Prostate Cancer and Prostatic Diseases
https://doi.org/10.1038/s41391-020-0238-y

ARTICLE

Clinical Research

Association between metformin medication, genetic variation and prostate cancer risk

Min Joon Lee[1] · Viranda H. Jayalath[1] · Wei Xu[2] · Lin Lu[2] · Stephen J. Freedland[3,4] · Neil E. Fleshner[1] · Girish S. Kulkarni[1] · Antonio Finelli[1] · Theodorus H. van der Kwast[5] · Robert J. Hamilton[1]

Received: 4 January 2020 / Revised: 15 April 2020 / Accepted: 6 May 2020

Abstract

Background The relationship between metformin use and prostate cancer risk remains controversial. Genetic variation in metformin metabolism pathways appears to modify metformin glycemic control and the protective association with some cancers. However, no studies to date have examined this pharmacogenetic interaction and prostate cancer chemoprevention.

Methods Clinical data and germline DNA were collected from our prostate biopsy database between 1996 and 2014. In addition to a genome-wide association study (GWAS), 27 single nucleotide polymorphisms (SNPs) implicated in metformin metabolism were included on a custom SNP array. Associations between metformin use and risk of high-grade (Grade Group ≥ 2) and overall prostate cancer were explored using a case-control design. Interaction between the candidate/GWAS SNPs and the metformin-cancer association was explored using a case-only design.

Results Among 3481 men, 132 (4%) were taking metformin at diagnosis. Metformin users were older, more likely non-Caucasian, and had higher body mass index, Gleason score, and number of positive cores. Overall, 2061 (59%) were diagnosed with prostate cancer, of which 922 (45%) were high-grade. After adjusting for baseline characteristics, metformin use was associated with higher risk of high-grade prostate cancer (OR = 1.76, 95% CI 1.1–2.9, $p = 0.02$) and overall prostate cancer (OR = 1.77, 95% CI 1.1–2.9, $p = 0.03$). None of the 27 candidate SNPs in metformin metabolic pathways had significant interaction with the metformin-cancer association. Among the GWAS SNPs, one SNP (rs149137006) had genome-wide significant interaction with metformin for high-grade prostate cancer, and another, rs115071742, for overall prostate cancer. They were intronic and intergenic SNPs, respectively, with largely uncharacterized roles in prostate cancer chemoprevention.

Conclusions In our cohort, metformin use was associated with increased risk of being diagnosed with prostate cancer. While SNPs involved in metformin metabolism did not have modifying effects on the association with disease risk, one intronic and one intergenic SNP from the GWAS study did, and these require further study.

Introduction

Prostate cancer is the most common non-cutaneous malignancy in men, affecting one in nine men in their lifetime [1]. Once diagnosed, the psychological and physical tolls of this malignancy are significant [2–4]. Screening, diagnosis, and treatment for prostate cancer are means of secondary and

Supplementary information The online version of this article (https://doi.org/10.1038/s41391-020-0238-y) contains supplementary material, which is available to authorized users.

✉ Robert J. Hamilton
rob.hamilton@uhn.ca

[1] Division of Urology, Department of Surgery, Princess Margaret Cancer Centre, University Health Network, University of Toronto, Toronto, ON, Canada

[2] Department of Biostatistics, Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada

[3] Division of Urology, Department of Surgery, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

[4] Division of Urology, Veterans Affairs Medical Center, Durham, NC, USA

[5] Department of Pathology, Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada

Published online: 18 May 2020

tertiary prevention with goals of minimizing suffering of already existing prostate cancer. Developing and improving methods of primary prevention or chemoprevention offers a great opportunity for public health, preventing healthy people from developing the disease altogether.

There are currently no approved means of prostate cancer chemoprevention. Emerging evidence suggests metformin may have activity in preventing prostate cancer. Metformin (1,1-dimethylbiguanide hydrochloride) is the most commonly prescribed oral hypoglycaemic agent [5]. It inhibits hepatic gluconeogenesis and stimulates glucose uptake in muscle and adipose tissue, thereby lowering fasting blood glucose and circulating insulin. In vitro and xenograft studies suggest metformin inhibits growth of prostate cancer cell lines (e.g., DU145, PC3, and LNCaP), and both insulin-dependent and insulin-independent antineoplastic effects via activation of AMP-activated protein kinase (AMPK) have been proposed [6–9].

Compared with other medications, however, fewer studies have examined metformin in prostate cancer, and there is considerable heterogeneity in associations reported in epidemiological studies of metformin and prostate cancer risk [10–16].

Studies have revealed germline variations of genes (i.e., single nucleotide polymorphism, SNP) may help predict metformin non-response. Gene candidates (e.g., those involved in the pharmacokinetics of metformin) include SLC22A, MATE1/2, SRR, and ATM, which are implicated in metformin absorption and clearance as well as success of glucose-lowering treatment [17–24]. Genetic variations of such genes may potentially alter the metabolic response to metformin and may contribute to the heterogeneity of associations with prostate cancer [25]. To date, however, no study has explored metformin pharmacogenomics in prostate cancer chemoprevention.

In this study, our primary aim was to study the association between metformin use and risk of high-grade prostate cancer and overall prostate cancer. Our secondary aim was to determine whether this association is modified by germline genetic variation. We hypothesized that metformin use is associated with decreased risk of prostate cancer, and that the observed heterogeneity in the effect of metformin in prostate cancer risk could be, in part, due to genetic polymorphisms.

Materials and methods

General information

We conducted an unmatched retrospective case-control study nested in the prospectively maintained prostate biopsy database at the University Health Network (UHN) in Toronto, Ontario, Canada. Blood samples were collected with consent from 3481 men undergoing prostate biopsy at the Princess Margaret Cancer Centre between 1996 and 2014. This study was approved by the UHN Research Ethics Board (REB#13-6476).

