Revisiting Prostate Cancer in India: A Genomic View

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ABSTRACT

In the recent past, there has been a rise in Prostate Cancer (PCa) in Asia, particularly India. Although systematic reviews on PCa have dealt on the genetics, genomics and the environmental influence in causal of PCa, no predictive analytics in comparing the PCa from Caucasian, American to Asian population was attempted. In this review article, we have attempted to elaborate this aspect of PCa and deliberated on challenges related to next generation sequencing methods of PCa’s manifestation when compared to the west.

1. Background

Concern for the global epidemiology of Prostate cancer (PCa) is substantially growing [1] as it accounts to the second most common cancer worldwide [2,3] and third most prevalent cancer in India [4]. PCa cases are diagnosed in over one million annually and the mortality rate has grown to more than 300,000 deaths per year. Incidence and mortality differ among geographic regions and populations showing multifactorial impacts of genetic variation, diet, lifestyle, environmental factors and use of prostate specific antigen-based screening policies [5]. In 2012, 1.1 million men were diagnosed with PCa worldwide, a total of 759,000 cases were recorded, (Figure 1) with Europe having the highest estimate of PCa cases (37.8%) followed by Northern America (28.4%), Asia (15.8%), Latin America and Caribbean (11.5%), Africa (4.0%) and Oceania (2.4%) [2]. Reported PCa incidence rates varied over 25-fold worldwide [6], even as many men not coming forward for the diagnosis itself. On the other hand, the Prostate-specific Antigen (PSA) screening serves as one of the most common non-invasive biomarkers to detect PCa[7]. As described by Chen et al. 2017, the world mortality-
incidence ratios (MIR) for PCa was 28.1% wherein less developed regions demonstrated high MIR for PCa with the highest MIR of 71.9% found in Africa. Countries with higher levels of human development and per capita gross domestic product (GDP) had been accounted with higher PCa incidence but not in mortality rates. In addition, the PCa incidence and mortality correlation with socio-economic development of country showed a simple linear regression between PCa incidence/mortality and human development index (HDI) [6]. On the other hand, Asians who immigrated to the western countries have been accounted for higher incidence of PCa when compared to the people in their native country. Reason for the higher occurrence of the PCa among Asian migrants could be due to different health care systems and more importantly the diet [8]. Therefore, it can be speculated that the westernized diet in Asian countries may have an influence on high risk of PCa, however it has been difficult to prove the relationship [9].

Figure 1. Estimated five year prevalence of PCa cases (*1000), adult population. Source: GLOBOCAN, 2012.

1.1. PCa in India and South Asian Population

The census of India which released on July 15, 2011 revealed interesting statistics relevant to the pattern of prevalence and other disease characteristics of PCa[10]. Though the prevalence and characteristics of PCa have been studied in India, its true incidence is limited, perhaps owing to the lack of exposure of patients to clinic and the unavailability of cancer registries. In the recent past, the population of India has displayed rapid changes in lifestyles, dietary practices and socio-economic milieu which have resulted in an enormous increase in the number of cancer cases. The most recent Population Based Cancer Registries (PBCRs) of different cities for the time period (2008–2011) shows that PCa has ranked among top ten leading sites of cancer in many cities including Bangalore, Barshi, Bhopal, Chennai, Delhi, Mumbai, Kamrup, Ahmadabad, Kolkata, Kollam, Nagpur, Pune, Trivandrum and Wardha (Figure 2). There are some reports which illustrate several genetic mutations and polymorphism of certain genes (for eg GSTM1 and GSTP1) associated with PCa[11]. Interestingly, higher prevalence of polymorphisms in DNA repair systems XRCC1 and XPD, is responsible for DNA repair which could lower cancer susceptibility, has been reported in south asian populations.
1.2. Identifying PCa mutations using Next Generation sequencing methods

In the recent past, next generation sequencing (NGS) has allowed the simultaneous identification of millions of short stretches of nucleic acids [13] screening a large number of genes with greater sensitivity and cost effectiveness [14]. The NGS studies branched towards understanding the cancer genome of several tumor types [15]. An attempt to perform diagnostic, prognostic, predictive biomarkers and biomarker-designed clinical trials is on the anvil [14]. Application of NGS has led towards easier identification of the PCa variants by exposing hidden information through genomic and transcriptomics landscape, especially identifying key biological and molecular components of progression and potential therapeutic opportunities of castration-resistant PCa (CRPC), a more advanced and metastatic form of PCa. [16]. CRPC was earlier known as hormone-refractory PC but different studies have shown that CRPC is not hormone-independent as androgen axis continues to play an important role in its growth.

