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ORIGINAL ARTICLE

BJUI COMPASS

Serum testosterone and prostate cancer in men with germline *BRCA1/2* pathogenic variants

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Abstract

Objectives: The relation of serum androgens and the development of prostate cancer (PCa) is subject of debate. Lower total testosterone (TT) levels have been associated with increased PCa detection and worse pathological features after treatment. However, data from the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) and Prostate Cancer Prevention (PCPT) trial groups indicate no association. The aim of this study is to investigate the association of serum androgen levels and PCa detection in a prospective screening study of men at higher genetic risk of aggressive PCa due to *BRCA1/2* pathogenic variants (PVs), the IMPACT study.

Methods: Men enrolled in the IMPACT study provided serum samples during regular visits. Hormonal levels were calculated using immunoassays. Free testosterone (FT) was calculated from TT and sex hormone binding globulin (SHBG) using the Sodergard mass equation. Age, body mass index (BMI), prostate-specific antigen (PSA) and hormonal concentrations were compared between genetic cohorts. We also explored associations between age and TT, SHBG, FT and PCa, in the whole subset and stratified by *BRCA1/2* PVs status.

Results: A total of 777 participants in the IMPACT study had TT and SHBG measurements in serum samples at annual visits, giving 3940 prospective androgen levels, from 266 *BRCA1* PVs carriers, 313 *BRCA2* PVs carriers and 198 non-carriers. The median number of visits per patient was 5. There was no difference in TT, SHBG and FT between carriers and non-carriers. In a univariate analysis, androgen levels were not associated with PCa. In the analysis stratified by carrier status, no significant

Please see Appendix A for full list of The IMPACT Study Collaborators and Steering Committee.

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association was found between hormonal levels and PCa in non-carriers, *BRCA1* or *BRCA2* PVs carriers.

Conclusions: Male *BRCA*1/2 PVs carriers have a similar androgen profile to non-carriers. Hormonal levels were not associated with PCa in men with and without *BRCA1/2* PVs. Mechanisms related to the particularly aggressive phenotype of PCa in *BRCA2* PVs carriers may therefore not be linked with circulating hormonal levels.

KEYWORDS
androgens, biomarkers, *BRCA1/BRCA2* pathogenic variant, prostate cancer, testosterone

1 | INTRODUCTION

Accumulating evidence has shown that *BRCA2* pathogenic variants (PVs) carriers are at higher risk of developing prostate cancer (PCa), estimated to be 2.5–8.6 fold, and that their disease progression is especially accelerated.[1–4] Despite representing a small fraction of PCa cases (0.45%–1% and 1.2% for *BRCA1* and *BRCA2*, respectively), studies have demonstrated that these patients present with a larger proportion of high-grade and metastatic disease.[5] Moreover, cancer-specific survival and metastasis-free survival are significantly higher in non-carriers, and carriers have a worse clinical outcome when treated with curative intent.[6] Lifetime risks for *BRCA1* PVs carriers have been described as higher than the overall population, but lower than for *BRCA2* carriers. However, for this genetic group, the evidence is inconsistent, with relative risks ranging from 0.3 to 4.[3,7]

The molecular mechanisms responsible for this aggressive disease behaviour in *BRCA2* carriers, and in particular, the clinical differences between *BRCA1* and *BRCA2* PVs carriers, are still largely unknown. *BRCA1/BRCA2* are tumour suppressor genes.[8] *BRCA1* acts in multiple pathways, including DNA damage response and repair, transcriptional regulation and chromatin remodelling. *BRCA2* is associated with DNA recombination and restoration, via interactions with RAD51 and PALB2.[9] *BRCA1* and *BRCA2* impairment results in a deficiency in repairing DNA double-stand breaks by homologous recombination (HR). Affected cells employ mutagenic pathways to repair these lesions, culminating in genomic instability and cancer predisposition.[10]