Case-control selection

Patients were categorized as cases ($N = 2061$) if diagnosed with prostate cancer at UHN or from a prior outside diagnostic biopsy. Patients whose prostate biopsies were negative for malignancy were considered controls ($N = 1420$). Patients completed a questionnaire that included medication use. Further clinical, demographic, and pathological information were collected from electronic medical records. Information obtained from chart review included age, race, date of diagnosis, clinical stage, biopsy Gleason score, prostate-specific antigen (PSA) level at diagnosis, treatment modality, use of metformin and dose of metformin closest to diagnosis, and other medication use.

Definitions

The "diagnostic biopsy" referred to either the first positive biopsy or the most recent negative biopsy for patients who have never been diagnosed with cancer (controls). For purposes of analyses, metformin medication use was classified as "Users/Never-users." Use of metformin up to 3 months prior to diagnostic biopsy was required to be classified a "User." Patients whose medication use could not be verified prior to the diagnostic biopsy were excluded from analysis. Medication use after diagnostic biopsy was not considered.

Metformin chemoprevention and analysis

Associations between metformin use and baseline clinico-demographic parameters were analyzed using the Chi-square test or Fisher's exact test for categorical variables and Wilcoxon rank sum test for continuous variables. Associations between prostate cancer status and baseline clinico-demographic parameters were assessed in the same manner.

To assess the main endpoint of association with prostate cancer status, we conducted logistic regression analyses. Univariable and multivariable logistic regression models were used to examine associations between metformin use and risk of high-grade prostate cancer (Grade Group ≥ 2; $N = 922$), adjusting for age, race, family history, BMI, medication use (statins, 5-ARI, alpha blockers, and NSAID), digital rectal examination (DRE), PSA prior to diagnostic biopsy, PSA level, American Urological Association Symptom Score (AUASS), prostate volume based

on transrectal ultrasound (TRUS) at the time of biopsy, and TRUS findings (e.g., nodules and seminal vesicle invasion), and number of cores obtained. Stepwise model selection using backward elimination was performed to reduce the number of variables in the final model to prevent overfitting. The control group ($N = 2559$) for this analysis was defined as either low-grade cancer (Grade Group = 1) or no cancer.

We also examined the association between metformin use and presence of overall prostate cancer using similar multivariable models as above.

Metformin pharmacogenomics and analysis

Background

We examined gene–medication interaction using a case-only design, which capitalizes power on the assumption that genotype is independent of metformin exposure among control patients [26]. Thus, the test for association between genotype and exposure (i.e., metformin use) among cases was a test for interaction. Prostate cancer cases were genotyped on a custom Illumina SNP array, using primers ordered from Sequenom Laboratories (San Diego, CA).

Candidate SNPs and genotyping

In addition to a genome-wide association study (GWAS), candidate SNPs were selected by reviewing studies examining genetic interactions with metformin drug concentrations or side-effects and effects on glycemic control and weight changes. Twenty-seven SNPs with significant interactions ($p < 0.05$) in the literature were chosen (Supplementary Table 1). Selected SNPs were submitted for inclusion on a custom SNP array designed by Illumina specifically for the PRACTICAL (Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome) consortium [27, 28]. In some cases, the specific SNP nominated was not included on the final OncoArray due to neighboring SNPs in perfect linkage disequilibrium. In such cases, for clarity, we show the data as if associated with the nominated SNP.

To examine the interaction between genotypes and metformin use, two multivariable logistic regression models were performed, simple model (included metformin use, one SNP of interest and their interaction term and principal components to account for ethnicity) and complex model (simple model and clinical covariates listed in the chemoprevention section).

Interaction was tested using the Wald test. The genome-wide significance level was set at $p < 5 \times 10^{-8}$. Statistical analyses were conducted using R (v3.1.3) and Plink (v1.07). SNPs with statistically significant interaction effects were then further explored by examining the metformin-cancer association within each genotype of the SNP.

Results

Baseline characteristics

The baseline characteristics of the cohort, stratified by metformin use, are shown in Table 1. At diagnosis, 132 patients (4%) reported using metformin. Metformin users were older, more likely to be non-Caucasian and had higher BMI. At biopsy among men with cancer, metformin users had more positive cores and higher Gleason scores, but no difference in clinical stage. Metformin users were more likely to report concomitant use of statins and NSAIDs.

Primary outcomes

Risk of high-grade prostate cancer

In a crude model, metformin use prior to diagnostic biopsy was significantly associated with an increased risk of high-grade prostate cancer (OR = 2.56, 95% CI 1.8–3.6, $p < 0.001$). In multivariable analyses adjusting for clinical factors, this association was attenuated somewhat, but remained significant (OR = 1.76, 95% CI 1.1–2.9, $p = 0.02$) (Table 2). Alpha blocker and NSAID use, age, AUASS, TRUS volume, prior biopsy, PSA level, and abnormal DRE were associated with high-grade prostate cancer risk.

Risk of prostate cancer overall

In a crude model, metformin use prior to diagnostic biopsy was significantly associated with an increased risk of overall prostate cancer (OR = 2.12, 95% CI 1.4–3.2, $p < 0.001$). In multivariable analyses adjusting for clinical factors, this association was attenuated somewhat, but remained significant (OR = 1.77, 95% CI 1.1–2.9, $p = 0.03$) (Table 3). 5-ARI use, age, race, AUASS, TRUS volume, prior biopsy, PSA level, and abnormal DRE were associated with overall prostate cancer risk.