As far as mutations are concerned, structural genomic rearrangements [17] due to deletion of tumor suppressors such as PTEN, TP53, NKX3-1 [18], CDKN1B [19] and BRCA mutations identified in patients with PCa were found to be somatic while ca. 15% of patients were known to be associated with metastatic CRPC (mCRPC) [16]. The genome-wide profiling in the plasma of patients having PCa revealed multiple copy numbers, such as losses in 8p and gains in 8q in addition to identification of TMPRSS2-ERG rearrangement associated 3-Mbp deletion on chromosome 21 [20]. Thus, determining somatic copy number alterations (SCNA) serving as mutational hotspots could be helpful. A novel mutational hotspot at the KCCAT42, FENDRR, CAT1886 and STCAT2 loci at the 16q23.1-q24.3 loss were identified as alterations of lincRNA sequences [21].

Tomlins 2005described that, in approximately 50% of all PCas' have gene fusions and rearrangements of ETS family of transcription factors (TMPRSS2-ERG). Moreover, other ETS family members such as TMPRSS2-ETV1, TMPRSS2-ETV4, TMPRSS2-ETV5, and SLC45A3-ERG are frequently rearranged and over-expressed in PCa. This phenomenon is specifically found only in prostatic tumors but occasionally present in high-grade prostatic intraepithelial neoplasia.

From in vitro and in vivo assessments of gene and protein expression, Ren et al. 2018 showed that the PLXNA1 protein as an effective therapeutic target to treat advanced PCa. Enhanced reduced representation bisulfite sequencing (ERRBS) can detect genome-wide DNA methylation at single-base resolution including CGI shores and allele-specific methylation (ASM) at various regions [24]. Based on the ChIP-Seq, Chen et al. 2013 showed that ERG restores an androgen receptor (AR) transcriptome in PTEN-deleted PCa. In addition, by using methylated DNA immunoprecipitation sequencing (MeDIP-Seq) epigenetic mechanisms such as DNA methylation and histone modification can be studied which are known to play an important role in
PCa development and progression associated with molecular and cellular alteration [26]. One of the most important DNA methylation markers which have been frequently reported in PCa is GSTP1 promoter hypermethylation, resulting in the loss of GSTP1 expression [27]. A number of methylation profiles have been developed and are being evaluated as potential markers for early diagnosis and risk assessment [25]. In the recent-past, an iCLIP-Seq method to infer RNA-Protein interactions at a higher resolution is made available [28].

1.3. Screening PCa using PSA

Prostate Specific Antigen (PSA) has been widely used as a tumor marker for PCa detection albeit has become a bit controversial in use [29]. The PSA is a glycoprotein with enzymatic protease activity secreted by prostatic epithelium and due to its enzymatic activity; the PSA liquefies the semen and increases the sperm motility [30]. PSA is a serine protease of approximately 33 kDa in size and is secreted by epithelial cells of prostate. In normal prostate, PSA is secreted into the luminal fluid whereas in case of PCa, it gets leaked into circulation due to disruption of basal cell layer resulting in an increase in PSA level [31]. The drawback of PSA test is its low specificity since PSA levels can be elevated in benign prostate hyperplasia (BPH), prostatic infarction and in prostatitis [32]. The other major drawback of PSA is that it does not differentiate between different stages of PCa[33]. To distinguish cancerous form from benign conditions and slow-growing from aggressive cancers, there have been certain modifications in the current PSA test in the form of PSA density, PSA velocity, detection assays for checking molecular forms of PSA, and precursor or pro-PSA, human glandular kallikrein 2 (hK2) and urinary marker uPM3 which are in regular use. [34]. However, the most widely accepted screening method is the Gleason grading system, which predicts the cancer behavior. [35].

