PROSTATE CANCER GERMLINE VARIATIONS AND IMPLICATIONS FOR SCREENING AND TREATMENT

Alexander Dias[1,2], Zsofia Kote-Jarai[1], Christos Mikropoulos[1], and Ros Eeles[1,2].
[1] The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG, UK

[2] Royal Marsden NHS Foundation Trust, Fulham Rd, London SW3 6JJ, UK

Corresponding author:

Prof Rosalind Eeles MA, FRCR, FRCP, PhD, FMedSci

The Institute of Cancer Research and Royal Marsden NHS Foundation Trust,

15 Cotswold Road, Sutton SM2 5NG, United Kingdom

Tel. +44 208 722 4094

Fax +44 208 722 4110

ros.eeles@icr.ac.uk

Dr. Alexander Dias

Clinical Fellow – The Institute of Cancer Research and Royal Marsden NHS Foundation Trust. Academic Urology Unit and The Oncogenetics Team

Alexander.Dias@icr.ac.uk

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Abstract

Prostate Cancer (PCa) is a highly heritable disease, and rapid evolution of sequencing technologies has enabled marked progression of our understanding of its genetic inheritance . A complex polygenic model that involves common low penetrance susceptibility alleles causing individually small, but cumulatively significant risk, and rarer genetic variants, causing greater risk, represent the current most accepted model. Through Genome Wide Association studies, more than 100 SNPs associated with PCa risk have been identified. Consistent reports have identified germline mutations in the genes *BRCA1*, *BRCA2*, *MMR*, *HOXB13, CHEK2, NBS1,* as conferring moderate risks, with some leading to a more aggressive disease behaviour. Considering this knowledge, several research strategies have been developed to determine if targeted prostate screening using genetic information can overcome the limitations of population-based PSA screening. Germline DNA-repair mutations are more frequent in men with metastatic disease than previously thought, and these patients have a more favourable response to therapy with poly(adenosine diphosphate [ADP]-ribose) polymerase (PARP) inhibitors. Genomic information is a practical tool that has the potential to enable the concept of precision medicine to become a reality in all steps of PrCa patient care.

1. Introduction

Family history, ethnicity and age are the only risk factors for prostate cancer (PCa) that have been consistently established. Some families display significant aggregation of PCa cases A man with a first degree relative affected by the disease has at least twice the chance of developing this condition compared with the general population [1]The risk increases even further if more than one relative is affected and also if the related case presented with early onset PCa [2]. For first degree relatives of cases diagnosed before the age of 65, the estimated relative risk (RR) is 3.4 (95% CI [Confidence Interval] 2.7-4.2) [3]. When 2 first degree relatives are affected, the pooled RR is 4.39 (CI 2.6-7.4) [4]. Studies analysing Nordic twin registries have demonstrated at least a 50% higher risk in monozygotic than dizygotic twins. This, associated with the higher incidence in African American men suggests that genetic, rather than shared lifestyle factors, are responsible for much of the familial aggregation [5-7]. A large prospective cohort study estimated that genetic variation can explain 58% of the liability to develop PCa [8]

2. Genetic Models

Genetic predisposition variants associated with PCa consist of a mixture of models, ranging from rare higher penetrance genes to common variants associated with a slightly increased risk per variant (although the cumulative risks can be substantial as there are many such variants whose risks are multiplicative). Segregation analyses of PCa family pedigrees have suggested a major genetic component as the causative factor. The hypothesis of a dominant pattern of inheritance was supported in some specific subsets of families [9, 10]. However, the genetic model on further studies has been shown to be complex and includes a recessive and X-linked form of inheritance in some modelling studies [11, 12]. The suggestion of a potential high risk genetic component has spurred the use of linkage studies in pedigrees with multiple affected individuals in an effort to identify these genes.

