Original Article

Heritable methylation marks associated with breast and prostate cancer risk[1]

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ABSTRACT

Background

DNA methylation can mimic the effects of germline mutations in cancer predisposition genes. Recently, we identified twenty-four heritable methylation marks associated with breast cancer risk. As breast and prostate cancer share genetic risk factors, including rare, high-risk mutations (e.g., in *BRCA2*), we hypothesized that some of these heritable methylation marks might also be associated with the risk of prostate cancer.

Methods

We studied 869 incident prostate cancers (430 aggressive and 439 non-aggressive) and 869 matched controls nested within a prospective cohort study. DNA methylation was measured in pre-diagnostic blood samples using the Illumina Infinium HM450K BeadChip. Conditional logistic regression models, adjusted for prostate cancer risk factors and blood cell composition, were used to estimate odds ratios and 95% confidence intervals for the association between the 24 methylation marks and the risk of prostate cancer.

Results

Five methylation marks within the *VTRNA2-1* promoter region (cg06536614, cg00124993, cg26328633, cg25340688 and cg26896946), and one in the body of *CLGN* (cg22901919) were associated with the risk of prostate cancer. In stratified analyses, the five *VTRNA2-1* marks were associated with the risk of aggressive prostate cancer.

Conclusions

This work highlights a potentially important new area of investigation for prostate cancer susceptibility and adds to our knowledge about shared risk factors for breast and prostate cancer.

Keywords: Prostate cancer, aggressive prostate cancer, DNA methylation, *VTRNA2-1*, *CLGN*.

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INTRODUCTION

A large fraction of the familial risk for prostate cancer remains unexplained (1). International efforts to identify genetic risk factors for prostate cancer have identified a limited number of rare mutations in prostate cancer predisposition genes, such as *BRCA2* and *HOXB13*, which

are associated with a moderately increased risk of prostate cancer. Together these rare mutations explain only a fraction of the overall heritability (2,3). An increasing number of common genetic variants, each individually associated with very small increases in prostate cancer risk, are being identified via genome-wide association studies (GWASs). Taken together, these variants are estimated to explain a further 33% of the familial risk for prostate cancer (4). Although susceptibility variants identified by further expansion of GWASs are likely to explain an additional proportion of the familial risk, research exploring other factors contributing to familial risk is urgently needed.

One area of research that could contribute new information is epigenetics, particularly DNA methylation, thanks to the development of molecular platforms that can be applied to the resources of large epidemiological studies (5). Of specific relevance to studies of methylation in the context of familial prostate cancer risk is the observation of heritable changes in DNA methylation, that may be functionally equivalent to the known deleterious germline mutations in cancer predisposition genes. This phenomenon has been observed for *MLH1* and *MSH2* in the context of Lynch syndrome, a hereditary condition in which genetic mutations in the key mismatch repair genes predispose individuals to colorectal, endometrial and other cancers (6). *MLH1* and *MSH2* have been demonstrated to be transcriptionally silenced through constitutional promoter methylation (7-9). Unlike common tissue-specific epigenetic marks, these constitutional methylation marks are soma-wide, possibly having escaped erasure during early embryogenesis or specifically reprogrammed after erasure [reviewed in (10)]. More recent studies have shown that some transgenerationally inherited *MLH1* and *MSH2* "epimutations" are in fact linked to nearby *cis*-acting genetic variants and consequently follow Mendelian inheritance patterns (11-13). Other *MLH1* "epimutations" occur sporadically and have not been linked to underlying genetic variations, and while these "epimutations" are often observed in a familial context, they do not follow completely Mendelian inheritance patterns (10).

Recently, we developed a novel statistical method to identify heritable methylation marks based on complex segregation analysis (14). The method was applied to 210 members of 25 families at high breast cancer risk, for which peripheral blood DNA methylation was measured at approximately 480,000 methylation sites using the Infinium HumanMethylation450 BeadArray. We identified 24 heritable methylation marks associated with breast cancer in high-risk families, and three of these marks were also associated with risk in an independent population-based study (14). As breast and prostate cancer share some

genetic risk factors (15-17), including rare mutations (e.g. in *BRCA2*), we aimed to assess whether the 24 heritable methylation marks associated with breast cancer risk were also associated with risk of prostate cancer.