Interestingly, patients with *BRCA1/2* mutations have different disease risks and progression patterns in two other hormonally driven cancers: ovarian and breast. In these cancers, there are also distinct clinical phenotypes between *BRCA1* and *BRCA2* PVs carriers. How these abnormalities in generic pathways translate in tissue-specific phenotypes is unknown, and the findings in these three hormonally driven cancers may indicate that other modifiers, such as androgen exposure, may be relevant. Recent studies linked DNA-repair mechanisms and androgen signalling.[11–13]

Many studies have interrogated the association between androgen levels and PCa risk. Low serum total testosterone (TT) levels have been associated with a higher risk of PCa[14,15] and worse pathological characteristics after surgery.[15–19] Additionally, others demonstrated that low serum TT levels may have implications for the long-term oncological outcomes, with worse 5-year biochemical free-survival.[20] However, several studies have shown discordant results, such as the secondary analysis of the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) and Prostate Cancer Prevention (PCPT) trials.[21,22]

Due to the controversy about the role of androgens in PCa risk and to recent evidence linking DNA-repair and androgen signalling, it is vital to explore the potential influence of hormonal levels in PCa risk in men with DNA-repair mutations. The IMPACT study (Identification of Men with a genetic predisposition to ProstAte Cancer: Targeted screening in men at higher genetic risk and controls) is a prospective multicentre PCa screening study involving men at increased genetic risk and controls.[23] More than 2000 participants were recruited, and the interim analyses have provided evidence favouring prostate-specific antigen (PSA) screening in men with *BRCA2* PVs.[24]

One study described serum androgen levels in a male cohort of *BRCA1/2* PVs carriers. This study did not include men with PCa and included only 29 *BRCA2* carriers.[25] Our objective is to interrogate whether serum levels of TT, SHBG and FT could vary by PVs status and if these levels could be associated with PCa among men enrolled in the IMPACT study, across different genetic categories.

2 | MATERIALS AND METHODS

2.1 | The IMPACT study

The IMPACT study methodology has been published previously.[23,26] The protocol was approved by the West-Midlands Research and Ethics Committee in the United Kingdom (reference 05/MRE07/25) and by each participating institution's committee. Men aged 40–69 were recruited from families with *BRCA1/2* PVs. These men could enter the study if they had tested positive or negative for the PVs. Men who had a negative test for *BRCA1* were not tested for *BRCA2* mutations and vice versa. In this analysis, the control group is

composed of participants who tested negative for the familial *BRCA1* or *BRCA2* PVs, in a combined control group.

Participants underwent annual PSA testing, and if the result was >3.0 ng/ml, a transrectal ultrasound guided prostate biopsy was recommended. We retrospectively reviewed data from patients participating in the IMPACT study that had at least one TT measurement, all taken previously to any PCa diagnosis or treatment.

At study entry, age and body mass index (BMI) were recorded. PSA levels were collected at baseline and prospectively. As part of study protocol, hormone levels were assessed in a subset of participants. Serum samples were obtained by venepuncture between 07:30 and 20:30 h.

2.2 | Testosterone assays and calculations

TT measurements were based on immunoassays for most IMPACT centres, with the exception of the Denmark Centre, which employed liquid chromatography/mass spectrometry.

The free testosterone (FT) levels were calculated using the Sodergard equation using a constant albumin concentration.[27]

2.3 | Statistical analysis

Individuals who were not diagnosed with PCa at the first screening round contributed more than one sample. TT levels can vary widely in the same individual.[28] Therefore, instead of using a single value of TT (such as baseline, pre-diagnostic or mean value), we included all the values available in each specific genetic cohort.

We analysed the sex hormone binding globulin (SHBG), TT and FT levels as continuous variables and TT dichotomised using a cut-off point of 8 nmol/L (230 ng/dl), the testosterone replacement threshold for most specialty societies.[29]

Two-way analysis of variance (ANOVA) was used to compare age and BMI at baseline between mutation carriers (*BRCA1* and *BRCA2* as separate groups) and non-carriers, controlling for study centre, included as a factor variable.