a. Evidence from Linkage Studies

Linkage is the co-segregation of genetic markers with a disease, and can be used to indicate the likely location of disease predisposition genes. The measure of the likelihood of linkage is defined as the "logarithm of odds" (LOD) [13]. Different linkage studies described associations with several candidate locus, including 1q42, 1p36, 8p22-23, 17q11, 20p13, Xq27-28, [14-20]. However, these findings were not consistently reproduced, casting doubt on their true association with PrCa risk. In 2005, the International Consortium for Prostate Cancer Genetics (ICPCG) reported the largest study to date, combining data from 1,233 families from 10 international groups [21], identifying only one locus with a LOD >3 located on 22q, and was only observed in families with high early-onset PCa aggregation. Recently, a successful approach involved the sequencing of a linkage region at 17q, which found mutations in the gene *HOXB13*, associated mainly with young onset and familial PCa [22]. Despite these isolated successful results, linkage analysis failed to provide definitive findings, suggesting a much more complex model of PCa susceptibility.

It is clear today that the inheritance model of a highly penetrant gene associated with a high risk is clearly not enough to account for all genetic burden of the disease, with multiple genes (polygenic inheritance) likely to be involved. A model based on several different common and low penetrance mutations is more adequate to account for the majority of PCa cases. Conversely, a small proportion of cases, especially those with early onset and strong familial aggregation, possibly harbour rarer, higher risk mutations. This came to light as the correct model for PCa predisposition (Fig. 1).

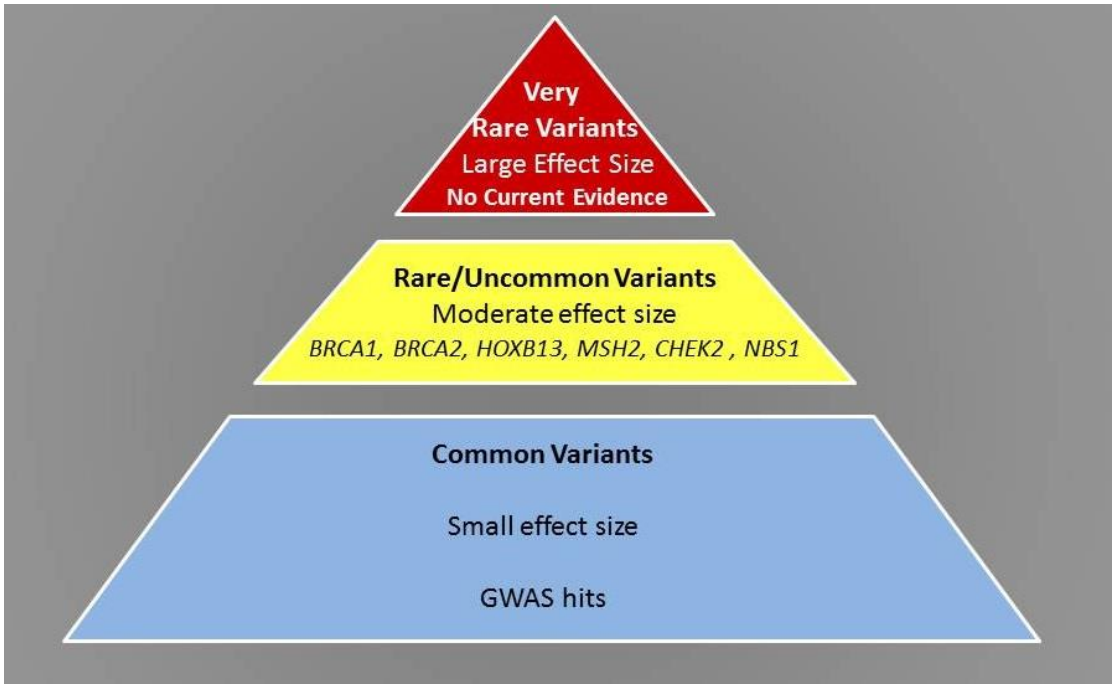

Figure1. Genetic predisposition to prostate cancer.

Modified From: Mikropoulos et al. [23]

a. Genome wide association studies and clinical implications

The pitfalls of the linkage analysis to search the genome, especially to identify low-penetrance and high frequency risk determinants, exposed the need for a different approach. In contrast with hypothesis-based previous studies, in Genome Wide Association Studies (GWAS) an agnostic approach is employed, comparing the frequency of Single Nucleotide Polymorphism (SNPs) between cases and controls throughout the genome.