MATERIALS AND METHODS

Participants

The Melbourne Collaborative Cohort Study (MCCS) is a prospective cohort study of 41,513 healthy adult volunteers (17,045 men) aged between 27 and 76 years (99% aged 40-69) recruited between 1990 and 1994 (18). Incident cases of invasive adenocarcinoma of the prostate (International Classification of Diseases 10th Revision (ICD-10), code C61) were identified up to 31 December 2010 by linkage with the Victorian Cancer Registry, which receives mandatory notification of all new cancer cases in Victoria, and the Australian Cancer Database, maintained by the Australian Institute of Health and Welfare. Aggressive cases were defined as: Gleason score 8-10; tumour stage T4, N+ or M+; and/or, died of prostate cancer (ICD-10 code: C61) during follow-up (up 31 December 2012). Controls were sampled from the MCCS and individually matched to cases on year of birth, country of birth and blood DNA source using incidence density sampling with age as the time scale (19).

Study participants provided written, informed consent in accordance with the Declaration of Helsinki, and the study was approved by Cancer Council Victoria's Human Research Ethics Committee.

DNA source and extraction

Peripheral blood specimens were collected at study enrolment (baseline) in the form of peripheral blood mononuclear cells (PBMC), buffy coats or dried blood spots on filter paper. DNA was isolated from PBMC and buffy coat specimens using QIAamp mini spin columns. DNA was prepared from twenty-one 3.2mm diameter punches from the dried blood spots after lysis in phosphate buffered saline using a TissueLyser. The supernatant was processed using Qiagen mini spin columns according to the manufacturer's instructions, as described previously (20).

DNA bisulfite conversion and hybridisation

Bisulfite conversion was performed on a minimum of 0.3µg DNA (assessed using the Quant-iT™ Picogreen® dsDNA assay measured on the Qubit® Fluorometer), using the Zymo Gold

single tube kit, according to manufacturer's instructions. Post-conversion quality control was performed using SYBR Green-based quantitative PCR, designed to determine the success of bisulfite conversion by comparing amplification of the test sample with unconverted and converted high-quality DNA controls.

Samples were processed on 96-well plates (eight HM450K BeadChips per plate). In order to minimise batch effects, matched cases and controls were processed together and run on the same BeadChip and cancer subtypes (non-aggressive and aggressive) were evenly distributed across the chips and plates. Paired cases and controls were randomly positioned on each BeadChip to reduce any possible position effects within chips. Two pairs of technical replicates and a reference duplicate (DNA prepared from the multiple myeloma cell line U266) were included on each plate. A total of 200ng of bisulfite converted DNA was whole-genome amplified and hybridised onto the BeadChips as per the manufacturer's instructions. The TECAN automated liquid handler was used for the single-base extension and BeadChip staining steps. The BeadChips were scanned using an iScan System (Illumina).

HM450K BeadChip pre-processing and quality control

Raw intensity signals were imported into R programming software (www.r-project.org) using the *minfi* Bioconductor package (21). Data were pre-processed and normalised to control probes using the "preprocessIllumina" *minfi* function. Subset-quantile within array normalization (SWAN) was performed to correct for type I and II probe bias (22). Samples were excluded if >5% of CpGs (excluding chromosome X and Y probes) had a detection p-value >0.01, while CpGs were excluded if they had missing values for >20% samples.

Statistical analyses

For each of the 24 CpG sites of interest, odds ratios (ORs) for prostate cancer risk per one standard deviation increase of the methylation M-values were estimated using conditional logistic regression models. We considered two models: a minimally adjusted model, with the adjustment provided by the case-control matching procedure and the inclusion of age at blood draw as a covariate; and a fully adjusted model, with additional adjustment for body-mass index (23), tobacco smoking (24), alcohol drinking (25), and white blood cell composition (estimated using the Houseman algorithm (26)). Our main results are based on the fully adjusted model, with the minimally adjusted analyses provided as a sensitivity analysis (Supplementary Material). Since the majority of the 24 sites showed bimodal (or, in some cases, trimodal) distributions, we categorized methylation values into high and low

categories, where the cutoffs were chosen by visual inspection of the Beta-value distributions (Figure 1), and the most frequent category was chosen as the reference category. Finally, we performed subgroup analyses by tumour aggressiveness. All p-values were two-sided and the p-value threshold for statistical significance was taken to be 0.05.