Differences in hormonal levels (TT, SHBG and FT) among mutation carriers and non-carriers were assessed using a nested two-way ANOVA on log-transformed values. Study centre was included as a factor variable, and a nested model was used to allow for repeated observations from each subject.

To explore associations between hormonal levels and PCa across PVs status, a binomial logistic regression model was used, with the covariates BMI, PSA and hormonal levels (TT, SHBG and FT), adjusted for study centre and age. All the available readings from each individual were included in the analysis. Cluster-robust estimators were employed to relax the assumption of independent observations, thus allowing for repeated observations from each subject.

A p-value of <0.05 was considered significant. All tests are two-sided. All analyses were performed in STATA 14, College Station, TX, StataCorp LP and SPSS Version 25, IBM.

3 | RESULTS

A total of 777 participants in the IMPACT study had at least one TT measurement (Table S1). A total of 3940 unique measurements were included. The median number of follow-up visits per patient was 5; 198 non-carriers, 266 *BRCA1* and 313 *BRCA2* PVs carriers were included. SHBG levels were available for 737 participants. Overall, 12.9% of the patients were diagnosed with PCa (100 out of 777), 27 non-carriers, 26 *BRCA1* and 47 *BRCA2* PVs carriers, respectively (Figure 1).

Demographic characteristics of participants (age and BMI), baseline PSA, hormonal levels and number of follow-up visits are shown in

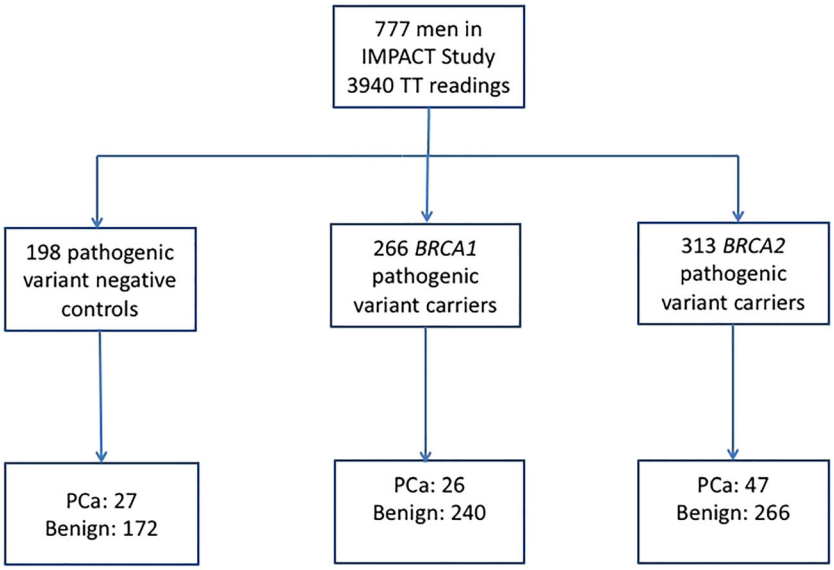

FIGURE 1 Consort diagram of study population. PCa, prostate cancer; TT, total testosterone

