

# Associations Between Genetically Predicted Plasma N-Glycans and Prostate Cancer Risk: Analysis of Over 140,000 European Descendants

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**Background:** Previous studies suggest a potential link between glycosylation and prostate cancer. To better characterize the relationship between the two, we performed a study to comprehensively evaluate the associations between genetically predicted blood plasma N-glycan levels and prostate cancer risk.

**Methods:** Using genetic variants associated with N-glycan levels as instruments, we evaluated the associations between levels of 138 plasma N-glycans and prostate cancer risk. We analyzed data of 79,194 cases and 61,112 controls of European ancestry included in the consortia of BPC3, CAPS, CRUK, PEGASUS, and PRACTICAL.

**Results:** We identified three N-glycans with genetically predicted levels in plasma to be associated with prostate cancer risk after Bonferroni correction. The estimated odds ratios (95% confidence intervals) were 1.29 (1.20–1.40), 0.80 (0.74–0.88), and 0.79 (0.72–0.87) for PGP18, PGP33, and PGP109, respectively, per every one standard deviation increase in genetically predicted levels of N-glycan. However, the instruments for these N-glycans only involved one to two variants. The proportions of variations that can be explained by the instruments range from 1.58% to 2.95% for these three N-glycans.

**Conclusion:** We observed associations between genetically predicted levels of three N-glycans PGP18, PGP33, and PGP109 and prostate cancer risk. Given the correlated nature of the N-glycans and that many N-glycans share genetic loci, pleiotropy is a major concern. Future work is warranted to better characterize the relationship between N-glycans and prostate cancer.

**Keywords:** plasma N-glycans, prostate cancer, risk

## Introduction

Prostate cancer is the second most commonly diagnosed cancer and the fifth leading cause of malignancy-related death among males worldwide.<sup>1</sup> It is projected that, in the United States, 191,930 new prostate cancer cases and 33,330 prostate cancer-related deaths will occur in 2020.<sup>2</sup> The survival rate tends to be much worse when cancer is diagnosed at a metastatic stage compared with localized stage.<sup>3</sup> The Prostate-specific antigen (PSA) blood test has been clinically used for prostate cancer screening.<sup>4</sup> However, using PSA screening is controversial due to the lack of a clear cutoff point for high sensitivity and specificity,<sup>5–7</sup> and unclear benefit in reducing mortality in specific populations.<sup>8–10</sup> Therefore, there are critical needs for better understanding the etiology and identifying effective biomarkers to improve the risk assessment of prostate cancer.

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A large proportion of proteins are modified by covalent addition of glycans. Glycosylation is a post-translational modification that is very important for normal physiological processes.<sup>11</sup> Multiple studies support potential important roles of glycans in cancer progression.<sup>12</sup> Alterations in glycosylation are shown to potentially change specific properties related to cancer, such as tumor growth, invasion and metastasis.<sup>13–18</sup> Different types of glycan alterations have been reported to be related to prostate cancer, such as changes to PSA glycosylation, sialylation and core fucosylation, O-GlcNacylation, cryptic and branched N-glycans, as well as galectins and proteoglycans.<sup>19</sup> As a major class of protein glycosylation besides O-glycans, N-glycans are known to influence many properties of glycoproteins, such as conformation, solubility, antigenicity, as well as recognition by glycan-binding proteins.<sup>20</sup> Basic research has suggested potential link between N-glycans and prostate tumorigenesis. For example,  $\beta$ -1,6-GlcNAc tri-branched and tetra-branched N-glycans were reported to be linked to prostate cancer metastasis in xenograft models.<sup>21</sup> In humans, changes in plasma and immunoglobulin G (IgG) N-glycosylation have been reported to be associated with colorectal cancer in a case-control study.<sup>22</sup> However, limited research has been performed to study the relationship between N-glycans and prostate cancer risk. Direct measurement of glycan levels in a large number of subjects is costly. Furthermore, conventional epidemiological studies could be influenced by several common limitations, such as selection bias, potential confounding, and reverse causation. Herein, to better characterize the relationship between plasma proteome N-glycosylation and prostate cancer, we used an approach of genetic instruments that mimic the design of randomized control trials (RCT).<sup>23–25</sup>