A SNP is one the most common type of human genetic variation. It is a difference in a single nucleotide base pair in the DNA sequence between members of the same species. The frequency of this allelic variation is usually at least 1% to be considered a polymorphic SNP (Pearson and Manolio 2008)[24]. Tag-SNPs' are SNPs in genomic regions of linkage disequilibrium with a causal variant. Tag-SNPs are useful as they can infer associations with other SNPs, but they are limited in that they might just be markers of the pathogenic SNPs [25, 26]. Frequently 300,000 to over two million SNPs can be genotyped in several thousands of samples on high- density microarrays. It has been an excellent tool to identify low penetrance susceptibility loci for many complex diseases. As so many data points are analysed at once, the level of genome-wide significance that needs to be achieved is P <of $5x10^{-8}$

8q24 was the first region identified [27] and is the region that has the highest number of independently associated variants, with different groups reporting 16

independent SNPs in genome wide searches, in diverse ethnic groups of men [28-32]. The contribution to the total risk seems to be larger in men of African ancestry [32, 33], and a recent report identified 2 risk variants only found in men of African ancestry and a novel signal, rs111906923, in this region [34]. These SNPs are located near or within many PCa associated long non-coding RNAs (lncRNAs). This finding reinforces the hypothesis that lncRNAs might have a role in ancestry-specific PCa predisposition [35, 36]. Additionally, the 8q24 region is located in the vicinity of *MYC,* a known oncogene. Using a chromosome conformational capture essay (3C), Ahmadyeh et al [37] presented evidence that support the role of 3 independent 8q24 subregion (region1: 128.54-128.62, region 2 128.14-128.28, region 3 128.47-128.54) as regulatory enhancers, with interaction with *C-MYC*.

Another SNP (rs11568818) with potential prognostic value is situated at 11q22 within a region containing the gene *MMP7*. *MMP7* encodes for a matrix metal-loproteinase, which is pivotal for tumour metastasis, and overexpression of *MMP7* is a potential biomarker for PCa aggressiveness and risk of metastatic disease [38]. Also high serum concentration of MMP-7 [38] and polymorphisms in this gene [39] are correlated with poorer outcome after radical treatment.

rs7141529 is within the locus of *RAD51B*. The RAD51 protein family is involved in DNA repair mechanisms [40], and disruption of the DNA repair pathway is now thought to be important in the development of many cancers [41].

SNP rs2735839 was identified between the *KLK2* and *KLK3* genes on chromosome 19 in which there is a kallikrein gene cluster [46]. Kallikreins are serine proteases and the most recognized member of this group is the prostate-specific antigen (PSA). Fine mapping of the region at 19q13 led to the identification of a coding SNP in *KLK3* that showed a stronger association with PrCa risk than the original SNP identified from GWAS, and this novel SNP potentially changes some characteristics of the PSA molecule [42].

Different groups reported the same hit on chromosome 10, rs10993994, close to the transcription start site of the microseminoprotein beta (*MSMB*) gene [30, 43]. The product of this gene is a 10.7 kD non-glycosylated cysteine-rich protein that is synthesized by epithelial cells in the prostate and secreted into seminal fluid [44]. It can be measured in the plasma and urine, and it is one of the three most common proteins secreted by the prostate gland [45]. Reduced levels are seen in early disease, in tumour tissue, as demonstrated by immunohistochemistry, and also in the urine [46]. Also, a comprehensive recent report assessed tissue and serum expression using immunohistochemistry, RT-qPCR and genotyping of rs10993994 in patients with benign hyperplasia, early and advanced PCa, demonstrated that decreased expression of MSMB parallels cancer progression, and adjusted MSMB serum levels correlates with PCa risk [47]. These characteristics make MSMB a potential screening target and

prognostic determinant, probably to be used in addition to PSA rather than replacing it [48].