TABLE 1 Characteristics of subjects at baseline/first visit

	Mutation negative carriers						
	BRCA1−	*BRCA2*−	Total	*BRCA1* PVs carriers	*BRCA2* PVs carriers	Total	*p*-value[a]
Subjects, *N*	103	95	*198*	266	313	777	
Age, years, median (IQR)	54 (46–61)	57 (49–64)	*55 (47–62)*	53 (46–60)	51 (45–59)	*53 (46–60)*	0.008
BMI, kg/m^2, mean (SD)	26.87 (3.75)	26.45 (3.54)	*26.67 (3.65)*	27.16 (3.97)	27.53 (5.22)	*27.18 (4.45)*	0.020
SHBG, nmol/L, median (IQR)[b]	36 (27.00–43.50)	38.5 (29.00–55.00)	*36 (28.00–48.00)*	35 (26.20–47.00)	36 (27.00–47.00)	*36 (27.00–47.00)*	0.298
Testosterone[b]							
Total testosterone, nmol/L, median (IQR)	12.5 (10.10–15.20)	13 (10.00–16.87)	*12.8 (10.00–16.00)*	13 (9.96–16.40)	13 (10.10–16.10)	*13 (10.00–16.20)*	0.916
Free testosterone, nmol/L, median (IQR)	0.25 (0.19–3.35)	0.23 (0.18–0.28)	*0.24 (0.19–0.29)*	0.24 (0.20–0.31)	0.25 (0.20–0.31)	*0.24 (0.20–0.30)*	0.825
PSA[b]							
PSA, ng/ml, median (IQR)	0.99 (0.61–1.60)	0.96 (0.60–1.60)	*0.98 (0.60–1.60)*	0.81 (0.54–1.40)	0.83 (0.50–1.40)	*0.87 (0.56–1.50)*	0.064
Free PSA, ng/ml, median (IQR)	0.30 (0.21–0.47)	0.29 (0.21–0.43)	*0.30 (0.21–0.44)*	0.25 (0.19–0.42)	0.24 (0.18–0.39)	*0.26 (0.19–0.42)*	0.191
Calculated fraction of PSA, %, median (IQR)	25 (21–35)	29 (20–38)	*27 (20.5–37)*	29 (21–40)	31 (20–39)	*29 (21–39)*	0.212
Gleason score in cancers, *N*							
6	9	9	*18*	15	24	*57*	
3 + 4		4	*4*	7	11	*22*	
4 + 3	1	2	*3*	2	1	*6*	
8+	1	1	*2*	2	10	*14*	
Missing					1	*1*	
Total follow-up visits, *N* (median)	528 (5)	453 (5)	981 (5)	1325 (5)	1638 (5)	3944 (5)	

Abbreviations: BMI, body mass index; IQR, inter-quartile range; PSA, prostate-specific antigen; PVs, pathogenic variants.

[a]Across mutation status (negative carriers as one group, *BRCA1*+, *BRCA2*+). Two-way analysis of variance with covariate and centre.

[b]Test performed on log-transformed values.

Table 1. *BRCA2* carriers were younger, with a median age at study entry of 51 years old (inter-quartile range [IQR] 45–59). The median age for non-carriers was 55 years old (IQR 47–62) and 53 years old for *BRCA1* carriers (IQR 46–60) ($p = 0.008$). BMI was higher in *BRCA2* carriers (26.67 vs. 27.16 vs. 27.53 kg/m^2, non-carriers, *BRCA1* and *BRCA2*, respectively, $p = 0.02$). This difference was more pronounced in patients who received a PCa diagnosis (Table S2). The number of study visits was similar between groups. We observed no differences in median baseline values of TT, SHBG and FT across the genetic categories. The majority of the samples were collected in the morning

TABLE 2 Univariate associations between covariates and prostate cancer risk, adjusted for study centre*

	Non-cancers (subjects (*N*), measurements (*N*))	Cancers (subjects (*N*), measurements (*N*))	OR (95% CI)	*p*-value
Age	658 (3684)	92 (218)	1.056 (1.02, 1.09)	0.002
SHBG	626 (3497)	89 (208)	1.025 (0.54, 1.95)	0.940
Testosterone**				
Total testosterone	658 (3684)	92 (218)	0.954 (0.45, 2.04)	0.904
Free testosterone	626 (3498)	89 (208)	0.879 (0.46, 1.66)	0.692
Testosterone (<8/≥8)***				
<8	185 (418)	15 (24)	1.018 (0.47, 2.18)	0.964
≥8	641 (3266)	88 (194)		