## Materials and Methods

### Genetic Instruments for N-Glycan Levels in Plasma

To determine genetic instruments for plasma levels of N-glycans, we conducted a systematic literature search up to April 30, 2020. We focused on genome-wide association studies (GWAS) for N-glycans attached to all plasma proteins or a single protein that is extensively studied, IgG. After careful assessment, we included two largest and most comprehensive studies, one for N-glycans from all proteins, and the other for IgG glycans.<sup>26,27</sup> For the assessed N-glycans, their quantification is relative in

that a glycan peak was defined as a proportion of glycans that fall into this specific (chromatographic) peak among all glycans. We determined the variant(s) associated with each glycan reported in these studies, and only kept single-nucleotide polymorphisms (SNPs) that were independent from each other for each glycan as genetic instruments. The N-glycome (for example, total plasma or IgG) is a result of the complex interplay between biochemical pathways of N-glycan biosynthesis.<sup>28</sup> The abundances of glycan species are determined by a partially overlapping set of N-glycan biosynthesis enzymes and availability of common substrates, leading to strong correlations between levels of many N-glycans. Therefore, if a locus harbors a particular gene, a product of which regulates the work of the N-glycan biosynthesis enzyme, this locus will likely show an effect on a wide spectrum of related glycan traits. This leads to the fact that one SNP can be a genetic instrument for a single or several glycan traits.

We selected genetic instruments for total plasma N-glycome traits from the results of a GWAS conducted by Sharapov et al, in 2763 TwinsUK samples.<sup>26</sup> Briefly, genotyping was conducted with combination Illumina SNP arrays (HumanHap300, HumanHap610Q, 1 M-Duo and 1.2MDuo 1 M). Standard quality control was applied with SNPs being filtered by sample call rate, minor allele frequency, SNP call rate as well as Hardy Weinberg Equilibrium. Variants were further imputed using IMPUTE2 software with 1000G Phase 1 version 3 as reference data. Plasma N-glycome quantification of samples were measured by ultra-performance liquid chromatography.<sup>29</sup> Normalization and batch correction were further performed on harmonized UPLC glycan data. Genome-wide Efficient Mixed Model Association algorithm (GEMMA) was further conducted to assess the kinship matrix and to run linear mixed model regression on variants assuming additive genetic effects. In this study, 14 loci were reported to be associated with levels of 68 different traits at the genome-wide significant level of  $P$ -value  $< 5 \times 10^{-8}/(29 + 1) = 1.66 \times 10^{-9}$ , where 29 is an effective number of tests (traits) that was estimated. Three other cohorts (QMDiab, SOCCS and PainOR) were further used to replicate the identified associations. We extracted genome-wide summary statistics for 113 studied total plasma N-glycome traits and more than 8 million SNPs from the Zenodo repository [<https://zenodo.org/record/1298406#X9-eQthKiUI>]. We selected the genetic instruments for each of 113 glycan traits separately. For each of the traits, we selected all SNPs that showed genome-wide

significant associations at the level of  $P$ -value  $< 1.66 \times 10^{-9}$ . (as reported in ref26). Selected SNPs were clumped by retaining only one SNP within a 250KB window. When there are more than one SNP located at the same chromosome for each glycan of interest, the correlations between these SNPs were estimated using the Pairwise LD function of SNIIPA ([http://snipa.helmholtz-muenchen.de/snipa/index.php?task=pairwise\\_ld](http://snipa.helmholtz-muenchen.de/snipa/index.php?task=pairwise_ld)). Only independent SNPs ( $R^2 < 0.1$  based on 1000 Genomes Project Phase 3 version 5 data for European descendants) were retained to create an instrument for each glycan. In total, we selected genetic instruments for 68 total plasma N-glycome traits. For most of the plasma N-glycan traits only one instrumental variable was available, therefore limiting the ability to distinguish between causal relationship and horizontal pleiotropy.