1.4 Enhancement of the PSA tests

Many efforts are being done to increase the diagnostic accuracy of PSA, including measurement of different molecular forms of PSA and rate of PSA increase. Total PSA (tPSA) refers to the sum of free PSA (unbound) and bound PSA (complexed predominantly to α-1-antichymotrypsin). The percentage free PSA test is approved for use in men which helps discriminate between the presence of PCa and BPH that serves as a predictor for biopsy [36]. In this process, laboratory tests in the form of Prostate Health Index (PHI), Digital Rectal Examination (DRE) have been helpful for primary screening the patients depending on the PSA test result [37].

2. Role of Metabolic diseases associated with PCa

Metabolic diseases such as diabetes have a major role to play towards an increased risk of several human malignancies such as cancers of the pancreas, colon, endometrium, breast, kidney, liver, biliary tract and esophagus [38]. However, association of diabetes with decreased risk of PCa in depth only long-term diabetes has a protective effect on PCa have been reported from several studies [38,39]. There are several mechanisms to describe the protective effect of diabetes on PCa whereas protection against the PCa is due to the hormonal alterations; insulin and testosterone (T) in diabetes patients [40]. Initially insulin level of diabetes patients seems to be higher, then on decrease gradually with disease progression due to progressive beta cell burn out while T and sex hormones binding globulins (SHBG) levels also drop with the time [41]. Numerous human and animal studies have shown that both androgens [42] and insulin [43] have an effect on prostate cell growth and malignant transformation. Therefore, it is believed to be low risk of PCa is due to the decline of T and or insulin in patient with diabetes. However, the results of all the epidemiological studies rely on sex hormones not consistent [41]. Baradaran et al. 2009 demonstrated that a small, albeit significant drop of PCa risk for increasing level of T/SHBG ratio and further Will et al. in 1999 observed PCa risk to have doubled more than 5 years of diabetes diagnosis. However, steroids are not only the key factors for protective effect on PCa in diabetes patients but there could be an influence of hormonal environment also apart from testosterone. Insulin, insulin like growth factors (IGF) and leptin have effect on this inverse relationship [39]. Alterations in serum testosterone and IGF-I concentrations result by diabetes mellitus, have influence on PCa risk reduction among men with genetic background of diabetes seems biologically plausible [45]. Another explanation based on the existence of a genetic factor that promotes diabetes risk and let fall the PCa risk. Genetic variation in peroxisome proliferator-activated receptor-gamma (PPARG) has association with a higher incidence of diabetes mellitus [46] and also expressed in human prostate adenocarcinomas and derived cell lines. Inhibition of PCa cell growth is expected to be the activation of this receptor with specific ligands [47]. As described by Hsing, Sakoda and Chua Jr., 2007 long term diabetes condition results in insulin resistance. Insulin is a potent mitogenic and anti-apoptotic factor and stimulates the prostate growth further, DNA polymorphisms in the insulin gene may be linked with increased PCa risk. Progressive insulin resistance and B-cell failure along with insulin depleting arising with long-standing diabetes may limit insulin actions and reduce the PCa risk.
However, declined androgen levels in severe diabetes cases are probably due to a toxic effect of hyperglycemia on the Leydig cells of the testis [49].

The PCa incidence and mortality rates around the world show high correlation with average level of fat consumption. It is speculated that, western diet, rich in polyunsaturated fats, results in an increase in PCa cases. There have been several other studies associating effect of diet and certain essential nutrients on PCa risk but it could not be verified/confirmed [50]. On contrary, vegetarian diets such as fresh vegetables, poly-nutrients have shown a protective effect against PCa[10][50]. Furthermore, some natural spices and food additives used by south Asians play an important role in protection against cancer. For example, disease prevention capability of Turmeric has been widely discussed revealing its anti-oxidant, anti-inflammatory and chemo-preventive capacity which has been experimentally validated. The mechanism of action of turmeric involves blocking the activity of nuclear factor kappa-B (NF-kB), responsible for cancer cell survival in many cell types. On other hand, screening behavior and access to care are followed by south Asians are important in cancer prevention [10].