Sensitivity analysis

As a sensitivity analysis to understand the potential confounding of prediabetic or diabetic phenotype on the metformin-cancer association, we analyzed patients who were not on metformin prior to their diagnostic biopsy but initiated metformin after their biopsy (i.e., eventual users). In these men, a trend toward increased risk of prostate cancer was observed (OR = 1.4, 95% CI 0.9–2.1). As this

Table 1 Demographic and clinico-pathological features of the study population ($n = 3481$).

Covariate	No metformin ($n = 3349$)	Metformin ($n = 132$)	p value[a]
Prostate cancer, *n* (%)	1962 (59)	99 (75)	**<0.001**
Demographics/health information			
Median age at biopsy (IQR)	64 (11)	66 (8)	**<0.001**
Family history, *n* (%)	672 (21)	22 (18)	0.43
Race, *n* (%)			
Caucasian	2530 (78)	78 (60)	
Asian	299 (9)	23 (18)	**<0.001**
Other	416 (13)	30 (23)	
Missing	104	1	
BMI, *n* (%)			
<25	1046 (31)	28 (21)	
25–29.9	1590 (47)	49 (37)	**<0.001**
30–34.9	586 (17)	47 (36)	
≥35	127 (4)	8 (6)	
Clinico-pathological information			
Median AUASS (IQR)	7 (10)	8 (10)	0.25
Median TRUS volume (mL) (IQR)	42 (25)	42 (25)	0.39
Previous negative biopsy, *n* (%)	207 (6)	5 (4)	0.35
Median PSA (ng/mL) (IQR)	5.8 (4)	6 (4.4)	0.56
Abnormal DRE, *n* (%)	870 (26)	34 (26)	1
Gleason score			
≤6	2489 (74)	70 (53)	**<0.001**
7	138 (4)	8 (6)	
>7	722 (22)	54 (41)	
Positive cores			
0	1387 (41)	33 (25)	
1	638 (19)	25 (19)	
2	345 (10)	16 (12)	**<0.001**
3	245 (7)	9 (7)	
≥4	732 (22)	49 (37)	
Clinical stage (%)			
T1	1022 (75)	45 (74)	
T2	320 (23)	16 (26)	0.68
T3/T4	26 (2)	0 (0)	
Missing/not applicable	1981	71	
Concomitant medication use before diagnostic biopsy			
Statin, *n* (%)	1003 (30)	101 (77)	**<0.001**
5-ARI, *n* (%)	459 (14)	20 (15)	0.61
Alpha blocker, *n* (%)	482 (14)	21 (16)	0.61
NSAID, *n* (%)	1105 (33)	90 (68)	**<0.001**

$p < 0.05$ is considered statistically significant.

IQR interquartile range, *AUASS* American Urology Association symptom score, *TRUS* transrectal ultrasound, *PSA* prostate-specific antigen, *DRE* digital rectal exam, *5-ARI* 5α-reductase inhibitor, *NSAID* nonsteroidal anti-inflammatory drug.

[a]Fisher's exact tests and Kruskal Wallis tests were used for comparisons of categorical variables and continuous variables, respectively.

point-estimate was intermediate to the point-estimate of those actually taking metformin at diagnostic biopsy, this suggests there may be a component of the metformin-cancer association explained by a diabetic or prediabetic phenotype. A similar, but much weaker trend was observed when looking at risk of high-grade disease (Supplementary Table 2).

As a second sensitivity analysis, we investigated the influence of metformin dosage and observed that increased metformin dosage was not significantly associated with high-grade or overall prostate cancer (Supplementary Table 3).

Secondary outcome: germline genetic variation

In exploring the 27 candidate SNPs implicated in metformin metabolism, there was a trend for interaction among some SNPs (i.e., rs622342 and rs391300), but given the number of candidate SNPs tested, it was likely that the SNPs were associated by chance (Supplementary Table 1).

The GWAS SNPs were explored using the simple model as described in the "Materials and methods." Among the GWAS SNPs, one SNP (rs149137006) reached genome-wide significance in interaction with the metformin-high-grade prostate cancer association (OR = 3.92, 95% CI 2.4–6.4, $p = 4.66 \times 10^{-8}$, additive model, Fig. 1). None reached genome-wide significance in interaction with the metformin-overall-grade cancer association in the simple model.

We next explored the GWAS SNPs using the complex model adjusting for baseline clinicopathologic features and principal components, as described in Materials and Methods. No SNPs reached significance in interaction with the metformin-high-grade prostate cancer association in the complex model. However, one SNP (rs115071742) modified the association between metformin and overall prostate cancer (OR = 15.8, 95% CI 5.9–42.2, $p = 3.82 \times 10^{-8}$, additive model, Fig. 1).

Table 4 explores the interaction of these two SNPs on the metformin-cancer association further. For rs149137006, metformin users with the Aa/aa genotype were at significantly increased risk of high-grade prostate cancer and overall prostate cancer. For rs115071742, metformin users with the AA genotype were at significantly increased risk of high-grade prostate cancer and overall prostate cancer, while metformin users with the Aa/aa genotype were at increased risk of high-grade prostate cancer only.

Discussion

The association between metformin use and prostate cancer risk remains uncertain. While genetic variation appears to modify the metabolism of metformin, and its glycemic and adverse effects, no study has looked at genetic modifiers of

Table 2 Association between clinico-demographic factors and high-grade prostate cancer.