We selected genetic instruments for IgG N-glycome traits from the published results of the GWAS of 77 IgG N-glycome traits conducted by Klaric et al. In this study, four cohorts including CROATIA-Korcula, CROATIA-Vis, ORCADES (Orkney Complex Disease Study), and TwinsUK were leveraged, with a combined sample size of 8090.<sup>25</sup> The HapMap2 (release 22) imputed genetic data were analyzed for their associations with 77 UPLC IgG N-glycan traits.  $P \leq 2.4 \times 10^{-9}$  was used to determine genome-wide significant associations (Bonferroni corrected for 21 independent glycan traits).<sup>30</sup> In addition, replication analysis was performed by analyzing data of EGCUT, FINRISK, COGS, and SDRNT1BIO (N=2388). For 19 of 27 significant loci identified, the SNP-glycan associations were replicated at  $P \leq 1.9 \times 10^{-3}$  (0.05/27). Based on this study, we selected genetic instruments for 70 IgG N-glycome traits.

## Association Analysis Between Genetically Predicted N-Glycan Levels and Prostate Cancer Risk

The prostate cancer GWAS summary statistics data were generated from 79,194 prostate cancer cases and 61,112 controls of European ancestry included in the consortia of BPC3, CAPS, CRUK, PEGASUS, and PRACTICAL.<sup>31–33</sup> Using the OncoArray platform, 46,939 prostate cancer cases and 27,910 controls were genotyped comprising 570,000 SNPs (<http://epi.grants.cancer.gov/oncoarray/>). The remaining included participants were from several GWAS, namely, BPC3, CaPS 1 and CaPS 2, iCOGS, NCI PEGASUS, and UK stage 1 and stage 2. More

detailed information for characteristics of the cases and controls, including classifications for aggressiveness in cases has been described elsewhere.<sup>32</sup> For example, the mean age ranged from 56 to 72 across the participating studies for cases, and ranged from 51 to 71 for controls. These genotype data were imputed using the 1000 Genomes Project data released in June 2014 as a reference. Summary statistics from logistic regression analyses were meta-analyzed using an inverse variance fixed effect approach further. Principal components and study-relevant covariates were controlled for in the OncoArray and each GWAS. Furthermore, country was stratified in the OncoArray, and study was stratified in the iCOGS analyses. It is worth noting that a key requirement of such type of analysis is that the dataset for estimating the SNP-exposure (in our case, SNP-glycan) associations do not have overlapped subjects with the dataset for estimating the SNP-outcome (in our case, SNP-prostate cancer) associations.<sup>34</sup> This is for considering that to use datasets with overlapped samples for deriving the SNP-exposure and SNP-outcome relationships may be prone to weak instrument bias towards the exposure-outcome estimate.<sup>34</sup>

The inverse variance weighted method (IVW), included in the MR-Base platform,<sup>35</sup> was used to assess the associations of genetically predicted N-glycan levels with prostate cancer risk.<sup>26,36,37</sup> In IVW method,  $\sum_i \beta_i, GX^* \beta_i, GY^* \sigma^2_i, GY / (\sum_i \beta^2_i, GX^* \sigma^2_i, GY)$  and  $1 / (\sum_i \beta^2_i, GX^* \sigma^2_i, GY)^{0.5}$  were used to estimate the beta coefficient of the association between genetically predicted N-glycan levels and prostate cancer risk, and the corresponding standard error, respectively.  $\beta_{i,GX}$  here represents the beta coefficient of the association between  $i$  th SNP and the glycan of interest reported in the literature;  $\beta_{i,GY}$  represents the beta coefficient of the association between  $i$  th SNP and prostate cancer risk in the prostate cancer GWAS; and  $\sigma_{i,GY}$  represents the standard error of the association of SNP-prostate cancer. The association odds ratio (OR), confidence interval (CI), and  $P$  value were estimated based on the calculated beta coefficient and standard error. A Bonferroni corrected threshold was used to determine significant associations. Given 80% power at the alpha of 0.05, the minimal detectable ORs per standard deviation of genetically predicted N-glycan levels ranged from 0.81 to 0.96 (from 1.04 to 1.23) based on power calculation using <https://shiny.cnsgeonomics.com/mRnd/>.<sup>38</sup>

