ESMO Clinical Research Fellowship
(October 2010 – September 2011)

Optimizing prostate cancer screening in high risk populations: Role and indications for prostatic biopsies and Germline Genetic profiling: The PROFILE study

Elena Castro

FINAL REPORT

Host Institute: The Royal Marsden Hospital & The Institute of Cancer, London, United Kingdom
Research Mentor: Rosalind Eeles

Home Institute: Centro de Investigacion del Cancer, Hospital Universitario de Salamanca, Spain
Head of department: Juan J. Cruz-Hernández

Introduction

Screening for PrCa in the general population is controversial [1-3]. Overdiagnosis and overtreatment are significant side-effects. However, targeted screening aimed at higher risk groups may have a greater impact [4]. Excluding age, and African-American ancestry, the strongest risk factor for PrCa is a family history (FH) [5]. First-degree relatives (FDR) of men with PrCa have approximately twice the risk of the general population, increasing to more than fourfold if diagnosed below age 60 [6]. Several germline single nucleotide polymorphisms (SNPs) have been associated with PrCa risk. Although the effect of each of these variants is small, they act multiplicatively and together explain ~30% of the genetic variance of PrCa [5]. Several studies have reported that Polygenic Risk Scores (PRS) based on the combined effects of the SNPs could be used to predict an individual's future PrCa risk [6, 7] and could be useful for targeted screening. However, it is unclear how they relate to the probability of detecting existing PrCa in asymptomatic men.
In this cross-sectional pilot study, the primary aim was to determine the feasibility of using PB in men with a FH of PrCa. The secondary aims were to evaluate the feasibility of collecting data on SNP profiles, PSA levels, and diffusion weighted MRI (DW-MRI) and assess whether they could be used as screening tools in this population.

Methods

Patients
Eligible men were identified through: (1) the cancer genetics and uro-oncology clinics at a cancer hospital in London; (2) men taking part in the UK Genetic Prostate Cancer study; UKGPCS (www.icr.ac.uk/ukgpcs); (3) adverts and newsletters. For (1) and (2) men with PrCa were approached either in clinic or by letter and invited to pass on an information sheet to eligible

relatives.
Eligibility criteria included 1) age 40 to 69 years; 2) FH of PrCa; 3) if previous PB it had to be negative and performed ≥1 year ago; 4) provision of informed consent. Individuals with a prior diagnosis of PrCa, currently being investigated for symptoms suggestive of PrCa, or diagnosed with cancer with a terminal prognosis of <5 years were excluded. FH was defined as having either i) one FDR with PrCa diagnosed <70 years; ii) 2 relatives (FDR or second degree relative) on the same side of the family with at least one diagnosed <70 years or iii) ≥3 relatives on the same side of the family diagnosed at any age.

Study Design
After written consent, participants completed a family and medical history questionnaire and provided blood samples for PSA measurement and DNA extraction. All men underwent a ten-core transrectal ultrasound guided (TRUS) PB regardless of baseline PSA level within 8 weeks of study entry. An expert pathologist reviewed all biopsies. Participants who rejected an initial PB were followed-up with six-monthly PSA measurements and have been excluded from these analyses. The study algorithm is detailed in figure 1.
The first 50 patients enrolled in the direct biopsy arm were also offered a DW-MRI prior to biopsy [8]. Images were assessed by an experienced observer using a combination of T2W and DW images and scored as positive or negative for tumour. This was not used to guide the biopsy as this component of the study was to gauge acceptability of this technique to inform the future main study design. The results of this component of the study have been reported separately.
For those patients diagnosed with PrCa at PB, management was offered either at the cancer centre or at their local hospital according to standard national guidelines. The outcome of different treatments in these patients will be followed for 5 years. Those patients who presented with atypical small acinar proliferation (ASAP) or high-grade prostatic intra-epithelial neoplasia (HG-PIN) were re-biopsied after 6 months. Men with a negative biopsy are being monitored with six monthly PSA testing and biopsy repeated if PSA increases by >50% (data not presented).

Genetic profiling
Participants' DNA samples were genotyped using the iCOGS array. iCOGS is a custom Illumina iSelect genotyping array [9,10]. We used data for 71 known PrCa susceptibility SNPs; 61 were directly genotyped and for 10 loci data for a proxy SNP with pairwise correlation r2> 0.75 were used (see table 1).