Variables	Comparisons	Adjusted[a] OR (95% CI)	*p* value	Global *p* value
Pharmacologic intervention before biopsy				
Metformin	Yes vs. No	1.76 (1.1,2.9)		**0.02**
Statin	Yes vs. No	1.05 (0.8,1.4)		0.71
5-ARI	Yes vs. No	0.89 (0.6,1.2)		0.49
Alpha blocker	Yes vs. No	0.63 (0.4,0.9)		**0.01**
NSAID	Yes vs. No	1.61 (1.3,2.0)		**<0.001**
PC1		0.98 (0.9,1.0)		0.57
PC2		0.99 (0.9,1.1)		0.82
PC3		1 (1.0,1.1)		0.79
Demographics/health information				
Age at biopsy	Continuous	1.07 (1.05,1.08)		**<0.001**
Family history	Yes vs. No	1.11 (0.9,1.4)		0.43
Race				
Caucasian	Reference	Reference		
Asian	Asian vs. Caucasian	0.62 (0.4,1.0)	**0.04**	0.07
Other	Other vs. Caucasian	1.12 (0.8,1.6)	0.52	
BMI				
<25	Reference	Reference		
25–29.9	25–29.9 vs. <25	1.12 (0.9,1.4)	0.37	0.55
30–34.9	30–34.9 vs. <25	1.24 (0.9,1.7)	0.2	
≥35	≥35 vs. <25	1.31 (0.7,2.3)	0.36	
Clinico-pathological information				
AUASS	Continuous	0.97 (0.96,0.99)		**0.001**
TRUS volume	Continuous	0.43 (0.2,0.8)		**0.004**
Prior biopsy	Yes vs. No	1.13 (1.1,1.2)		**<0.001**
PSA	Continuous	2.47 (2.0,3.1)		**<0.001**
Abnormal DRE	Yes vs. No	0.96 (0.96,0.97)		**<0.001**

$p < 0.05$ is considered statistically significant.

IQR interquartile range, *AUASS* American Urology Association symptom score, *TRUS* transrectal ultrasound, *PSA* prostate-specific antigen, *DRE* digital rectal exam, *5-ARI* 5α-reductase inhibitor, *NSAID* nonsteroidal anti-inflammatory drugs, *PC1–3* principal components.

[a]Adjusted for age, race, family history of prostate cancer, 5-ARI use, digital rectal examination findings, PSA prior to diagnostic biopsy, free-to-total PSA ratio, prostate volume based on transrectal ultrasound (TRUS) at the time of biopsy, TRUS findings, and number of cores obtained.

its impact on prostate chemoprevention. In this study, in contrast to our hypothesis, metformin did not have a chemopreventive association. In fact, we observed metformin was associated with increased risk of being diagnosed with prostate cancer. Two SNPs appeared to modify the metformin-cancer association and these SNPs were within the intergenic and intronic regions with largely uncharacterized roles.

Our results suggest that metformin may not be an effective chemopreventive agent. This is contrary to several previous in vitro and in vivo studies, which examined antineoplastic effects of metformin in prostate cancer tumorigenesis [6, 7, 29]. The postulated mechanisms of metformin involve both insulin-dependent and insulin-independent pathways. Insulin-dependent effects are believed to be mediated through activation of AMPK which lowers circulating insulin [30, 31]. Insulin has shown mitogenic and pro-survival effects and has been implicated in prostate cancer development [32, 33]. The insulin-independent effects of metformin are also mediated through activation of AMPK by inhibiting mammalian target of rapamycin (mTOR) and modulating the phosphatidylinositol-3-kinase/protein kinase B/Akt (PI3K-PKB/Akt) pathway [34]. This signaling pathway has been implicated in prostate cancer development and progression [35–37]. Currently there is no clear biological mechanism to support our result.

Previous clinical studies investigating metformin for chemoprevention of prostate cancer observed mixed results. Four retrospective studies observed a protective association between metformin use and risk [10–13]. One of the largest

Table 3 Association between clinico-demographic factors and prostate cancer overall.

Variables	Comparisons	Adjusted[a] OR (95% CI)	*p* value	Global *p* value
Pharmacologic intervention before biopsy				
Metformin	Yes vs. No	1.77 (1.1,2.9)		**0.03**
Statin	Yes vs. No	0.92 (0.7,1.2)		0.47
5-ARI	Yes vs. No	0.6 (0.5,0.8)		**<0.001**
Alpha blocker	Yes vs. No	1.18 (0.9,1.6)		0.24
NSAID	Yes vs. No	0.9 (0.7,1.1)		0.31
PC1		1.04 (1,1.1)		0.14
PC2		1.1 (1,1.2)		**0.001**
PC3		0.98 (0.9,1.0)		0.51
Demographics/health information				
Age at biopsy	Continuous	1.06 (1.05,1.08)		**<0.001**
Family history	Yes vs. No	1.24 (1,1.55)		0.06
Race				
Caucasian	Reference	Reference		
Asian	Asian vs. Caucasian	0.58 (0.4,0.8)	**0.002**	**0.01**
Other	Other vs. Caucasian	0.88 (0.7,1.2)	0.40	
BMI				
<25	Reference	Reference		
25–29.9	25–29.9 vs. <25	1.11 (0.9,1.4)	0.35	0.54
30–34.9	30–34.9 vs. <25	1.21 (0.9,1.6)	0.18	
≥35	≥35 vs. <25	1.25 (0.8,2.1)	0.39	
Clinico-pathological information				
AUASS	Continuous	0.98 (0.97,1)		**0.03**
TRUS volume	Continuous	0.98 (0.97,0.98)		**<0.001**
Prior biopsy	Yes vs. No	0.37 (0.3,0.6)		**<0.001**
PSA	Continuous	1.06 (1.04,1.09)		**<0.001**
Abnormal DRE	Yes vs. No	1.67 (1.3,2.1)		**<0.001**

$p < 0.05$ is considered statistically significant.