## Results

We evaluated the associations between 138 N-glycan traits (of which 68 reflect total plasma N-glycome features, and 70 reflect IgG N-glycome) and prostate cancer risk. Detailed information of the genetic instruments used was shown in [Supplementary Table 1](#). Aggregating independently associated variants together, 0.57% to 20.32% of the variation of N-glycan traits can be explained by the corresponding instrument variants ([Supplementary Table 1](#)). The largest proportion of variation was explained for IGP29 (20.32%). The smallest one was for IGP27 (0.57%) with rs7621161 as the instrument.

The results for the associations between genetically predicted N-glycan levels and prostate cancer risk are shown in [Table 1](#). Among all N-glycans tested, we identified three N-glycans with genetically predicted levels to be associated with prostate cancer risk at  $P \leq 3.62 \times 10^{-4}$ , a Bonferroni-corrected significance level (0.05/138). Higher predicted levels of PGP18 (OR=1.29; 95% CI, 1.20–1.40;  $P = 8.32 \times 10^{-9}$ ) while lower predicted levels of PGP33 (OR=0.80; 95% CI, 0.74–0.88;  $P = 1.33 \times 10^{-6}$ ) and PGP109 (OR=0.79; 95% CI, 0.72–0.87;  $P = 5.49 \times 10^{-7}$ ) were associated with an increased risk of prostate cancer ([Table 1](#)). The instruments for these three N-glycans are rs3115663 for PGP18, rs1866767 for PGP33, and rs11223780 and rs140053014 for PGP109 ([Supplementary Table 1](#)).

## Discussion

Leveraging a large GWAS dataset for prostate cancer risk, we evaluated the relationship between genetically predicted plasma N-glycan levels and prostate cancer risk. This study, to our knowledge, is the first study to characterize potential roles of N-glycans in risk of prostate cancer using genetic instruments. We observed that lower predicted levels of PGP18 (The percentage of Man9 N-glycan in total plasma glycans) as well as higher predicted levels of PGP33 (the percentage of A4G4S[3,3,3,3]4 in total plasma glycans) and PGP109 (the ratio of disialylated and trisialylated trigalactosylated structures in total plasma glycans) were associated with lower risk of prostate cancer. Man9-mannosidase is a high mannose glycan, which is an alpha 1,2 specific enzyme located in the endoplasmic reticulum, and plays a key role in the processing of N-linked oligosaccharides.<sup>39</sup> The A4G4S [3,3,3,3]4 is a highly branched galactosylated sialylated glycan (attached to alpha1-acid protein - an acute-phase