RESULTS

Altogether, 869 prostate cancer cases (430 with aggressive and 439 with non-aggressive tumors) and 869 matched controls were included in the analysis (Table 1). The median age at diagnosis was 69.1 years and the interquartile range (IQR) was 63.9 to 74.7 years. The median time from blood draw to diagnosis was 10.3 years (IQR: 6.1 to 14.0 years). Figure 1 presents the distributions of methylation measures (beta-values) for the 24 CpG sites of interest.

Six of the 24 CpGs showed a linear association with risk of prostate cancer after adjustment for white blood cell composition and other potential confounders (Table 2). Five were located at *VTRNA2-1* (cg06536614, cg00124993, cg26328633, cg25340688 and cg26896946; P=0.03 to 0.05) and one in *CLGN* (cg22901919; P=0.02). As shown in Figure 2, all eleven HM450K CpGs located across the *VTRNA2-1* locus showed a consistent methylation pattern spanning over 665bp. Results were very similar when no adjustment was made for white blood cell composition or other potential confounders (Supplementary Table 1).

Table 2 also shows stratified analyses by aggressiveness of prostate cancer tumours for the 24 heritable methylation marks. In these stratified analyses, for CpG sites in the *VTRNA2-1* region, associations were observed with aggressive disease only (e.g. cg25340688, OR=0.84, 95% CI: 0.73-0.97), including at an additional unannotated neighbouring methylation site (cg11608150, OR=0.83, 95% CI: 0.72-0.96).

Table 3 shows the estimated odds ratios for the less frequent category of methylation compared with the more frequent category. Slightly stronger evidence of association for CpG sites in the promoter of *VTRNA2-1* (all P=0.01). The risk of aggressive prostate cancer was increased by an estimated 53 to 58% for individuals with methylation lower than 0.3 at *VTRNA2-1* (cg25340688: aggressive cases OR=1.55, 95% CI: 1.10-2.20; all cases, OR=1.30, 95% CI: 1.03-1.64).

DISCUSSION

Some genetic risk factors are common to breast and prostate cancer, including many that are associated with homologous recombination deficiency (e.g. rare variants in *BRCA2*). Our results show that breast and prostate cancer also share some epigenetic risk factors, namely some heritable methylation marks. In particular, we observed an association between prostate cancer risk and methylation at *CLGN*, which encodes calmegin, an endoplasmic reticulum chaperone protein expressed in the testis (27) and required for sperm fertility (28), and a cluster of associations at *VTRNA2-1*, which encodes an RNA polymerase III transcript.

These results, and our previous results on breast cancer susceptibility, demonstrate a strong regional methylation pattern at the *VTRNA2-1* site consistent with allele-specific methylation. Work by us and by others also shows that a high proportion (73-80%) of non-tumour tissues have monoallelic methylation at *VTRNA2-1* (14,29,30). The *VTRNA2-1* RNA molecule can act as a tumour suppressor by downregulating the RNA-activated protein kinase R (31). A disruption to the *VTRNA2-1* allele-specific methylation is common in cancer tissues (29) and promoter methylation has been associated with cervical cancer (32), lung cancer (33) and acute myeloid leukemia (30). A recent study that included data from the MCCS reported associations of the *VTRNA2-1* cluster with the risk of lung cancer and mature B-cell neoplasm, in the opposite direction to that of the present study (34). In our previous study of breast cancer, *CLGN* and *VTRNA2-1* were found to be associated with breast cancer risk in multiple-case breast cancer families only (14); these associations were in the same direction as those with prostate cancer risk in the MCCS.

The *VTRNA2-1* region has been reported as a polymorphic or atypically imprinted region with allele-specific methylation that acquires a methylated allele maternally (29,35). Imprinted regions are often associated with tissue growth and a loss of imprinting (LOI) can be linked to tumorigenesis as shown for the *H19/IGF2* region (36). This locus has also been described as a metastable epiallele as the imprinting of this region can be modulated by periconceptional environment and persist through adulthood (37-39). Van Dijk et al have reported that hypomethylation in the *VTRNA2-1* region in newborns was associated with a 2.1-fold increased risk of childhood obesity compared with monoallelic methylation (95% CI 1.3-3.4, P=0.002) (40).

Some transgenerationally heritable DNA methylation marks have been associated with nearby genetic variation (14,41) but allele-specific methylation at *VTRNA2-1* has not yet been

linked to any underlying genetic variation (14,29,35). This suggests that the allele-specific methylation pattern at *VTRNA2-1* is unlikely to be an mQTL and further supports polymorphic epigenetic imprinting of the region.