IQR interquartile range, *AUASS* American Urology Association symptom score, *TRUS* transrectal ultrasound, *PSA* prostate-specific antigen, *DRE* digital rectal exam, *5-ARI* 5α-reductase inhibitor, *NSAID* nonsteroidal anti-inflammatory drugs, *PC1–3* principle components.

[a]Adjusted for age, race, family history of prostate cancer, 5-ARI use, digital rectal examination findings, PSA prior to diagnostic biopsy, free-to-total PSA ratio, prostate volume based on transrectal ultrasound (TRUS) at the time of biopsy, TRUS findings, and number of cores obtained.

observational studies by Margel at al. found no association between metformin use and risk of any prostate cancer [14]. However, disproportionally older, and high-grade cancer distribution in their cohort limits generalizability of the study. A randomized controlled study by Feng et al. similarly found no association between metformin use and prostate cancer diagnosis [16]. On the other hand, Azoulay et al. found an association between increasing number of metformin prescriptions and prostate cancer risk [15].

Wu et al. performed a meta-analysis of case-control and cohort studies from January 1966 to February 2014, looking only in diabetic patients [38]. The pooled relative risk of prostate cancer among metformin users from ten studies was non-significant. However, when stratified by study design (case-control or cohort), a significantly reduced risk was observed in cohort studies, but not in case-control studies. Limitations of these observational studies may include time-related biases [39], in which misclassification of exposure or measurement of exposure over unequal time can result in skewed risk reductions.

We analyzed whether the observed adverse association between metformin use and prostate cancer risk was modified by variations in genes involved in metformin metabolism. Overall, the candidate SNPs we selected did not significantly modify the metformin-cancer association. When performing the GWAS SNP analysis, the only significant SNP when high-grade prostate cancer cases were analysed was rs149137006 that modified the metformin-cancer association. This was located in the intronic region of *HERPUD1* (Homocysteine Inducible ER Protein With

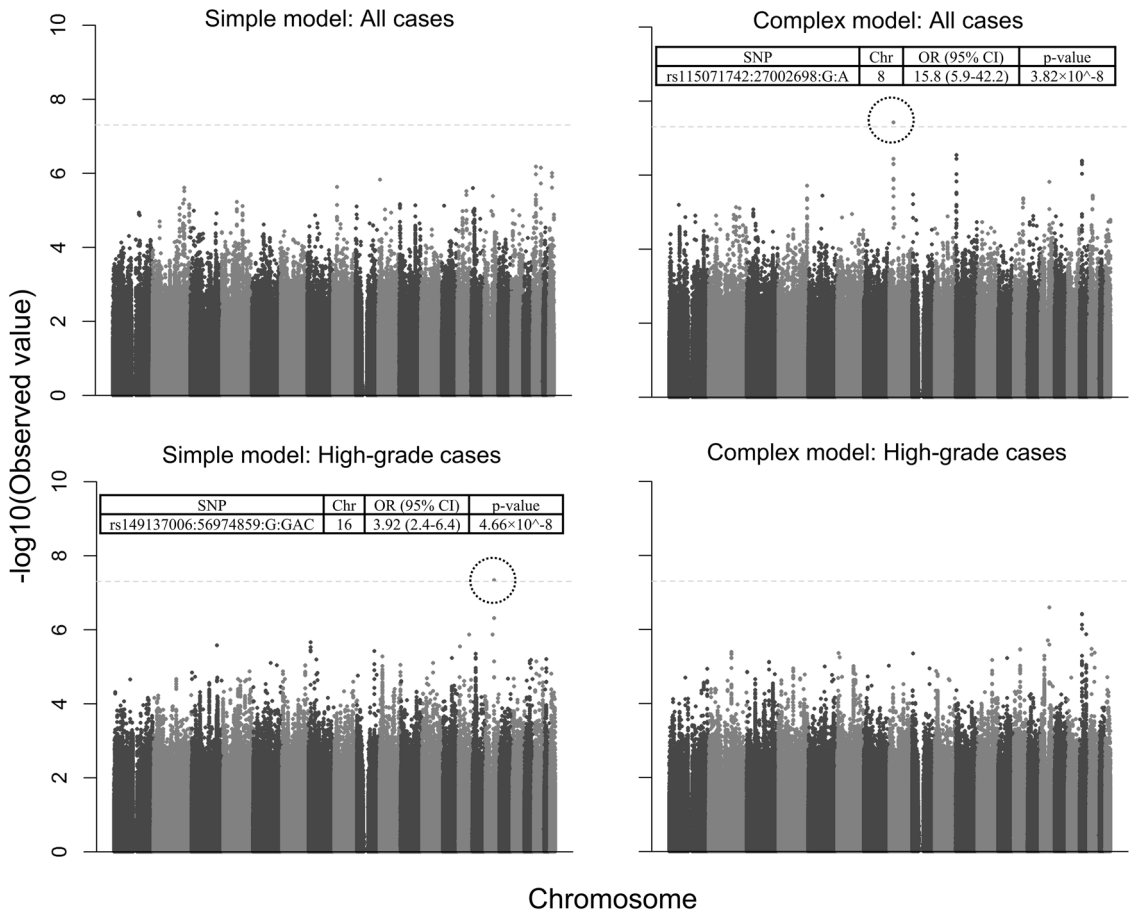

Fig. 1 Manhattan plot of the high-grade prostate cancer case only and all prostate cancer case-only analysis. Both simplified and complex models were used as described in the "Materials and methods." Genotypes were analyzed using a genetic additive model. For the simple model, interaction between metformin use and genotype was assessed by testing for association between genotype and exposure (i.e., metformin use) using a multivariable logistic regression including metformin use, one SNP of interest and their interaction term while adjusting for principal components. For the complex model, in addition to the variables in the simple model, included clinical variables and adjusted for age, race, family history, BMI, medication use (statins, 5-ARI, NSAID), AUASS, abnormal DRE, PSA level, TRUS volume and finding, number of cores obtained, prior biopsy, and principal components. For the genetic association, multiple comparisons were adjusted by using the genome-wide significant level with $p < 5 \times 10^{-8}$.