**Table 1** Associations Between Genetically Predicted Plasma N-Glycan Levels and Prostate Cancer Risk

Trait	OR (95% CI) <sup>a</sup>	P value <sup>b</sup>
PGP18	1.29 (1.20–1.40)	$6.40 \times 10^{-11}$
PGP109	0.79 (0.72–0.87)	$5.49 \times 10^{-7}$
PGP33	0.80 (0.74–0.88)	$1.33 \times 10^{-6}$
PGP2	0.92 (0.87–0.98)	$5.47 \times 10^{-3}$
PGP3	0.95 (0.88–1.02)	0.18
PGP6	0.95 (0.88–1.03)	0.23
PGP13	1.03 (0.98–1.08)	0.29
PGP17	1.02 (0.97–1.07)	0.48
PGP20	1.01 (0.95–1.08)	0.68
PGP22	0.90 (0.74–1.1)	0.32
PGP23	0.97 (0.91–1.02)	0.26
PGP24	1.01 (0.97–1.05)	0.55
PGP25	1.00 (0.94–1.06)	0.95
PGP27	0.98 (0.94–1.04)	0.55
PGP28	0.97 (0.89–1.05)	0.43
PGP30	1.01 (0.97–1.04)	0.69
PGP32	1.00 (0.96–1.05)	1.00
PGP34	1.00 (0.95–1.06)	0.96
PGP36	1.03 (0.94–1.13)	0.55
PGP37	0.98 (0.91–1.07)	0.71
PGP38	1.05 (0.97–1.15)	0.23
PGP39	0.97 (0.89–1.05)	0.44
PGP40	1.05 (0.97–1.15)	0.23
PGP41	1.03 (0.98–1.09)	0.28
PGP42	1.01 (0.9–1.14)	0.82
PGP43	0.98 (0.92–1.04)	0.55
PGP45	1.07 (0.96–1.2)	0.23
PGP46	0.89 (0.74–1.07)	0.23
PGP47	1.02 (0.96–1.08)	0.54
PGP50	1.04 (0.95–1.14)	0.43
PGP51	1.03 (0.95–1.12)	0.43
PGP52	1.03 (0.96–1.11)	0.43
PGP56	1.02 (0.98–1.06)	0.37
	1.02 (0.98–1.05)	0.43
	1.02 (0.97–1.07)	0.52
	1.09 (0.82–1.46)	0.67
PGP57	1.01 (0.97–1.06)	0.57
PGP59	0.94 (0.87–1.01)	0.09
PGP60	1.00 (0.93–1.08)	0.97
PGP61	1.06 (0.97–1.16)	0.23
PGP62	1.01 (0.93–1.1)	0.80
PGP63	0.95 (0.88–1.03)	0.23
PGP67	1.05 (0.99–1.11)	0.08
PGP70	0.93 (0.85–1.01)	0.09
PGP72	1.06 (0.99–1.14)	0.09
PGP74	0.99 (0.93–1.05)	0.74
PGP78	1.04 (0.97–1.12)	0.23
PGP79	1.08 (0.99–1.18)	0.09
PGP80	0.95 (0.88–1.03)	0.23
PGP82	0.96 (0.89–1.03)	0.23

(Continued)

Table I (Continued).

Trait	OR (95% CI) <sup>a</sup>	P value <sup>b</sup>
PGP83	0.95 (0.87–1.03)	0.23
PGP84	0.95 (0.86–1.04)	0.23
PGP85	0.95 (0.87–1.03)	0.23
PGP86	1.05 (0.97–1.15)	0.23
PGP87	1.00 (0.92–1.09)	0.97
PGP88	0.95 (0.87–1.03)	0.23
PGP89	0.95 (0.87–1.03)	0.23
PGP90	1.05 (0.97–1.14)	0.23
PGP92	0.96 (0.88–1.04)	0.34
PGP99	0.94 (0.84–1.05)	0.30
PGP102	0.97 (0.91–1.04)	0.43
PGP103	1.02 (0.96–1.09)	0.55
PGP105	0.98 (0.92–1.04)	0.55
PGP106	1.02 (0.96–1.08)	0.55
PGP107	0.95 (0.88–1.03)	0.23
PGP110	0.93 (0.86–1.02)	0.12
PGP112	1.00 (0.95–1.05)	0.99
IGP2	0.98 (0.93–1.03)	0.43
IGP3	0.98 (0.87–1.11)	0.75
IGP5	1.03 (0.96–1.11)	0.40
IGP6	0.97 (0.87–1.1)	0.66
IGP7	1.04 (0.94–1.16)	0.45
IGP8	1.01 (0.94–1.08)	0.88
IGP9	1.01 (0.96–1.06)	0.66
IGP10	1.01 (0.94–1.09)	0.78
IGP11	0.97 (0.91–1.03)	0.36
IGP12	0.99 (0.92–1.07)	0.84
IGP13	0.97 (0.91–1.03)	0.28
IGP14	0.96 (0.91–1.02)	0.17
IGP15	1.04 (0.96–1.13)	0.33
IGP17	1.04 (0.99–1.1)	0.14
IGP18	0.97 (0.87–1.07)	0.55
IGP19	0.98 (0.85–1.13)	0.78
IGP21	0.96 (0.87–1.07)	0.49
IGP22	1.03 (0.97–1.09)	0.32
IGP23	1.05 (0.98–1.13)	0.18
IGP24	1.01 (0.96–1.06)	0.72
IGP25	1.10 (0.96–1.26)	0.18
IGP26	1.02 (0.97–1.08)	0.41
IGP27	1.11 (0.95–1.28)	0.18
IGP28	1.05 (0.94–1.16)	0.40
IGP29	1.01 (0.97–1.05)	0.70
IGP30	1.02 (0.96–1.09)	0.51
IGP31	1.00 (0.93–1.07)	0.93
IGP32	1.03 (0.99–1.07)	0.19
IGP34	1.00 (0.93–1.08)	0.96
IGP35	0.97 (0.94–1.01)	0.18
IGP36	1.00 (0.8–1.24)	0.98
IGP37	0.98 (0.86–1.12)	0.77
IGP38	0.98 (0.86–1.11)	0.76