In cancer cells, hypermethylation of the *VTRNA2-1* promoter leads to transcriptional repression of *VTRNA2-1* and cell proliferation (30,31). In this study, we have shown that hypomethylation at *VTRNA2-1*, measured in DNA from blood collected prior to the diagnosis of prostate cancer, is associated with prostate cancer risk (as had been observed for breast cancer risk). Further work is required to understand if heritable *VTRNA2-1* DNA hypomethylation is present in other tissues and if this is biologically related to *VTRNA2-1* tumour DNA hypermethylation observed in tumour progression.

Strengths of our study include its prospective design and the case-control matching on relevant variables, including random positioning of case-control pairs on a same chip of the assay (42). We additionally adjusted for potential case-control differences in lifestyle or the composition of blood cells, which may both affect methylation profiles. Of note, the results were very similar in minimally adjusted analyses, which is consistent with the 24 methylation marks being heritable, and therefore stable across blood cell types and indicates that the findings were not driven by modification of methylation marks by lifestyle exposures. To our knowledge, no genome-wide association study has reported associations between SNPs in or near *CLGN* and *VTRNA2-1* and the risk of prostate cancer, which may support that our associations at these loci are caused by epigenetic effects (4).

CONCLUSION

We have identified heritable methylation marks in *VTRNA2-1* and *CLGN* that are associated with the risk of prostate cancer and, in previous work, breast cancer. This highlights a potentially important new area of investigation for heritable prostate cancer susceptibility and it adds to our knowledge about shared risks factors for breast and prostate cancer.

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Figure Legends

Figure 1
Beta-value distribution for 24 CpGs, and cutoffs used for categorization of the methylation variable.

Figure 2
Box plots showing beta-value distributions for all CpGs annotated to VTRNA2-1, separately for aggressive and non-aggressive cases and their matched controls

Table 1. Study sample characteristics

		Cases (N=869)	**Controls (N=869)**
Matching variables			
Age at blood draw; median (interquartile range)		60.6 (53.9-65.4)	60.5 (53.9-65.4)
DNA source	Dried blood spot	687 (79%)	687 (79%)
	PBMC	173 (20%)	173 (20%)
	Buffy coat	10 (1%)	10 (1%)
Country of birth	Australia/ NZ/ UK	708 (81%)	708 (81%)
	Italy	91 (11%)	91 (11%)
	Greece	70 (8%)	70 (8%)
Other risk factors			
Smoking	Never	385 (44%)	365 (42%)
	Current	77 (9%)	97 (11%)
	Former	407 (47%)	407 (47%)
Alcohol consumption*	None	163 (19%)	161 (19%)

	Low	579 (67%)	578 (66%)
	Moderate	70 (8%)	74 (9%)
	High	57 (7%)	56 (6%)
Body-mass index (kg/m^2)	≤ 25	232 (27%)	249 (29%)
	25-30	472 (53%)	472 (54%)
	>30	165 (19%)	165 (18%)
Clinical variables			
Age at diagnosis; median (interquartile range)		69.1 (63.9-74.7)	
Tumor aggressiveness	Aggressive	430 (49%)	
	Non-aggressive	439 (51%)	

Abbreviations: NZ: New-Zealand, UK: United-Kingdom

* Alcohol consumption: None: 0 g/day, Low: 1–39 g/ day (males) or 1–19 g/day (females), Moderate: 40–59 g/day (males) or 20–39 g/day (females), High: ≥60 g/day (males) or ≥40 g/day (females))

Table 2. Association with prostate cancer risk for 24 heritable methylation marks previously found to be associated with breast cancer risk (methylation modelled as a continuous variable)