Abbreviations: CI, confidence interval; OR, odds ratio; SHBG, sex hormone binding globulin.* Robust variance estimators to allow for sampling of subjects at multiple timepoints.** Log-transformed values.*** Subjects can contribute data to both categories

TABLE 3 Associations between androgens and prostate cancer status, stratified by *BRCA* status, adjusted for centre and age[a]

	BRCA status	Non-cancers (subjects (*N*), measurements (*N*))	Cancers (subjects (*N*), measurements (*N*))	OR (95% CI)	*p*	*p*-het
SHBG[b]						
	Negative	158 (859)	23 (47)	1.343 (0.29, 6.27)	0.707	
	BRCA1+	219 (1193)	24 (56)	2.585 (0.97, 6.87)	0.057	*0.464*
	BRCA2+	249 (1445)	42 (105)	0.345 (0.15, 0.77)	0.009	*0.119*
Testosterone[b]						
Total testosterone						
	Negative	170 (921)	24 (53)	2.640 (0.20, 34.80)	0.461	
	BRCA1+	227 (1245)	25 (57)	1.339 (0.42, 4.29)	0.623	*0.633*
	BRCA2+	261 (1518)	43 (108)	0.696 (0.33, 1.47)	0.343	*0.330*
Testosterone (<8/≥8)[c]						
<8	Negative	49 (90)	2 (3)	1.459 (0.25, 8.49)	0.674[d]	
≥8		167 (831)	23 (50)			
<8	*BRCA1*+	69 (162)	2 (4)	2.330 (0.44, 12.20)	0.317[d]	
≥8		214 (1083)	25 (53)			
<8	*BRCA2*+	67 (166)	11 (17)	0.858 (0.30, 2.43)	0.773[d]	
≥8		260 (1352)	40 (91)			

Abbreviations: CI, confidence interval; OR, odds ratio; SHBG, sex hormone binding globulin.
[a]Robust variance estimators to allow for sampling of subjects at multiple timepoints.
[b]Log-transformed values.
[c]Subjects can contribute data to both categories.
[d]No significant interactions.

period (Table S4). There was no significant correlation between PSA and TT levels (Figure S1).

Considering all the prospective samples, we observed no differences in median values of TT, SHBG and FT across the genetic categories, in both participants with and without a PCa diagnosis (Table S3). The median TT level in the non-carrier group was 12.6 nmol/L (IQR 9.8–15.6), 12.9 nmol/L in *BRCA1* mutation carriers (IQR 9.7–15.9) and 13.0 nmol/L in *BRCA2* mutation carriers (IQR 10.00–16.0) ($p = 0.670$). *BRCA1/2* status showed no influence on hormonal levels (Table 1 and Tables S2 and S3).

On univariate logistic regression, age was a significant predictor of PCa (odds ratio [OR] 1.085, 95% confidence interval [CI] 1.02, 1.09, $p = 0.002$). Considering the entire cohort, the OR (and 95% CI) for SHBG, TT and FT was 1.025 (0.54, 1.95), 0.95 (0.45, 2.04) and 0.87 (0.46, 1.66), respectively, all with $p > 0.1$ (Table 2). The association remained non-significant when using TT as a dichotomous variable.

We then assessed if the associations between androgen levels and PCa could vary accordingly with genetic status. A second regression model, stratified by *BRCA1/2* gene category and adjusted for age, mostly showed no significant associations between hormonal levels and PCa. In the non-carriers' group, the OR for the association of TT readings and a PCa diagnosis was 2.640 (95% CI 0.20, 34.80). In *BRCA1* carriers, it was 1.339 (95% CI 0.42, 4.29), and in *BRCA2* carriers, the OR was 0.696 (95% CI 0.33, 1.47). Similar findings were observed for FT. For SHBG levels, only in *BRCA2* carriers did the association with PCa reach the level of statistical significance (OR 0.345, 95% CI 0.15, 0.77, $p = 0.009$), but this was not significant in the genetic category comparison analysis (*p*-het 0.11; Table 3).