Statistics

For each patient, the PRS was calculated as the weighted sum of the number of risk alleles at the 71 loci where the weights were the estimated log-odds ratios (OR) associated with each allele, obtained from published studies [11]. For this purpose, we predicted cumulative risk of developing PrCa by age 80 (range 0-1) at the age at recruitment. In exploratory analysis, we used a logistic

regression to evaluate associations with PB and the genetic score to predict for positive PB. IBM SPSS version 22 was used for statistical analysis.

Results

From December 2010, 897 men aged 40-69 with FH of PrCa were invited into the study and 32% (285) replied. Enrolment was closed in January 2013 when 115 men had entered the study and 100 accepted to undergo PB (table 2). Fifteen men declined PB and were followed up with PSA only. There was no difference in the recruitment approach between those accepting and declining the invitation to undergo a PB.

The median age of participants was 53 years (40-69) with a median PSA of 1.98ng/ml (0.2-9.8). Ten-core PB identified 11 cases of HG-PIN, 7 ASAP and 26 cancers. Median age of PrCa diagnosis was 61.5 yrs (range: 44-69) with median PSA 3.2 ng/ml (0.35-9.8). The median age of men diagnosed with PrCa was higher than that of those with negative biopsies (61.5 vs 52.0 yrs, P=0.009). Fourteen tumours were low risk (54%), 10 were intermediate (38%) and 2 (8%) were high risk as classified by the NICE criteria [12] (see table 3, tumour characteristics).

Complications occurred in 5 out of 100 participants (5%), with four (4%) infections reported, one requiring hospitalisation. The fifth participant was kept under observation after fainting post-biopsy.

The analysis showed that the PSA was associated with PrCa diagnosis (P value=0.005; OR 1.56 (95%CI:1.15-2.14)). Age at study entry was also significant (P value=0.012; OR 1.10 (95%CI:1.02-1.19)), but PRS was not significant (P value = 0.30).

When stratified by Gleason Score of the tumour (7 or above vs lower than 7) no association was observed between the PRS and tumours with Gleason Score of 7 or above (P value=0.13). PSA was associated with high GS (P value = 0.02; OR: 1.90 (95%CI=1.11-3.25)).

We also evaluated the association between the PRS for different levels of presenting PSA. In men presenting with PrCa who had a PSA<3ng/ml (usually considered 'normal'), the PRS was no different in men with a benign biopsy (P value=0.5). Furthermore, for men presenting at enrolment with a PSA <1ng/ml (35 men with 5 PrCas diagnosed at PB), SNP profiling was similarly not associated with PrCa (P value=0.247).The median age of PrCa diagnosis for the subgroup with the very low PSA was 57 (range 44 to 69).

The majority of patients diagnosed with PrCa presented with a low risk tumour (and of these all but one 52% opted for Active Surveillance. For those opting for a radical treatment radical prostatectomy was chosen by 37% of men. One patient received radiotherapy treatment with Cyberknife and one man with metastatic disease received hormone treatment alone.

Discussion

The results of this study provide evidence that it is feasible to screen for PrCa using upfront PB in men with a FH of the disease, and about a third of men uptake the offer of such screening. A

quarter of men were diagnosed with PrCa and of these 46% had disease which is in intermediate/high risk categories which would warrant treatment on NICE guidelines. There were no severe side effects.

Interestingly of the 26% of study participants found to have PrCa, 54% had a PSA <3 ng/ml and these patients would most likely have not undergone PB within the traditional PSA-based screening schedules. This is a finding that raises questions about the reliability of PSA, especially for men with a significant FH. In comparison the Prostate Cancer Prevention Trial (PCPT) reported an overall PrCa detection rate of 15.2% in 2950 men with a PSA <4.0ng/ml and unsuspicious DRE. Importantly they found that only 19.6% of tumours had high grade disease (defined as GS >7) and men were older with an age range 62-91 years [13]. While the two studies are not directly comparable using both different classifications of disease risk and different PSA levels, there was a remarkable difference in the incidence of clinically significant tumours. Little is known about the effect of FH on PrCa progression and the benefits of early diagnosis in this group [14]. All but one man diagnosed with low-risk PrCa accepted active surveillance which highlights the growing understanding of the natural history of PrCa and the increasing acceptance of a structured monitoring strategy..