Site	Chr.	Position	Gene Name	All cases			Aggressive cases			Non-aggressive cases		
				OR[a]	95% CI	p	OR[a]	95% CI	p	OR[a]	95% CI	p
cg18584561	2	11682017	GREB1	1.02	0.93-1.12	0.67	1.09	0.95-1.25	0.22	0.96	0.84-1.10	0.57
cg01741999	2	219137824	PNKD	0.99	0.87-1.13	0.91	1.00	0.82-1.22	0.99	1.00	0.82-1.20	0.96
cg11035303	3	43465503	ANO10	0.94	0.86-1.04	0.24	0.91	0.79-1.04	0.18	1.01	0.87-1.17	0.91
cg25188166	3	119420208	unannotated	0.96	0.87-1.06	0.44	0.87	0.76-1.01	0.07	1.03	0.90-1.18	0.70
cg02096220	4	129212177	unannotated	0.98	0.90-1.08	0.75	0.92	0.80-1.06	0.25	1.07	0.94-1.22	0.30
cg22901919	4	141317067	CLGN	**0.88**	**0.79-0.98**	**0.02**	**0.93**	**0.80-1.07**	**0.30**	**0.84**	**0.72-0.99**	**0.03**
cg11608150	5	135415948	unannotated	**0.91**	**0.83-1.01**	**0.08**	**0.83**	**0.72-0.96**	**0.01**	**0.98**	**0.85-1.12**	**0.76**
cg06536614	5	135416381	VTRNA2-1 (MIR886)	**0.90**	**0.82-1.00**	**0.05**	**0.85**	**0.73-0.98**	**0.03**	**0.94**	**0.82-1.08**	**0.39**
cg26328633	5	135416394	VTRNA2-1 (MIR886)	**0.90**	**0.81-0.99**	**0.03**	**0.84**	**0.72-0.97**	**0.02**	**0.94**	**0.82-1.07**	**0.34**
cg25340688	5	135416398	VTRNA2-1 (MIR886)	**0.91**	**0.82-1.00**	**0.05**	**0.84**	**0.73-0.97**	**0.02**	**0.94**	**0.82-1.08**	**0.40**
cg26896946	5	135416405	VTRNA2-1 (MIR886)	**0.90**	**0.81-0.99**	**0.03**	**0.84**	**0.72-0.97**	**0.02**	**0.94**	**0.82-1.07**	**0.35**
cg00124993	5	135416412	VTRNA2-1 (MIR886)	**0.90**	**0.81-0.99**	**0.03**	**0.83**	**0.72-0.96**	**0.01**	**0.94**	**0.82-1.08**	**0.41**
cg18110333	6	292329	DUSP22	0.98	0.89-1.07	0.61	0.95	0.83-1.09	0.48	1.00	0.87-1.14	0.98
cg03916490	7	1080558	C7orf50	1.03	0.93-1.13	0.61	1.00	0.87-1.14	0.96	1.05	0.91-1.21	0.49
cg10306192	11	102576374	MMP27	0.97	0.89-1.07	0.60	1.08	0.94-1.24	0.28	0.90	0.79-1.03	0.14
cg26773954	13	111969980	unannotated	0.98	0.88-1.08	0.66	1.03	0.89-1.20	0.65	0.93	0.81-1.08	0.33
cg23947138	13	114782778	RASA3	0.98	0.89-1.08	0.68	1.00	0.87-1.15	0.95	0.96	0.84-1.11	0.61
cg23012654	14	97493395	unannotated	0.99	0.90-1.09	0.82	0.97	0.85-1.11	0.69	1.00	0.87-1.14	0.96
cg05865327	14	102274741	PPP2R5C	0.93	0.84-1.02	0.13	0.92	0.80-1.06	0.22	0.94	0.82-1.07	0.34
cg27639199	15	81666528	TMC3	1.02	0.93-1.13	0.65	1.02	0.88-1.17	0.81	1.03	0.90-1.18	0.68
cg01074083	16	17516862	XYLT1	0.99	0.89-1.09	0.77	0.94	0.81-1.08	0.39	1.06	0.92-1.22	0.44
cg04417708	17	4043867	ZZEF1	0.99	0.90-1.09	0.88	1.01	0.88-1.15	0.93	0.97	0.85-1.11	0.69
cg05187003	21	34641507	IL10RB	0.97	0.87-1.08	0.55	0.90	0.77-1.05	0.19	1.03	0.89-1.20	0.70
cg18514595	22	49579968	unannotated	1.04	0.94-1.15	0.42	1.07	0.93-1.23	0.36	0.99	0.85-1.14	0.86

[a] Odds ratio from conditional logistic regression of the risk of prostate cancer on M-values (per 1 standard deviation), adjusting for body-mass index, tobacco smoking, alcohol drinking, age at blood draw, and white blood cell composition. Cases and controls were individually matched on year of birth, year of blood draw, country of birth, and sample type.