(Continued)

Table I (Continued).

Trait	OR (95% CI) <sup>a</sup>	P value <sup>b</sup>
IGP39	1.01 (0.97–1.05)	0.66
IGP40	1.00 (0.96–1.04)	0.93
IGP42	0.98 (0.93–1.03)	0.41
IGP43	0.98 (0.86–1.11)	0.75
IGP45	1.03 (0.96–1.1)	0.42
IGP46	0.97 (0.87–1.1)	0.66
IGP47	1.01 (0.93–1.11)	0.76
IGP48	1.08 (0.9–1.28)	0.41
IGP49	1.01 (0.96–1.06)	0.66
IGP50	1.01 (0.94–1.09)	0.77
IGP51	0.97 (0.9–1.04)	0.40
IGP52	1.01 (0.94–1.08)	0.84
IGP53	0.97 (0.91–1.04)	0.38
IGP54	0.97 (0.89–1.05)	0.41
	1.00 (0.92–1.09)	0.98
IGP55	0.96 (0.84–1.09)	0.50
IGP57	0.97 (0.9–1.04)	0.34
IGP58	1.03 (0.98–1.08)	0.31
IGP59	1.02 (0.98–1.07)	0.32
IGP60	1.03 (0.98–1.08)	0.31
IGP61	1.03 (0.98–1.08)	0.27
IGP62	0.98 (0.93–1.04)	0.60
IGP63	1.00 (0.95–1.06)	0.98
IGP64	0.99 (0.94–1.05)	0.76
IGP65	1.01 (0.95–1.06)	0.79
IGP66	1.01 (0.96–1.07)	0.60
IGP67	1.00 (0.95–1.05)	0.92
IGP68	1.01 (0.96–1.06)	0.76
IGP69	1.00 (0.95–1.05)	0.86
IGP70	1.01 (0.96–1.07)	0.60
IGP71	1.01 (0.96–1.07)	0.60
IGP72	0.98 (0.94–1.03)	0.52
IGP73	1.01 (0.94–1.08)	0.85
IGP74	1.00 (0.95–1.04)	0.88
IGP75	1.00 (0.95–1.04)	0.89
IGP76	1.00 (0.95–1.05)	0.98
IGP77	0.99 (0.92–1.06)	0.72

**Notes:** <sup>a</sup>OR represents odds ratio per one standard deviation increase in genetically predicted levels of N-glycan; CI represents confidence interval of the odds ratio; <sup>b</sup>P value: Derived from association analyses of 79,194 cases and 61,112 controls.

protein,<sup>40</sup> known to be associated with chronic low-grade inflammation.<sup>41,42</sup> The observed inverse association of A4G4S[3,3,3,3]4 with prostate cancer risk is not entirely consistent with known knowledge of A4G4S[3,3,3,3]4 with higher level of low-grade inflammation and future work is needed to better understand the relationship between these two. Our work provides new information to understand the relationship between N-glycans and

prostate cancer. Due to the potential pleiotropy bias, additional work is needed to better understand whether changes in N-glycosylations could influence the risk of prostate cancer.