Although each susceptibility allele is known to confer only a small increased risk individually their risks act multiplicatively [49, 50]. From the current 41 GWAS, more than 100 SNPs are independently associated with PCa risk and explain approximately 33% of the familial risk of the disease (Table 1). Fine-mapping of these GWAS region also proved to be very important as it can not only discover multiple independent hits in a region but also often can replace the original tag SNPs with a better, more significantly associated variant, hence risk prediction will also be more precise using these newly discovered variants [51]. Men in the top 1% of the genetic risk profile of a population typed for these SNPs would have a 5.7-fold increased relative risk of PCa development in comparison with the average of the population [52]. In the near future, the novel findings from the Oncoarray Consortium will add new and relevant information about the association of common genetic variants and PCa.

This risk stratification approach can lead to a new way to select men for early detection programmes, potentially overcoming many of the limitations of PSA-based screening. Using 26 SNPs and family history, MacInnis et al. [53] developed an algorithm that has the potential to predict individual PCa development risk. Newly discovered SNPs can be added to the equation, as long as they contribute multiplicatively to the risk, allowing updated SNP-based risk stratification as new discoveries are confirmed. Once validated in prospective series, this can become a useful tool in screening decisions in clinical practice.

Recently, the results of the Stockholm 3 (STHLM3) study were published. This prospective screening cohort involved 58,818 participants, aged 50–69 years. A PSA-only based detection protocol was compared with the STHLM3 model, which employed plasma protein biomarkers (PSA, free PSA, intact PSA, hK2, MSMB, MIC1), genetic polymorphisms (232 SNPs), and clinical variables (age, family history, previous prostate biopsy, prostate examination). The model outperformed PSA alone for detection of cancers with a Gleason score ≥7 ($P<0.0001$), with an area under the curve (AUC) of 0.74 (95% CI 0.72–0.75), compared with an AUC of 0.56 (95% CI 0.55–0.60) for PSA alone [54].

A study investigated targeted screening in men with family history. The PROFILE feasibility study involved 115 men with a PrCa family history and who were of Caucasian origin. One hundred patients underwent a TRUS prostate biopsy at study enrollment, and the results were correlated with clinical variables and a polygenic risk score (PRS), based on 71 SNPS. Twenty-five PrCa cases were detected, with 12 (48%) classified as intermediate or high-risk disease, requiring active treatment. This number is almost twice as expected based on the Prostate Cancer Prevention Trial data [55]. Age at study entry and PSA were predictors of PrCa diagnosis. No significant association was found between the PRS and biopsy outcome. However, this initial pilot was not powered

to detect this difference [56]. A more extensive main PROFILE study of 700 men is in progress and has incorporated multiparametric MRI into the screening algorithm. The conclusion of the main study will provide invaluable evidence as to whether a SNP profile will be helpful in the screening of higher risk men.

The routine use of risk SNPs profiling in public health would require further evidence. In the next few years large studies in this area will be crucial to determine the role of this tool in population-based screening programs [57].

Copy number variations (CNV) are a distinct class of germline polymorphisms and recently have been associated with cancer predisposition [58]. In an analysis of 1900 Caucasian men, Demichelis et al, reported 2 low-frequency CNV strongly associated with PCa [59]. The firs locus, mapping to 15q21.3, overlaps a noncoding element that hold multiple activator protein 1 (AP-1) transcription factor binding sites. The second on 12q21.31, maps directly to α-1,3-mannosyl-glycoprotein 4-β-N acetylglucosaminyltransferase C (MGAT4C) gene. This glicosyltransferase is involved in regulationg cell-cell adhesion in epithelia. These findings were replicated in an independent cohort, and illustrate that germline CNV may be involved in mechanisms of carcinogenesis regulation and progression.

2. Rare Variants

The evidence from GWAS allowed clarification of a significant proportion of the inherited PCa risk. This technique is powered to detect common variants, with frequency >5%, not detecting less common risk alleles. Studies employing direct testing in a large number of cases and controls using next-generation sequencing have consistently reported a few rare genes, with moderate to highly penetrance that confer moderate to high individual risk.

a. *BRCA1/2* and implications for targeted screening

The most reproducible results came from analysis of germline mutations in breast cancer predisposition cancer genes 1 and 2 (*BRCA1* and *BRCA2*). *BRCA1* and *BRCA2* are tumour suppressor genes, coding large proteins, inherited in a dominant fashion with incomplete penetrance [60]. BRCA1 is a protagonist in cellular control mechanisms, acting in DNA damage response and repair, transcriptional regulation and chromatin modelling. BRCA2 is likely to be related to DNA recombination and restoration processes, via interactions with RAD51 and PALB2 [61]. *BRCA1/2* impairment results in a deficiency in repairing DNA double-stand breaks by the conservative approach of homologous recombination (HR) [62]. Affected cells start to employ potentially mutagenic pathways to repair these lesions [63].