4 | DISCUSSION

In this work, we provide the largest report of androgen levels in a cohort of male *BRCA1/2* PVs carriers. We have found no association between *BRCA1/2* variant and baseline hormonal levels. We also investigated the association of TT, SHBG, FT and a PCa diagnosis during the IMPACT screening study. We found that pre-diagnostic TT, FT and SHBG were not associated with PCa risk in this cohort.

Circulating androgens have a clear relationship with PCa progression, as illustrated by the therapeutic effect of androgen suppression in metastatic disease, maintained even in later stages of disease.[30,31] Serum androgens are required for the normal prostate growth and for the development of PCa, but how they act in the initial carcinogenesis is unknown, with distinct models being proposed.[32–35] The wide range of results in the literature is probably due to the complexity of androgen physiology and assessment. There is a significant intra-individual variability on TT levels.[36] In our study, only baseline BMI data were available, not allowing for adjustment for this variable in the regression analysis. At baseline, men with a *BRCA2* PV showed a higher mean BMI level. However, the observed absolute difference was small, with a narrow SD, with mean values around normal/overweight BMI classification for all genetic categories. This means that most men in the study are not in the superior extreme of BMI distribution, where a clinically relevant effect on TT levels would be expected.[37] Additionally, most middle aged men do not experience a major change on BMI levels over a 5-year period.[38] Therefore, we do not expect that this could have had a major influence on the observed results.

There are limited data on androgens in men with genetic predisposition to PCa. In 2017, Goldberg et al. reported androgen levels in 87 *BRCA1/2* PVs carriers and 43 non-carriers.[25] They reported higher TT and free androgen index and lower prolactin levels in carriers. Our series, with a larger sample size (579 carriers and 198 non-carriers), failed to reproduce these results. Despite showing a higher level of TT in carriers, in the Goldberg et al. study, the mean levels in carriers and controls are within the considered physiologic range and were based on a single sample from each individual. It is well known that a day-to-day variation within the physiologic range occurs.[36] Most specialty societies[29] do not recommend any hormonal intervention should be considered based on a single hormonal level. The differences in the results are probably explained by the larger sample size and the availability of multiple samples from each individual in IMPACT.

The aim of the study is looking at the association of serum androgens in a population of men at higher risk of aggressive PCa due to *BRCA* status, and a stratified analysis between low- and high-grade cancer would be highly relevant. However, this study was conducted using data collected after the initial screening rounds of the IMPACT study, representing 777 participants. The number of PCa cases detected at that stage was small, not allowing for a proper statistical analysis using subgroups based on grade. The IMPACT study is ongoing, with more than 2000 participants,[24] and the interim analysis showed higher numbers of aggressive disease in *BRCA2* PV carriers. The final analyses will allow statistical power for a follow-up publication with stratification between low- and high-grade cancer.

Our findings should not be interpreted as a definitive evidence of no interaction between the hormonal and DNA-repair pathways as a disease modifier in *BRCA1/2* PVs carriers. There is growing evidence linking DNA damage and androgen signalling pathways in PCa.[11] *BRCA1* and *BRCA2* are believed to act as androgen receptor (AR) coactivators. Yeh et al. demonstrated that *BRCA1* enhances transcription of AR target genes and performs an important role in androgen-induced apoptosis by upregulating the expression of p21, because dihydrotestosterone (DHT) dramatically induces the expression of p21 in PCa cells expressing *BRCA1* and the AR.[39] *BRCA2* has been demonstrated to associate with the AR and enhance its transcriptional activity.[40] On the other hand, AR has been shown to regulate expression of DNA-repair genes and related proteins. DNA-dependent protein kinase catalytic subunit (DNAPKcs), Ku70 and Ku80, are key components of the non-homologous end joining (NHEJ) double strand break DNA repair pathway. In a PCa model, AR was found to directly regulate DNAPKcs expression, as androgen stimulation elicited upregulation of both mRNA and protein levels of DNAPKcs.[12]