Another major concern with using PB for PrCa screening is safety. Although haematuria and haematospermia are common, severe post-procedural infections have been reported to occur in approximately 1% of cases with major complications being rare [15,16]. Similar incidences were observed in our series with a 4% infection rate, 1% requiring hospitalisation, and no major complications reported. Therefore the potential negative consequences of PrCa screening, including biopsy complications and cancer diagnosis, were regarded as acceptable to be able to proceed to the main study which will be powered to detect the effect of the PRS in the screening algorithm. Men in the PROFILE study might be more predisposed to accept more screening related side-effects than the general population due to their previous experience of relatives suffering from PrCa.

However, a recent study evaluating the psychological impact of prostate biopsy in 1147 men that participated in the Prostate Testing for Cancer and Treatment (ProtecT) trial reported that post-biopsy symptoms such as discomfort, pain or bleeding were experienced relatively commonly, and for the majority were tolerated as a minor problem or no problem [17].

In this pilot study we have carried out exploratory analyses to assess the performance of age, PSA, and PrCa risk SNPs in predicting biopsy outcome in this setting. It is important to note that SNP risk scores and PSA were considered here as tools for predicting PB outcome rather than predicting future risk of developing PrCa. The latter cannot be evaluated in the present study design, but as prospective data are accumulated this will be assessed in the future.

In this small pilot study using univariate analyses the SNP profile was not associated with prostate cancer at PB. However, much larger studies are required to address this question reliably. Furthermore, this may be improved as further common variants are identified which will increase the discrimination of the risk strata in populations. A possible confounder that may have caused this is that men in this study have a strong FH and their genetic risk will be high, therefore resulting in poor discrimination. Others have previously reported similar results, with AUCs that ranged from 0.57 to 0.67, although their prediction models included fewer genetic variants than

ours [7, 18-21].

The predictive value of SNP profiling in men presenting with a PSA of 1ng/ml to 3 ng/ml was assessed by Nordstrom et al [22]. Based on current clinical practice if these men were following a PSA screening protocol, they would not undergo PB. A risk score based on 49 SNPs was a significant predictor of a positive biopsy (P value=0.028). The OR for PrCa was 1.6 with increasing SNP score. In the PROFILE study we analysed the predictive value of the genetic score for men with a FH and a very low presenting PSA<= 1ng/ml.

These men would have normally been reassured by the PSA result and we found no statistically significant association of PRS with PrCa diagnosis (P value=0.247. However, the number of PrCas diagnosed in asymptomatic young men with a very low PSA is significant (14% Pr Ca incidence).

SNP profiling is an important tool in PrCa risk prediction algorithms. Risk prediction models serve to identify those men who could potentially benefit from screening, through early diagnosis and treatment [23]. Nam et al reported a prediction model using FH, PSA and 4 of the published GWAS risk SNPs, which improved the positive predictive value of PSA [19]. Zheng et al included 11 SNPs in their model and showed that the prediction of the combination of genetic variants and FH was similar to that of PSA [24]. Pashayan et al reported the use of a polygenic risk model to personalize screening, and compared this with a theoretical model in which only age was used to determine whether to screen a population and showed that SNPs reduced the risk of overdiagnosis [25]. MacInnis et al developed a model for predicting the probability of developing PrCa in the future based on 26 SNPs and FH [26]. Kader et al (2012) developed a multivariable predictive model for patients who have previously had a negative PB [7]. The predictive performance of the model, that included age, FH, PSA, prostate volume and number of cores at PB, was improved after the addition of the genetic score based on 33 PrCa risk-associated SNPs.

The proposed PROFILE study offers a unique opportunity to prospectively determine the benefit of the model, as participants will be followed up for 5 years.