Table 4 Adjusted association between metformin use and high-grade prostate cancer and prostate cancer overall, stratified across genotype.

SNP: rs149137006	$N =$ 3020	Metformin OR for high-grade prostate cancer (95% CI), p value	Metformin OR for prostate cancer overall (95% CI), p value
AA	2330	1.61 (1,2.6), 0.06	1.41 (0.9,2.3), 0.2
Aa	654	6.64 (3.4,13.1), $\mathbf{3.98 \times 10^{-8}}$	4.23 (1.9,10.2), **0.001**
aa	36		

SNP: rs1150717423	$N =$ 049	Metformin OR for high-grade prostate cancer (95% CI), p value	Metformin OR[a] for prostate cancer overall (95% CI), p value
AA	2967	2.43 (1.6,3.6), $\mathbf{1.05 \times 10^{-5}}$	1.71 (1.1,2.6), **0.01**
Aa	82	4.18 (1.1,16.5), **0.04**	Not estimable
aa	0		

The ORs are from subgroup analysis. $p < 0.05$ is considered statistically significant.

SNP single nucleotide polymorphism, *N* number of alleles used in the model, *Not estimable* too few samples of metformin users in this group analysis.

[a]For example, estimated OR = 1.71 (1.1,2.6) is obtained from the logistic regression of metformin, users vs. non-users as reference, on prostate cancer as the outcome given genotype AA for rs115071742. N with genotypes Aa and aa were combined as there were not enough events observed in Aa and/or aa subgroup analysis.

Ubiquitin Like Domain 1), a gene in chromosome 16 that encodes an androgen-inducible protein involved in the endoplasmic reticulum stress response pathway. Although its role is largely unknown, reduced HERPUD1 expression has been implicated in primary prostate tumorigenesis [40]. The only significant SNP when all prostate cancer cases were analyzed was rs115071742, an intergenic SNP residing in chromosome 8. The nearby gene, *LOC105379340*, ~40 kb downstream from this SNP is a non-coding RNA with an uncharacterized role.

Although both SNPs are not exonic, it is possible they are transcribed into functional non-coding RNA molecules or *cis*- or *trans*-regulatory elements that may have biological implications yet discovered. For example, activities of long non-coding RNAs have recently been observed in tumor initiation and progression, as well as cancer therapy response; and such markers may predict therapeutic outcome in cancer patients, specifically through drug-metabolism or drug targeting pathways [41–49]. Function of the newly identified SNPs and the associated gene/nearby gene are largely unknown in the context of prostate cancer or metformin pharmacogenomics; this requires further investigation.

Several limitations of our work must be acknowledged. The lack of robust genetic interaction in our study may be multifactorial. Despite our large sample size, only a small proportion reported metformin use. However this observation was consistent with the study by Wright et al. [11]. Stratifying this use (4%) across SNP genotypes to find an interaction effect, however, leads to substantial decreases in power. Moreover, our cohort reported a heterogenous mixture of metformin duration and dose and thus it is possible any genetic signal may be diluted. On a similar note, data on multiple anti-diabetic agent or other concurrent medication use (e.g., Ondansetron/Cephalexin/Cimetidine/Trimethoprim) were not available, which could have contributed as confounders to our study. It is also possible that the genetic variation we studied did have a significant impact on metformin concentration and efficacy. This could have led the clinician to alter the dose to compensate, which would nullify the effect of the genetic modification. Finally, given that the combined reported genetic variation to date appears to only explain a small proportion of the variability in responses to metformin, it may be that the common genetic variation we studied is not sufficient to substantively alter the chemopreventive effects of metformin.

Another source of heterogeneity among this body of literature, our study included, is due to unmeasured confounding. Patients taking metformin clearly differ from those that do not. One such example is diabetes—other than medication use, we do not have a capture of date of diagnosis or severity of diabetes (e.g., HbA1c/glucose level), and our sensitivity analysis capturing the prediabetic phenotype (i.e. eventual users: men who started metformin after diagnostic biopsy) suggests there is at least a component of this confounding our results.

In addition to limitations in genetic analysis, it is possible that metformin use was misclassified if this was not captured at the time of biopsy. Thus, some users may have been recorded as non-users and this can bias the observed association. As mentioned previously, due to the retrospective nature of this study, despite our attempts to capture and adjust for covariates, there may be residual unmeasured confounding driving the positive association we observed between metformin and prostate cancer.

This study does have a number of important strengths. First, this case-control retrospective study of the prospectively maintained participants included a large sample size of those diagnosed with prostate cancer in one of the largest cancer centers in North America. Our study included stratification of patient population by disease severity (i.e., low grade vs. high grade) and it is one of the first studies looking at their relevance in the context of metformin pharmacogenomics in prostate cancer chemoprevention. We also were able to control for many potential confounders including race, BMI, and concurrent medication use.

As stated above, the uniqueness of this study is the methodology, examining the importance of genetic variation in chemoprevention and cancer pharmacology. There are several drugs that have been previously tested to be inconclusive or have yet been tested for prostate cancer chemoprevention (e.g., statin). Our method of studying genetic polymorphisms can be utilized to investigate such drugs' effect on prostate cancer chemoprevention and/or other cancer types.