Table 3. Association with prostate cancer risk for 24 heritable methylation marks previously found to be associated with breast cancer risk (methylation modelled as a categorical variable, with most frequent category chosen as reference)

Site	Chr.	Position	Gene Name	Smaller peak definition	All cases			Aggressive cases				Non-aggressive cases		
					OR[a]	95% CI	p	OR[a]	95% CI	p	p		OR[a]	OR[a]
cg18584561	2	11682017	*GREB1*	beta < 0.2	0.93	0.76-1.13	0.48	0.83	0.62-1.10	0.20		1.02	0.77-1.35	0.88
				beta > 0.6	0.88	0.62-1.24	0.45	0.90	0.54-1.49	0.67		0.84	0.52-1.35	0.47
cg01741999	2	219137824	*PNKD*											
cg11035303	3	43465503	*ANO10*	beta > 0.2	1.24	0.85-1.80	0.27	1.40	0.85-2.29	0.18		0.95	0.52-1.74	0.87
cg25188166	3	119420208	*unannotated*	beta < 0.3	1.14	0.75-1.74	0.53	1.68	0.88-3.21	0.12		0.85	0.48-1.51	0.58
				0.3 < beta < 0.7	1.01	0.79-1.30	0.91	1.05	0.73-1.50	0.80		1.00	0.70-1.41	0.98
cg02096220	4	129212177	*unannotated*	beta < 0.2	0.83	0.48-1.43	0.50	1.36	0.59-3.16	0.47		0.51	0.24-1.08	0.08
				0.2 < beta < 0.8	1.08	0.88-1.32	0.46	1.13	0.84-1.51	0.42		1.03	0.77-1.37	0.86
cg22901919	4	141317067	*CLGN*	beta < 0.7	**1.31**	**0.99-1.73**	**0.06**	**1.16**	**0.78-1.75**	**0.46**		**1.40**	**0.94-2.08**	**0.10**
cg11608150	5	135415948	*unannotated*	beta < 0.3	**1.20**	**0.96-1.49**	**0.11**	**1.40**	**1.02-1.93**	**0.04**		**1.08**	**0.78-1.48**	**0.64**
cg06536614	5	135416381	*VTRNA2-*	beta < 0.3	**1.29**	**1.02-1.64**	**0.03**	**1.55**	**1.09-2.20**	**0.01**		**1.16**	**0.83-1.62**	**0.39**
cg26328633	5	135416394	*VTRNA2-1*	beta < 0.3	**1.28**	**1.01-1.62**	**0.04**	**1.55**	**1.09-2.19**	**0.01**		**1.14**	**0.82-1.59**	**0.44**
cg25340688	5	135416398	*VTRNA2-1*	beta < 0.3	**1.30**	**1.03-1.64**	**0.03**	**1.55**	**1.10-2.20**	**0.01**		**1.17**	**0.84-1.63**	**0.35**
cg26896946	5	135416405	*VTRNA2-1*	beta < 0.3	**1.30**	**1.03-1.65**	**0.03**	**1.58**	**1.11-2.25**	**0.01**		**1.15**	**0.83-1.61**	**0.40**
cg00124993	5	135416412	*VTRNA2-1*	beta < 0.3	**1.28**	**1.01-1.61**	**0.04**	**1.53**	**1.09-2.14**	**0.01**		**1.14**	**0.82-1.58**	**0.45**
cg18110333	6	292329	*DUSP22*	beta < 0.3	1.03	0.82-1.28	0.82	1.07	0.77-1.47	0.69		0.99	0.72-1.36	0.95
cg03916490	7	1080558	*C7orf50*	beta < 0.85	0.92	0.74-1.14	0.44	0.92	0.68-1.24	0.57		0.98	0.72-1.35	0.92
cg10306192	11	102576374	*MMP27*	beta > 0.3	1.02	0.83-1.25	0.86	0.77	0.57-1.04	0.09		1.26	0.94-1.69	0.12
cg26773954	13	111969980	*unannotated*	beta < 0.8	1.05	0.82-1.33	0.71	0.93	0.66-1.31	0.69		1.15	0.81-1.64	0.43
cg23947138	13	114782778	*RASA3*	beta < 0.7	0.97	0.78-1.21	0.80	0.86	0.62-1.19	0.37		1.08	0.78-1.48	0.65
cg23012654	14	97493395	*unannotated*	beta < 0.8	1.02	0.77-1.34	0.91	0.93	0.62-1.41	0.73		1.12	0.76-1.63	0.57
cg05865327	14	102274741	*PPP2R5C*	beta < 0.7	1.24	0.99-1.55	0.07	1.29	0.93-1.79	0.12		1.18	0.85-1.63	0.31
cg27639199	15	81666528	*TMC3*	0.2 < beta < 0.8	0.98	0.80-1.20	0.85	1.01	0.76-1.35	0.93		0.93	0.70-1.24	0.61
				beta > 0.8	1.23	0.84-1.81	0.29	1.14	0.63-2.09	0.66		1.35	0.80-2.25	0.26
cg01074083	16	17516862	*XYLT1*	beta < 0.8	1.01	0.81-1.25	0.93	1.08	0.78-1.49	0.64		0.88	0.65-1.21	0.44
cg04417708	17	4043867	*ZZEF1*	beta < 0.75	0.92	0.75-1.13	0.42	0.85	0.63-1.15	0.28		1.00	0.74-1.35	0.99
cg05187003	21	34641507	*IL10RB*											
cg18514595	22	49579968	*unannotated*	0.2 < beta < 0.8	1.07	0.87-1.31	0.55	1.16	0.87-1.55	0.32		0.89	0.66-1.21	0.47