Recently, studies suggested that changes to N-glycans such as the branching of complex biantennary glycans to triantennary and tetraantennary structures and the emergence of cryptic N-glycans could be related to prostate cancer.<sup>12,43–46</sup> Kawahara et al found that a panel of 56 intact N-glycopeptides could discriminate prostate cancer from benign prostatic hyperplasia.<sup>43</sup> Several other studies observed that changes to branched N-glycans could also help distinguish prostate cancer from benign prostatic hyperplasia.<sup>44,45</sup> Moreover, it was identified that tetraantennary and tetrasialylated N-glycans could potentially serve as biomarkers to improve the stratification of patients with indolent and aggressive prostate cancer.<sup>46</sup> Matsumoto et al identified that castration-resistant prostate cancer (CRPC) patients had significantly higher levels of terminal  $\alpha$ -2.3 sialylated glycan,  $\alpha$ -2.6 sialylated glycan, and terminal galactose while lower levels of branched-LacNAc structure compared with non-CRPC patients.<sup>47</sup> Ishibashi et al found that tri- and tetra-antennary N-glycans were significantly higher in CRPC patients than other groups including healthy volunteers, subjects with benign prostatic hyperplasia, early-stage prostate cancer patients, as well as prostate cancer patients with androgen deprivation therapy.<sup>48</sup> Also, the expression of N-glycan branching enzyme genes (*MGAT1*, *MGAT2*, *MGAT4B*, *MGAT5A*, and *MGAT5B*) were significantly higher in the CRPC-like cell lines DU145 and PC-3 compared with androgen-dependent LNCaP cells and normal prostate epithelial RWPE-1 cells. In addition, IgG antibodies targeting the Man9- or Tri-/m-II-autoantigens were detectable and these antibody activities were selectively increased in prostate cancer patients compared with benign prostatic hyperplasia patients.<sup>49</sup> Ishikawa et al developed an automated system for measuring cancer-associated  $\alpha$ -2,3-linked sialyl N-Glycan-carrying prostate-specific antigen (S2, 3PSA). The area under the curve (AUC) for the S2,3PSA ratio was 0.83 which was much higher than conventional PSA testing (AUC=0.51), in their analyses differentiating 50 prostate cancer patients from 50 benign prostatic hyperplasia patients.<sup>50</sup> Llop et al conducted a study to assess the core fucosylation and the sialic acid linkage of PSA N-glycans in serum samples from 29 benign prostatic hyperplasia patients and 44 prostate cancer patients with different degrees of aggressiveness. They

found significant increased levels of the  $\alpha$ -2,3-sialic acid and decreased levels of the core fucose percentage of PSA N-glycans in aggressive prostate cancer compared with benign prostatic hyperplasia and nonaggressive prostate cancer.<sup>51</sup> As the precursors, cores, and internal sequences of N-glycans, cryptic N-glycans are reported to be closely related to development of prostate cancer.<sup>21,49,52,53</sup> Wang et al reported the detection of cryptic N-glycan Man9 in the serum of prostate cancer patients, and its antibodies could help differentiate high-grade prostate cancer.<sup>54</sup> It would be interesting to investigate whether specific N-glycans could provide useful information for prognosis of advanced prostate cancer patients such as CRPC according to therapy.<sup>55–57</sup> Beyond the scope of glycans, research has supported potential roles of additional markers in risk prediction of prostate cancer. For example, the prostate health index (PHI) was reported as a better predictor of positive first biopsy in men with total PSA “gray” levels of 2–10 ng/mL compared with %fPSA (free to total PSA ratio  $\times 100$ ).<sup>58</sup> Future work assessing whether a combination of N-glycans and PHI can further improve the prediction may be warranted.