3. Genetic Biomarkers for PCa

Urine, a waste product of kidney, has become one of the most attractive bio-fluids in clinical proteomics [51]. Urine is non-invasive, harmless and can be collected in large quantities without any significant proteolytic degradation [52,53]. Since prostate cells can be detected in urine [54], different biomarkers specific to PCa has been identified through urine and used as serological marker in diagnostic test, which are described below:

3.1 Non-coding RNA as biomarkers

MicroRNAs (miRNAs) are naturally-occurring, small (18-22 nucleotides) non-coding RNAs [55] that control the expression of more than 60% of protein-coding genes, have regulatory function on various molecular signaling pathways in the cell and are therefore potential diagnostic indicators of tumor formation and metastasis [56]. Differential expression of miRNA in PCa can be firmly correlated with its clinical expression suggesting that miRNAs are promising potential biomarkers and can be used in the detection of PCa [57]. At present, over 10000 human miRNAs have been reported out of which more than 200 common miRNAs have been analyzed from urine exosomes. There have been a large number of studies to prove the usefulness of urinary miRNAs in combination with clinical parameters for enhancing the accuracy of classification of PCa. Among all the miRNAs reported, miR-141, miR-21, miR-200b, miR221, miR-106b and miR-375 have been the most frequently investigated in urine from PCa patients [58][59]. Further analysis revealed that all these miRNAs were overexpressed in PCa serum samples compared with healthy controls which are in agreement with other studies. Together all these data suggest that use of miRNAs for non-invasive and specific detection of PCa can be very promising and can significantly improve the prediction level of the presence of PCa.

Prostate cancer antigen 3 (PCA3), also known as Differential Display code 3 (DD3), a prostate-specific long non-coding RNA is dramatically overexpressed in human PCa tissue relative to normal prostate tissue [60]. The PCA3 score is calculated as the ratio of PCA3 to PSA mRNA (PCA3 mRNA/PSA mRNA x 1000) [61]. Compared to PSA test which gives false positives, PCA3 is more accurate in predicting clinically significant PCa and could be used as a diagnostic tool for PCa screening, grading and recurrence monitoring [62,63]. The limiting factor with PCA3 is that it does not correlate with Gleason score and clinical tumor staging which restricts its use in the medical field. Recent studies, however has enabled identification of other urinary long non-coding RNAs such as metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1) a multiple cancer-associated lncRNA [64], and FR0348383, a PCa-associated lncRNA [65]. Whereas MALAT-1 or FR0348383 have great potential as independent predictors of PCa, a large multi-center study has validated the clinical utility of a 3 protein-coding gene panel (HOXC6, TDDEL1, and DLX1) in urine [66]. Surprisingly, these three gene panels were known to have higher accuracy compared with urinary PCA3 or PSA in predicting aggressive PCa and combining them with PSA further improved the predictive accuracy. lncRNAs in plasma do not exist in their full-length form, though few stable fragments can be highly expressed and detectable in human plasma [67].

3.2 Gene fusion biomarkers

Gene fusion is the process of combining two or more distinct genes into a single chimeric gene or transcript and a major mechanism in driving carcinogenesis [68]. The TMPRSS2-ERG fusion gene is a PCa-specific fusion gene comprising androgen-related transmembrane protease serine 2 gene (TMPRSS2) and ETS-related gene (ERG), which results in aberrant expression of the transcription factor ERG and inhibits normal prostate differentiation [69]. The diagnostic accuracy of combining TMPRSS2-ERG and PCA3 into a urine test
Current challenges for diagnosis of PCA

The significant factors related with PCA are PSA level, Gleason score, smaller prostate volume, abnormal DRE findings and, age besides ethnicity (National Collaborating Centre for Cancer (UK), 2008). Four third of all PCA patients are above 65 years old and it is rarely diagnosed in men under 50 [88]. The initial diagnosis date has been risen up approximately 5 years with the use of PSA testing [89]. Onset in familial PCA is usually reported in men under 55 years old however, sporadic PCA rarely diagnose at this age [90]. From all cases approximately 10% have been reported as familial PCA whereas approximately 40% of patients under 55 years old account for familial PCA. The two most important genes; BRCA1 and BRCA2, which are responsible for breast cancer, are associated with high risk of PCA in men under 65 years old [91].

Current recommended normal reference range for PSA is 0–4.0 ng/mL. However efforts to increase the sensitivity of cancer detection in younger men and decrease the number of unnecessary biopsies in older men (improve specificity) led to establishing age and race specific PSA range suggested recommendations have been developed based of the results of several researches that demonstrated the PSA levels correlation with patient’s race, age and prostate volume (Table 1) [30]. Since the use of PSA is limited and controversial, the search for novel PCA-specific biomarkers, especially from non-invasive bio-fluids is an important task [92]. Due to the
heterogeneity of the disease, no single biomarker will be diagnostic and prognostic for every patient [93]. Based on this, it can be concluded that the next diagnosis or PSA test will most likely be an assay comprising multiple biomarkers that are differentially expressed in PCa. Also, success of these biomarkers will depend on their validation in large cohort of patients and translation of these findings to clinical practice.