Conclusions

In our cohort, metformin use was associated with an increased risk of being diagnosed with prostate cancer. While we observed two SNPs that may modify the metformin-cancer association, the mechanisms of this interaction remain unclear, and warrant further investigation. Our study found insufficient evidence to promote metformin as a personalized chemotherapeutic for the prevention of prostate cancer. Given our study and the heterogenous epidemiological literature about metformin published to date, a prospective randomized trial of metformin in primary prevention is not advised.

Acknowledgements RJH funding from CCSRI (Grant#702108) and PCC (Grant#D2013-17) and CUASF Career Development for Research Grant 2017-2018.

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. American Cancer Society. Key statistics for prostate cancer. Atlanta, Georgia, United: American Cancer Society; 2019.
2. Carlson LE, Angen M, Cullum J, Goodey E, Koopmans J, Lamont L, et al. High levels of untreated distress and fatigue in cancer patients. Br J Cancer. 2004;90:2297–304.
3. Balderson N, Towell T. The prevalence and predictors of psychological distress in men with prostate cancer who are seeking support. Br J Health Psychol. 2003;8(Pt 2):125–34.
4. Resnick MJ, Koyama T, Fan KH, Albertsen PC, Goodman M, Hamilton AS, et al. Long-term functional outcomes after treatment for localized prostate cancer. N. Engl J Med. 2013;368:436–45.
5. Saenz A, Fernandez-Esteban I, Mataix A, Ausejo M, Roque M, Moher D. Metformin monotherapy for type 2 diabetes mellitus. Cochrane Database Syst Rev. 2005;3:CD002966.
6. Ben Sahra I, Laurent K, Giuliano S, Larbret F, Ponzio G, Gounon P, et al. Targeting cancer cell metabolism: the combination of metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells. Cancer Res. 2010;70:2465–75.
7. Ben Sahra I, Laurent K, Loubat A, Giorgetti-Peraldi S, Colosetti P, Auberger P, et al. The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level. Oncogene. 2008;27:3576–86.
8. Tsutsumi Y, Nomiyama T, Kawanami T, Hamaguchi Y, Terawaki Y, Tanaka T, et al. Combined treatment with Exendin-4 and metformin attenuates prostate cancer growth. PLoS ONE. 2015;10:e0139709.
9. Zaidi S, Gandhi J, Joshi G, Smith NL, Khan SA. The anticancer potential of metformin on prostate cancer. Prostate Cancer Prostatic Dis. 2019;3:351–61.
10. Murtola TJ, Tammela TL, Lahtela J, Auvinen A. Antidiabetic medication and prostate cancer risk: a population-based case-control study. Am J Epidemiol. 2008;168:925–31.
11. Wright JL, Stanford JL. Metformin use and prostate cancer in Caucasian men: results from a population-based case-control study. Cancer Causes Control. 2009;20:1617–22.
12. Ruiter R, Visser LE, van Herk-Sukel MP, Coebergh JW, Haak HR, Geelhoed-Duijvestijn PH, et al. Lower risk of cancer in patients on metformin in comparison with those on sulfonylurea derivatives: results from a large population-based follow-up study. Diabetes Care. 2012;35:119–24.
13. Preston MA, Riis AH, Ehrenstein V, Breau RH, Batista JL, Olumi AF, et al. Metformin use and prostate cancer risk. Eur Urol. 2014;66:1012–20.
14. Margel D, Urbach D, Lipscombe LL, Bell CM, Kulkarni G, Austin PC, et al. Association between metformin use and risk of prostate cancer and its grade. J Natl Cancer Inst. 2013;105:1123–31.
15. Azoulay L, Dell'Aniello S, Gagnon B, Pollak M, Suissa S. Metformin and the incidence of prostate cancer in patients with type 2 diabetes. Cancer Epidemiol Biomark Prev. 2011;20:337–44.
16. Feng T, Sun X, Howard LE, Vidal AC, Gaines AR, Moreira DM, et al. Metformin use and risk of prostate cancer: results from the REDUCE study. Cancer Prev Res. 2015;8:1055–60.
17. Wang DS, Jonker JW, Kato Y, Kusuhara H, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. J Pharmacol Exp Ther. 2002;302:510–5.
18. Shu Y, Brown C, Castro RA, Shi RJ, Lin ET, Owen RP, et al. Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. Clin Pharm Ther. 2008;83:273–80.
19. Zhou K, Donnelly LA, Kimber CH, Donnan PT, Doney AS, Leese G, et al. Reduced-function SLC22A1 polymorphisms encoding organic cation transporter 1 and glycemic response to metformin: a GoDARTS study. Diabetes. 2009;58:1434–9.
20. Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH. Genetic variation in the multidrug and toxin extrusion 1 transporter protein influences the glucose-lowering effect of metformin in patients with diabetes: a preliminary study. Diabetes. 2009;58:745–9.
21. Stocker SL, Morrissey KM, Yee SW, Castro RA, Xu L, Dahlin A, et al. The effect of novel promoter variants in MATE1 and MATE2 on the pharmacokinetics and pharmacodynamics of metformin. Clin Pharmacol Ther. 2013;93:186–94.
22. Choi JH, Yee SW, Ramirez AH, Morrissey KM, Jang GH, Joski PJ, et al. A common 5'-UTR variant in MATE2-K is associated with poor response to metformin. Clin Pharmacol Ther. 2011;90:674–84.
23. Dong M, Gong ZC, Dai XP, Lei GH, Lu HB, Fan L, et al. Serine racemase rs391300 G/A polymorphism influences the therapeutic efficacy of metformin in Chinese patients with diabetes mellitus type 2. Clin Exp Pharmacol Physiol. 2011;38:824–9.
24. GoDarts, Group UDPS, Wellcome Trust Case Control Consortium, Zhou K, Bellenguez C, Spencer CC, et al. Common variants near ATM are associated with glycemic response to metformin in type 2 diabetes. Nat Genet. 2011;43:117–20.
25. Pacanowski MA, Hopley CW, Aquilante CL. Interindividual variability in oral antidiabetic drug disposition and response: the role of drug transporter polymorphisms. Expert Opin Drug Metab Toxicol. 2008;4:529–44.
26. Clarke GM, Morris AP. A comparison of sample size and power in case-only association studies of gene-environment interaction. Am J Epidemiol. 2010;171:498–505.
27. Kote-Jarai Z, Olama AA, Giles GG, Severi G, Schleutker J, Weischer M, et al. Seven prostate cancer susceptibility loci identified by a multi-stage genome-wide association study. Nat Genet. 2011;43:785–91.
28. Kote-Jarai Z, Easton DF, Stanford JL, Ostrander EA, Schleutker J, Ingles SA, et al. Multiple novel prostate cancer predisposition loci confirmed by an international study: the PRACTICAL Consortium. Cancer Epidemiol Biomark Prev. 2008;17:2052–61.
29. Akinyeke T, Matsumura S, Wang X, Wu Y, Schalfer ED, Saxena A, et al. Metformin targets c-MYC oncogene to prevent prostate cancer. Carcinogenesis. 2013;34:2823–32.
30. Dowling RJ, Goodwin PJ, Stambolic V. Understanding the benefit of metformin use in cancer treatment. BMC Med. 2011;9:33.
31. Cusi K, Consoli A, DeFronzo RA. Metabolic effects of metformin on glucose and lactate metabolism in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 1996;81:4059–67.
32. Frasca F, Pandini G, Sciacca L, Pezzino V, Squatrito S, Belfiore A, et al. The role of insulin receptors and IGF-I receptors in cancer and other diseases. Arch Physiol Biochem. 2008;114:23–37.
33. Lima GA, Correa LL, Gabrich R, Miranda LC, Gadelha MR. IGF-I, insulin and prostate cancer. Arq Bras Endocrinol Metab. 2009;53:969–75.
34. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell. 2003;115:577–90.
35. Markman B, Atzori F, Perez-Garcia J, Tabernero J, Baselga J. Status of PI3K inhibition and biomarker development in cancer therapeutics. Ann Oncol. 2010;21:683–91.