Polkinghorn et al. identified 32 DNA-repair genes that comprise part of the complex AR-regulated transcriptome. These genes were

both induced by androgen and exhibit AR peaks in their enhancer or promoter. Many of these genes, such as *RAD51* and *CHEK1*, are involved in BRCA-mediated DNA repair.[13] Interestingly, the same authors also reported that the NHEJ is affected by androgen deprivation. NHEJ has been accepted as an error-prone DNA-repair pathway. However, recent evidence has shown that a more precise NHEJ modality exists in eukaryotic cells and that this is dependent on BRCA/DNA PKCs machinery.[9,41]

In view of the evidence linking DNA-repair and androgen signalling, showing that AR activation can trigger and enhance DNA-repair, we postulated that a mechanism to justify the clinical behaviour in *BRCA2* PVs carriers could be a cumulative carcinogenic effect between lower TT and a deficient DNA-repair pathway. However, we found no association between serum levels of TT, FT and PCa risk and this was not influenced by *BRCA1/2* status.

The lack of association between FT and PCa in this cohort may also be explained by the method of assessment of this variable. We used the Sodergard calculation, reliable clinically, but may not be accurate enough for the question posed here. Also, the interactions between AR signalling and DNA-repair pathways may rely on more diverse mechanisms, and albumin–testosterone or SHBG–testosterone complexes may play an independent role.

We found that in *BRCA2* carriers, lower levels of SHBG reached statistical significance for an association with PCa in univariate analysis, but this was not sustained in the genetic group comparison. Data on association of SHBG and PCa are scarce, even when considering sporadic PCa cases.[42] In addition to the well-known function of binding oestrogens and androgens of SHBG, different authors proposed an independent role for SHBG in the prostate, through interaction with a specific membrane receptor, leading to activation of AMPc and increase in PSA expression and apoptosis. How this pathway could interact with the DNA-repair mechanisms is yet to be explored.[43]

The IMPACT population represents an opportunity to address the hypothesis that androgens can lead to distinct disease behaviour in *BRCA1/2* PVs carriers. To date, it is the largest cohort recruited of *BRCA1/2* carriers, which are predisposed to aggressive disease at a younger age.[5] Identification of mechanisms of disease progression in this group could help to understand the biological mechanisms of sporadic tumours that share the same phenotype.[10] Secondly, due to the longitudinal design, multiple samples are collected and recorded prospectively, minimising influence of physiological variation.

The main limitation of our study is that androgen analysis is not an a priori defined hypothesis of the IMPACT study. Standardisation of sample collection and analysis can further improve future/follow-up studies. Additionally, the levels of circulating androgens may not reflect the intraprostatic hormonal milieu.[44] The small numbers of PCa cases could also be relevant. Finally, the inclusion of cases detected in the first screening round limit our ability to draw conclusions about the causal relationship between hormonal levels and PCa, with reverse causation being a possibility.

5 | CONCLUSION

Men with and without *BRCA1/2* PVs have similar baseline androgen levels. We found that pre-diagnostic TT, FT and SHBG are not relevant predictors of PCa risk and this was not affected by *BRCA1/2* status in this cohort. The mechanisms of disease could differ between these distinct genetic cohorts, and specific pathways are not fully understood. Our study suggests that circulating androgens are not associated with PCa risk in *BRCA1/2* PVs carriers and cannot explain the distinct biological behaviour of *BRCA*-related prostate tumours.

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AUTHOR CONTRIBUTIONS

Rosalind A. Eeles had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Eeles, Dias, Brook, Bancroft, Kote-Jarai, Mikropoulos, Page, and Saya. Acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; Administrative, technical, or material support: All authors. Statistical analysis: Alex, Brook, Page, Bancroft, and Kote-Jarai. Obtaining funding: Eeles and all IMPACT collaborating sites obtained their own funding for running the study at their site. Supervision: Eeles.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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