Moreover, our biopsy results could have been impacted by the biopsy criterion (PSA >4 and/or positive DRE) that were decided at the time of the protocol conception to stick to French recommendations about PCa diagnosis 2 years ago. PCa also may have been underdiagnosed in the current population, owing to the number of cores taken during prostate biopsy, since current guidelines suggest to take at least 10–12 cores. Another limitation concerning the relationship between SNPs and Gleason score is the lack of pathological review. Indeed, if all specimens were analyzed by the same pathologist in each center, interobserver variability of Gleason score may have impacted the results. These results also suffer from the absence of validation of the final pathological stage, only obtained after radical prostatectomy. Biopsies can underestimate Gleason score and thus our findings need to be confirmed by prospective studies including radical prostatectomy cases.

Concerning our multivariable model, free/total PSA ratio was not included in the analysis because of some missing data concerning this parameter. Moreover, current recommendations point out that this dosage is not useful in all clinical situations, but only in a 4 to 10 ng/mL range of PSA.

Despite these limitations, our study shows for the first time, with a rigorous prospective evaluation and extensive complete data collection that risk genotypes of SNPs located at 8q24 are related to PCa risk (rs6983267) and aggressiveness (rs1447295) in French Caucasian men. It overcomes limitations of previous work based on retrospective population studies and should inspire further work evaluating genotyping in a clinical setting. These results highlight that knowledge of SNP genotypes can bring critical information at the early detection phase. However, some previously described associations between genetic variations with PCa risk and characteristics were not reproduced in this clinical context in multivariable analyses. Evaluation in a prospective clinical setting thus ap-

pears mandatory for validation of genotyping approaches in PCa diagnosis and management, before their integration in nomograms and prognostic tools.

DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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