36. Sarker D, Reid AH, Yap TA, de Bono JS. Targeting the PI3K/AKT pathway for the treatment of prostate cancer. Clin Cancer Res. 2009;15:4799–805.
37. Colquhoun AJ, Venier NA, Vandersluis AD, Besla R, Sugar LM, Kiss A, et al. Metformin enhances the antiproliferative and apoptotic effect of bicalutamide in prostate cancer. Prostate Cancer Prostatic Dis. 2012;15:346–52.
38. Wu GF, Zhang XL, Luo ZG, Yan JJ, Pan SH, Ying XR, et al. Metformin therapy and prostate cancer risk: a meta-analysis of observational studies. Int J Clin Exp Med. 2015;8:13089–98.
39. Suissa S, Azoulay L. Metformin and cancer: mounting evidence against an association. Diabetes Care. 2014;37:1786–8.
40. Segawa T, Nau ME, Xu LL, Chilukuri RN, Makarem M, Zhang W, et al. Androgen-induced expression of endoplasmic reticulum (ER) stress response genes in prostate cancer cells. Oncogene. 2002;21:8749–58.
41. Schmitt AM, Chang HY. Long noncoding RNAs in cancer pathways. Cancer Cell. 2016;29:452–63.
42. Wang D, Ding L, Wang L, Zhao Y, Sun Z, Karnes RJ, et al. LncRNA MALAT1 enhances oncogenic activities of EZH2 in castration-resistant prostate cancer. Oncotarget. 2015;6:41045–55.
43. Marques Howarth M, Simpson D, Ngok SP, Nieves B, Chen R, Siprashvili Z, et al. Long noncoding RNA EWSAT1-mediated gene repression facilitates Ewing sarcoma oncogenesis. J Clin Invest. 2014;124:5275–90.
44. Agrelo R, Souabni A, Novatchkova M, Haslinger C, Leeb M, Komnenovic V, et al. SATB1 defines the developmental context for gene silencing by Xist in lymphoma and embryonic cells. Dev Cell. 2009;16:507–16.
45. Ling H, Spizzo R, Atlasi Y, Nicoloso M, Shimizu M, Redis RS, et al. CCAT2, a novel noncoding RNA mapping to 8q24, underlies metastatic progression and chromosomal instability in colon cancer. Genome Res. 2013;23:1446–61.
46. Wang Y, Wang Z, Xu J, Li J, Li S, Zhang M, et al. Systematic identification of non-coding pharmacogenomic landscape in cancer. Nat Commun. 2018;9:3192.
47. Parasramka M, Yan IK, Wang X, Nguyen P, Matsuda A, Maji S, et al. BAP1 dependent expression of long non-coding RNA NEAT-1 contributes to sensitivity to gemcitabine in cholangiocarcinoma. Mol Cancer. 2017;16:22.
48. Prensner JR, Chen W, Iyer MK, Cao Q, Ma T, Han S, et al. PCAT-1, a long noncoding RNA, regulates BRCA2 and controls homologous recombination in cancer. Cancer Res. 2014;74:1651–60.
49. Bester AC, Lee JD, Chavez A, Lee YR, Nachmani D, Vora S, et al. An integrated genome-wide CRISPRa approach to functionalize lncRNAs in drug resistance. Cell. 2018;173:649–64 e620.