5. **Comparative NGS analyses of PCa datasets:**

There has been a consistent need to understand the genetics behind PCa although a large number of cohort studies have been instrumental in identifying the causal genes and differentially expressed genes (DEG). From our pilot analyses, we surveyed four datasets (supplementary table 1) across Caucasians, American and Asian (Chinese) [94–96] and tried to predict the common genes across them (figure 3). This gives a subtle reason to demonstrate the effect of DEGs and the nature of the genes observed from transcriptome and whole exome sequencing (WES) respectively (supplementary information). While comparing exomes between Caucasian and Asian population, we observed eleven common genes between them, *viz.* ITGA7, ZNF691, ZSWIM5, NRG4, KRBA2, ECT2, FAM91A1, PDZD8, PYGL, EDN2 and TP53I3. On the other hand, CRISP, CSRP3, COL2A1, UGT1A6, UGT1A1, LRRN1, UGT1A3, B4GALNT4 and KCNC2, a total of nine genes were common between transcriptome of Caucasian and Asians, and we observed eight common genes in between "DEGs", "Exome Caucasian" and "Exome Asian" in the form of ITGA7, ZNF691, ZSWIM5, NRG4, KRBA2, FAM91A1, PDZD8, PYGL and three common between the DEGs and exome Caucasian, IGDCC4, LIG4 and EZH2.

![Figure 3](image_url)
Figure 3. (a) The Venn diagram shows eleven common genes across Caucasian and Asian exome datasets (ITGA7, ZNF691, ZSWIM5, NRG4, KRBA2, ECT2, FAM91A1, PDZD8, PYGL, EDN2 and TP53I3). (b) Nine common genes in Caucasian and Asian transcriptome (CRISP3, CSRIP3, COL2A1, UGT1A6, UGT1A1, LRRN1, UGT1A3, B4GALNT4, KCNC2) out of total 3169 genes from Asian dataset and 105 genes from the Caucasian dataset and (c) Eight common genes (ITGA7, ZNF691, ZSWIM5, NRG4, KRBA2, FAM91A1, PDZD8, PYGL) across Asian exome, Caucasian exome and DEGs were found. However, three common genes (IGDCC4, EZH2, and LIG4) were found across DEGs and Caucasian exome.

6. Palliative Care

Palliative care is specialized medical care that is catered for serious illness. While it focuses on providing relief from the symptoms, pain and stress, several attempts have been made to improve quality of life for the patient and family members [97, 98]. PCa leads to various problems such as interrupted flow of urine, difficulty starting or stopping urination and painful or burning sensation during urination. Other symptoms might include pain in bones, lower back, hips or upper thighs; difficulty having an erection; pain with ejaculation; and blood in the urine or semen. Early PCa, however, does not usually cause such extreme symptoms. Several options for treating PCa include surgery, radiation therapy and hormone therapy [99].

Abiraterone acetate is a type of hormone therapy given to patients with metastatic PCa when they stop responding to other types of hormone therapy as the drug stops the testosterone production. [100]. The survival however, for post-palliative care is assumed to be nine to twelve months.

7. Conclusions

Cancer prostate is alarming and considered third most significant cancers in Asia alone. There is an increase in prevalence and incidences in India which begs us a question whether any specific mutations are characteristics for Indian phenotype. This could ideally in pursuance of the fact that the diet and lifestyle conditions are different to that of west. In this review, while providing a summary of causative genes responsible for various diseases, we have attempted to discuss the reasons pertaining to the lack of PCa diagnoses in Indian population while reviewing the challenges, methods and histopathological aspects of PCa. However, one definitive need for post treatment regimen is palliative care which is certainly lacking with PCa diagnoses. With the NGS datasets uprising, one question still remains elusive: Is there an end to the problems of early detection of PCa?

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SUPPLEMENTARY FILES

1. Supplementary Information (Document)
2. Supplementary Table (Dataset information)

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