The proportions of variance of N-glycans that can be explained by the summed GWAS-identified genetic variants are relatively high for many of the assessed N-glycans, suggesting that many of the instrumental variables used in this study are relatively strong. The main association analysis of our study leveraging a large number of prostate cancer cases and controls provided high statistical power to detect the glycan-prostate cancer associations. The design of using genetic instruments could reduce selection bias, potential confounding, and reverse causation bias that are commonly imbedded in conventional observational studies. On the other hand, there are potential limitations of our study. Given the correlated nature of the N-glycans themselves and that many N-glycans share genetic loci, pleiotropy will be a major concern. While observed association of a variant with two traits could be because of pleiotropy of a single functional variant, it could as well be because of linkage disequilibrium between two distinct functional variants. Thus, our findings should be taken with caution and further studies are needed to validate them. Second, our analysis may be constrained by the variants identified in previous GWAS of plasma levels of N-glycans. In the study of Sharapov et al, the discovery analysis was based on TwinsUK dataset, which consisted of 89% females and 11% males. On the other hand, prior to GWAS, the glycan traits were adjusted

or age and sex. Furthermore, as described in the original paper, for a majority of the GWAS identified associations, they were further replicated in three additional datasets (PainOR, QMDiab, and SOCCS), which contain relatively higher proportions of males (45%, 50%, and 54%, respectively). It is expected that if we only focus on datasets of males in these studies for determining glycan associated variants, the statistical power would be relatively low. Considering that the replication analysis using data involving much higher proportions of males largely support the variant-glycan associations identified in the discovery analysis, we think that the current strategy using instruments based on analyses of combined sex may be more appropriate. In the Klaric et al, the discovery analyses were performed in four cohorts, CROATIA-Korcula (38% males), CROATIA-Vis (46% males), ORCADES (41% males), and TwinsUK (all females). The glycan traits were corrected for age, sex, cohort-specific covariates and cryptic relatedness before analyses. It is expected that additional N-glycan biomarkers could be identified using newly identified genetic variants (preferably in studies of males only with sufficient sample sizes) in the future. Furthermore, future work using comprehensive genetic prediction models of N-glycans aggregating effects of multiple variants could further improve the statistical power with increased variance of N-glycan levels that could be explained by genetic instruments. Third, the present work primarily focuses on studying prostate cancer risk comparing cases versus controls. Future work considering clinical and pathological features of prostate cancer (such as stage and Gleason score) would be needed to identify glycans that are potentially related to prostate cancer aggressiveness. It would also be useful to incorporate family history information into analyses to differentiate glycans playing a different role in familiar versus sporadic prostate cancer. Further studies are also needed to verify our findings with directly measured levels of these glycans. Functional investigations are also warranted to understand the biological roles of the identified N-glycans in prostate tumorigenesis.

## Conclusions

In this large-scale study, we identified three N-glycans with genetically predicted levels in plasma to be associated with prostate cancer risk. However, potential biases could have influenced these observed associations. Future work is needed to better characterize the relationship between N-glycans and prostate cancer.

## Summary

Prostate cancer is the second most commonly diagnosed cancer among males worldwide. Prostate-specific antigen screening is controversial due to the lack of a clear cutoff point for high sensitivity and specificity, and unclear benefit in reducing mortality in specific populations. Therefore, there are critical needs for better understanding the etiology and identifying effective biomarkers to improve the risk assessment of prostate cancer. The purpose of this study is to better characterize the relationship between glycosylation and prostate cancer, using genetic instruments. In this work, we identified several N-glycan biomarker candidates for prostate cancer risk. It not only provides new data regarding novel candidate risk biomarkers for prostate cancer but also demonstrates the utility of integrating genomics and glycomics data in biomarker research.

## Data Availability

For the prostate cancer GWAS data in the PRACTICAL consortium, the OncoArray genotype data and relevant covariate information (ie, ethnicity, country, principal components, etc.) are available in dbGAP (Accession #: phs001391.v1.p1). In total, 47 of the 52 OncoArray studies, encompassing nearly 90% of the individual samples, are available. The previous meta-analysis summary results and genotype data are currently available in dbGAP (Accession #: phs001081.v1.p1). The overall meta summary association statistics as described in Schumacher et al 2018 are available at [http://practical.icr.ac.uk/blog/?page\\_id=8164](http://practical.icr.ac.uk/blog/?page_id=8164).

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## Disclosure

The authors declare no potential conflicts of interest.

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