Tumorigenesis in individuals with germline mutations in *BRCA* genes usually demands somatic inactivation of the remaining wild type allele in a tumour suppressor

model, although there are rarer reports of haplo-insufficiency from loss of the mutant copy [64]. The genomic instability generated is believed to be the mechanism for the cancer predisposition observed

Clear evidence has shown that mutations of the tumour suppressor *BRCA2* gene predispose men to PCa. This predisposition was reported by the Breast Cancer Linkage Consortium (BCLC), which analysed men in families with a history of breast and ovarian cancers (also *BRCA*-driven malignancies). Their data estimated that men with germline *BRCA2* mutations have an approximately five-fold higher relative risk of PCa than men without *BRCA2* mutations. This risk increases to over seven-fold in families with men with early onset PCa (<65 years) [65, 66]. Subsequent studies pointed to an even higher risk with an estimated lifetime absolute PCa risk around 15% at the age 65, corresponding to an increased relative risk of approximately 8.6-fold [67].

By contrast, the relative risk of prostate cancer in young *BRCA1* mutation carriers is controversial. These patients have been shown to have a smaller, though consistent, increased relative risk, with an estimate of 1.8- fold to 4.5-fold, representing a cumulative risk at 65 years of up to 8.6% [68, 69].

Despite representing a small fraction of PCa cases (0.45% and 1.2% for *BRCA1* and 2, respectively) [67, 68], *BRCA* mutation carriers have repeatedly been shown to have aggressive disease [70-73]. In the largest analysis of clinical characteristics of PCa patients who are *BRCA* carriers to date, Castro et al. [74] reported a larger proportion of high grade disease, Gleason score >8, nodal involvement and metastatic presentation amongst carriers than controls. Moreover, cancer specific survival and metastasis free survival were both significantly higher in noncarriers. It is possible that this occurs because there is a higher incidence of copy number variation in PCa tumour and normal prostatic tissue in germline *BRCA* mutation carriers [75].The sum of all evidence points toward the rationale for a more aggressive early detection strategy and management of PCa in *BRCA* mutated patients.

Currently, a large multicentre international study is investigating the use of targeted PSA screening in *BRCA* mutation carriers. The IMPACT study (Identification of Man with a genetic predisposition to ProstAte Cancer: Targeted screening in men with *BRCA1/2* mutations) [76, 77] has recruited to date 2481 mutation carriers and controls. The results of the first screening round, published in 2014, showed a predictive positive value (PPV) of biopsy using a PSA threshold of 3 of 37.5% in *BRCA1* carriers and 23.3% in controls; 48% in *BRCA2* carriers and 33.3% in *BRCA2* controls. Furthermore, the PPV for detection of clinically significant disease (intermediate and high grade) was higher in the *BRCA2* carrier group when compared to controls, 2.38% and 0.71%, respectively (Pearson p=0.04) [78]. The final report will present the results of 5 screening rounds.

b. Lynch Syndrome

A patient group in which a higher PCa risk has been reported is men with Lynch syndrome. Lynch syndrome is a multicancer syndrome caused by germline mutations in the MMR genes; *MLH1*, *MSH2*, *MSH6*, and *PMS2*. Colorectal cancer and endometrial cancer are a predominant feature, with a 70% and 50% lifetime risk, respectively [79]. There is increasing evidence that PCa risk is also increased in Lynch syndrome. From the Manchester Regional Lynch Syndrome Database, enrolling 821 men, Barrow et al [80] described a 10.41-fold increase in PCa risk (95% CI 2.8–26.65). In one of the largest cohorts to date, Engel et al. [81] analysed 2,118 MMR gene mutation carriers, and found a cumulative incidence of 9.1% with a standard incidence ratio of 2.5 (95% CI 1.4–4.0). Both of these studies agreed that there is a preponderance of *MSH2* mutations among PCa patients with Lynch syndrome. In a recent meta-analysis, Ryan et al [82] estimates a 2.28-fold increased PCa risk (95% CI, 1.37–3. 29) for all men from mutation-carrying families.