We acknowledge that the present pilot study has several weaknesses. PROFILE was designed as a feasibility study and therefore has a small sample size that does not allow definitive conclusions. For that reason, we did not test the ability of the genetic score to distinguish between the risk of indolent as opposed to aggressive disease. There is debate whether the SNPs identified to date are able to distinguish between these two forms of PrCa [27]. Secondly, this analysis only included men of European ancestry as several GWAS included only men of this ethnicity and it is not clear to what extent these results are applicable to other ethnic groups. Furthermore, all men had a FH of PrCa and therefore had an increased risk of PrCa compared with the general population, which may make them more receptive to undergoing PB and may also explain the high incidence of PrCa and pre-cancerous findings in this series. Moreover, by definition all the participants in the present are expected to have higher PRSs compared to the general population, which would limit the ability of the PRS to discriminate between those with PrCa at PB and those without PrCa.

The PROFILE study will start recruiting in 2014, aiming to recruit 350 men of European ancestry with FH and 350 men of Afro-Caribbean ancestry. As well as DW-MRI other predictive biomarkers will be incorporated such as urinary PCA3 and TMPRSS-ERG translocation status. We aim to use the most up to date SNP set available to calculate the risk score by ethnic group.

Conclusion

PROFILE is the first study conducted in men with a FH of PrCa to incorporate biopsy, genetic profiling, imaging and biomarkers in an innovative screening model. The feasibility study reported here showed that a quarter of the men who underwent a PB were found to have a PrCa and 46% of these had disease which should be treated radically on NICE guidelines. Our results indicate that direct PB is feasible and acceptable as a means of PrCa screening in men with FH of PrCa. A larger study is underway for the development of a prediction model combining clinical variables and PrCa risk-associated SNPs that would help to determine which men at high risk of PrCa due to their FH should undergo a PB.

References

[1] Carlsson S, Vickers AJ, Roobol M, Eastham J, Scardino P, Lilja H, et al. Prostate cancer screening: facts, statistics, and interpretation in response to the US Preventive Services Task Force Review. Journal of clinical Oncology: official journal of the American Society of Clinical Oncology. 2012;30:2581---4.
[2] Schroder FH. Landmarks in prostate cancer screening. BJU international. 2012;110 Suppl 1:3---7.
[3] Andriole GL, Jr. PSA screening and prostate cancer risk reduction. Urologic oncology. 2012;30:936---7. [4] Basch E, Oliver TK, Vickers A, Thompson I, Kantoff P, Parnes H, et al. Screening for prostate cancer with prostate---specific antigen testing: American Society of Clinical Oncology Provisional Clinical Opinion. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2012;30:3020---5. [5] Eeles R, Goh C, Castro E, Bancroft E, Guy M, Olama AA, et al. The genetic epidemiology of prostate cancer and its clinical implications. Nature reviews Urology. 2013.
[6] Xu J, Sun J, Kader AK, Lindstrom S, Wiklund F, Hsu FC, et al. Estimation of absolute risk for prostate cancer using genetic markers and family history. The Prostate. 2009;69:1565---72.
[7] Kader AK, Sun J, Reck BH, Newcombe PJ, Kim ST, Hsu FC, et al. Potential impact of adding genetic markers to clinical parameters in predicting prostate biopsy outcomes in men following an initial negative biopsy: findings from the REDUCE trial. European urology. 2012;62:953---61.
[8] deSouza NM, Morgan VA, Bancroft E, Sohaib SA, Giles SL, Kote---Jarai Z, Castro E, Hazell S, Jafar M, Eeles R. Diffusion- -weighted MRI for detecting prostate tumour in men at increased genetic risk. European Journal of Radiology Open 1 (2014) 22–27.
[9] Eeles RA, Olama AA, Benlloch S, Saunders EJ, Leongamornlert DA, Tymrakiewicz M, et al. Identification of 23 new prostate cancer susceptibility loci using the iCOGS custom genotyping array. Nature genetics. 2013;45:385---91, 91e1---2.
[10] Bahcall OG. iCOGS collection provides a collaborative model. Foreword. Nature genetics. 2013;45:343.
[11] Eeles R, Goh C, Castro E, Bancroft E, Guy M, Al Olama AA, et al. The genetic epidemiology of prostate cancer and its clinical implications. Nature reviews Urology. 2014;11:18---31.
[12] NICE.
[13] Thompson I, Pauler DK, Goodman PJ, Tangen CM, Lucia MS, Parnes HL, Minasian LM, Ford LG,