c. HOXB13

Another gene currently of interest is the *HOXB13,* Homeodomain-containing proteins (HOX) are a large class of sequence-specific transcription factors. Humans have 39 *HOX* genes, arranged in 4 chromosomal clusters, named *HOXA, HOXB, HOXC,* and *HOXD* [83]. The core function is to specify the identity of body segments along the AP axis during embryonic development, and HOXB13 plays a crucial role in the prostate development [84]. Interaction of HOXB13 and Androgen receptor has been demonstrated in normal and PrCa cells [85], with functions of different androgen targets. Therefore, HOXB13 seems to be an important regulator of cellular response to androgens [84, 86].

Ewing et al. [22] reported the association of a rare germline mutation (G84E) in the *HOXB13* gene with an increased risk of hereditary PCa. This mutation (G84E) was genotyped in 2443 PCa families recruited by the International Consortium for Prostate Cancer Genetics and was found in 4.6% of families, all of European descent. It was more common in Nordic countries and less common in North America and Australia. And within *HOXB13* carrier families the G84E mutation was more common in men with a diagnosis of PCa [70]. A meta-analysis of 24 trials with 97,844 participants by Shang *et al.* identified that the frequency of the G84E mutation was higher among cases with younger age at onset with an OR (odds ratio) of 10.1 (95% CI: 5.97–17.12) and among patients with family history, with an OR of 5.01 [71]. The meta-analysis by Huang et al. of 11 studies with 120,617 men measured the relative risk of PCa as 4.51-fold for *HOXB13* G84E carriers and also highlighted that this variant is associated with early-onset (OR: 9.73), familial (OR: 7.27) and high-risk (OR: 5.81) PCa [72]. A large case-control study involving Caucasian British men reported an incidence of 1.5% of the variant among PCa cases, associated with a 2.93-fold increased risk [87].

The clinical utility of this finding remains to be determined.

d. CHEK2

In response to genotoxic insults causing double strand DNA breaks, *CHEK2* is activated and propagates the checkpoint signal along diverse pathways, leading to cell cycle arrest, activation of DNA repair mechanisms and eventually apoptotic cell death [88]. *CHEK2* variants have been associated with PCa risk. A recent report from the Copenhagen general population study found that *CHEK2**1100delC heterozygotes had an adjusted hazard ratio for PCa of 1.6. A pooled analysis of 5 studies described an OR of 1.98 (95%CI 1.23–3.18) in unselected cases and 3.39 (95%CI 1.78–6.47) in familial cases. [89]

e. NBS1

The product of the gene Nijmegen Breakage Syndrome NBS1 is a component of the *BRCA1* DNA-damage response pathway [90]. A candidate gene analysis involving 1861 individuals reported a higher frequency of the founder mutation 657del5 in patients with familial PCa, when compared with patients with sporadic PrCa and controls (OR = 16, P=0.0001). In a study involving 7706 patients, the same founder mutation was more frequent in cases than controls with an OR = 2.5 (P=0.0003). Additionally, this variant was associated with a worse prognosis [91].

3. Germline Genetic variants and treatment outcomes

Evidence is emerging that germline variants can influence diverse treatment modalities has been accumulating in recent years, making the use of genetic information to guide treatment decisions a realistic perspective.

Using the 23 SNPs discovered with the Collaborative Oncological Gene-Environment Study array (iCOGS) Kearns et al. described that rs11568818 was associated with pathological upgrading in a prospective cohort of surgically treated patients. This finding was confirmed in a second cohort of patients on active surveillance (AS), in which rs11568818 was associated with pathological upgrading on surveillance biopsies [92]. It has been shown that on average 30% of patients on AS will move to definitive treatment in the first 5 years, and genetic information that could inform a more precise stratification before AS enrolment would be of great value.