Lippman SM, Crawford ED, Crowley JJ, Coltman CA Jr. Prevalence of prostate cancer among men with a PSA level < or =4.0ng per millilitre. NEJM. 2004; 350(22): 2239---46.
[14] Epstein JI, Walsh PC, Carmichael M, Brendler CB. Pathologic and clinical findings to predict tumor extent of nonpalpable (stage T1c) prostate cancer. JAMA: the journal of the American Medical Association. 1994;271:368---74.
[15] Goh CL, Saunders EJ, Leongamornlert DA, Tymrakiewicz M, Thomas K, Selvadurai ED, et al. Clinical implications of family history of prostate cancer and genetic risk single nucleotide polymorphism (SNP) profiles in an active surveillance cohort. BJU Int. 2013;112:666---73.
[16] Loeb S, Carter HB, Berndt SI, Ricker W, Schaeffer EM. Is repeat prostate biopsy associated with a greater risk of hospitalization? Data from SEER---Medicare. The Journal of urology. 2013;189:867---70.
[17] Wade J, Rosario DJ, Macefield RC, Avery KN, Salter CE, Goodwin ML, et al. Psychological impact of prostate biopsy: physical symptoms, anxiety, and depression. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013;31:4235---41.
[18] Salinas CA, Koopmeiners JS, Kwon EM, FitzGerald L, Lin DW, Ostrander EA, et al. Clinical utility of five genetic variants for predicting prostate cancer risk and mortality. The Prostate. 2009;69:363---72.
[19] Nam RK, Zhang WW, Trachtenberg J, Seth A, Klotz LH, Stanimirovic A, et al. Utility of incorporating genetic variants for the early detection of prostate cancer. Clinical cancer research: an official journal of the American Association for Cancer Research. 2009;15:1787- -93.
[20] Akamatsu S, Takahashi A, Takata R, Kubo M, Inoue T, Morizono T, et al. Reproducibility, performance, and clinical utility of a genetic risk prediction model for prostate cancer in Japanese. PloS one. 2012;7:e46454.
[21] Klein RJ, Hallden C, Gupta A, Savage CJ, Dahlin A, Bjartell A, et al. Evaluation of multiple risk---associated single nucleotide polymorphisms versus prostate---specific antigen at baseline to predict prostate cancer in unscreened men. European urology. 2012;61:471---7.
[22] Nordström T, Aly M, Eklund M, Egevad L, Grönberg H. A genetic score can identify men at high risk for prostate cancer among men with prostate---specific antigen of 1---3 ng/ml. Eur Urol. 2014 Jun;65(6):1184---90.
[23] Freedman AN, Seminara D, Gail MH, Hartge P, Colditz GA, Ballard---Barbash R, et al. Cancer risk prediction models: a workshop on development, evaluation, and application. Journal of the National Cancer Institute. 2005;97:715---23.
[24] Zheng SL, Sun J, Wiklund F, Gao Z, Stattin P, Purcell LD, et al. Genetic variants and family history predict prostate cancer similar to prostate---specific antigen. Clinical cancer research: an official journal of the American Association for Cancer Research. 2009;15:1105---11.
[25] Pashayan N, Pharoah P, Tabar L, Neal DE, Martin RM, Donovan J, et al. Validation of a modelling approach for estimating the likely effectiveness of cancer screening using cancer data on prevalence screening and incidence. Cancer epidemiology. 2011;35:139---44.
[26] Macinnis RJ, Antoniou AC, Eeles RA, Severi G, Al Olama AA, McGuffog L, et al. A risk prediction algorithm based on family history and common genetic variants: application to prostate cancer with potential clinical impact. Genetic epidemiology. 2011;35:549---56.
[27] Teerlink CC, Thibodeau SN, McDonnell SK, Schaid DJ, Rinckleb A, Maier C, et al. Association analysis of 9,560 prostate cancer cases from the International Consortium of prostate Cancer Genetics confirms the role of reported prostate cancer associated SNPs for familial disease. Human genetics. 2014;133:347---56.

Prof. Rosalind Eeles (The Institute of Cancer Research) visited my Home Institution (Hospital Universitario de Salamanca) in October 2010.

The ESMO Clinical Research Fellowship is supported by an Educational Grant from Novartis.