The mechanisms involved in radiation-induced tissue damage are complex, and it is likely that genetic variation is one of determinants of individual response. A three-stage GWAS identified association between a locus comprising *TANC1* at 2q24.1 and late radiotherapy toxicity, with a combined P value = 4.64×10^{-11} [93]. An additional GWAS enrolling 663 patients treated with radical radiotherapy described more association between common variants and treatment toxicity than expected by chance, at a significance level of p= 5×10^{-7} [94]. In a study of 1560 patients from 4 radiotherapy cohorts, Ahmed et al demonstrate that 75 SNPs associated with PrCa risk are not

predictors of radiotherapy toxicity, showing that men with genetic predisposition due to common variants can be safely treated with current radiotherapy regimens, and illustrating that many of the individual determinants of radiation toxicity are yet to be identified [95].

Castro et al. investigated the influence of *BRCA* mutations on PCa treatment outcomes in a cohort of 1302 patients, including 67 *BRCA* mutation carriers. The authors reported that carriers when treated with curative intent modalities (either radiotherapy or radical prostatectomy) develop metastasis sooner and had shorter survival than noncarriers [96]. *BRCA* mutation carriers with organ confined PCa were treated on protocols developed for noncarriers, and this study provided the first evidence that this may not be the ideal approach.

A recent multicentre study described that deleterious germline DNA-repair mutations are more frequent in men with metastases than described in the overall PCa population, including patients with localized disease. These mutations were identified in 11.8% (82) of 692 patients with documented metastatic disease. *BRCA2* (5.3%), *ATM* (1.6%) and *CHEK2* (1.9%) were the most frequent (Fig. 2) [97], A previous report in 191 familial PCa cases showed a frequency of 7.3% of mutations in at least one of 22 crucial tumour suppressor genes (BROCA panel) [98].

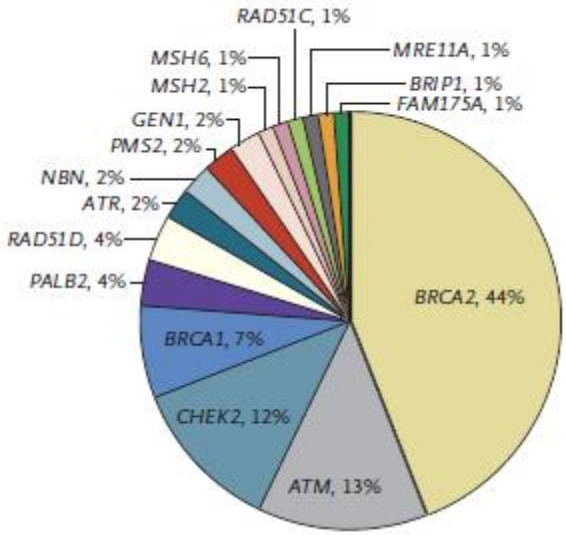

Figure 2. Distribution of DNA-repair germline mutations among 82 patients with metastatic prostate cancer. From Pritchard et al. [97]

In a phase 2 study of castration-resistant PCa patients, Mateo et al. described that patients with somatic or germline mutations in DNA-repair genes had a significantly higher response to poly(adenosine diphosphate [ADP]-ribose) polymerase (PARP) inhibitors. From 16 patients which such mutations, 88% responded, including 100% of patients with *BRCA2* loss [99]. Also, *BRCA2* biallelic loss appears to be related with platinum-based chemotherapy sensitivity [100] . These findings, associated with the

increased incidence of germline mutations in the metastatic setting, illustrate the potential for a genomic-based approach to treatment. The presence of an increased frequency of germline mutation in the metastatic setting has been demonstrated mainly for breast and prostate, however, there is a clear rationale for its role as a common feature of aggressive cancer.

4. Conclusion

Our understanding of PCa genetics is growing at an accelerated pace. The potential clinical implications are applicable at every crucial step of the patient care pathway: from selection of patients for screening, guiding treatment decisions at diagnosis to targeted systemic therapy in the metastatic setting, thus making genomic information as a practical tool to enable the concept of precision medicine to become a reality.

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