	beta > 0.8	1.14	0.77-1.68	0.52	1.06	0.60-1.87	0.83	1.19	0.68-2.10	0.54

[a] Odds ratio from conditional logistic regression of the risk of prostate cancer on categorized methylation values, adjusting for body-mass index, tobacco smoking, alcohol drinking, age at blood draw, and white blood cell composition. Cases and controls were individually matched on year of birth, year of blood draw, country of birth, and sample type.

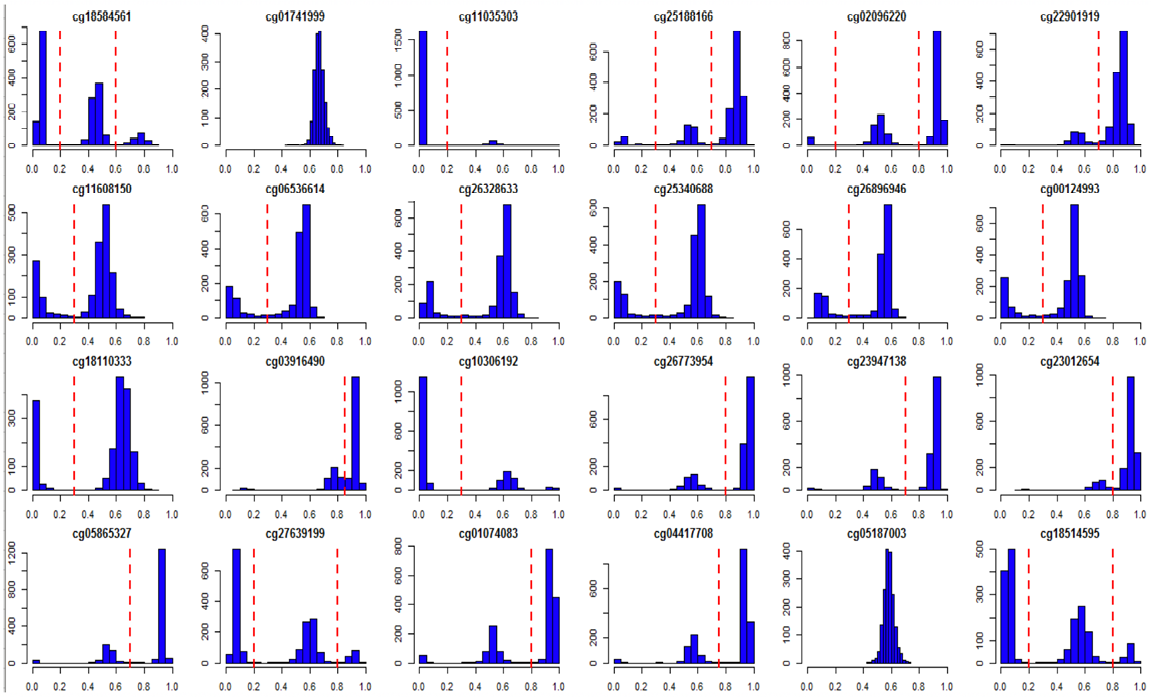

Figure 1 .

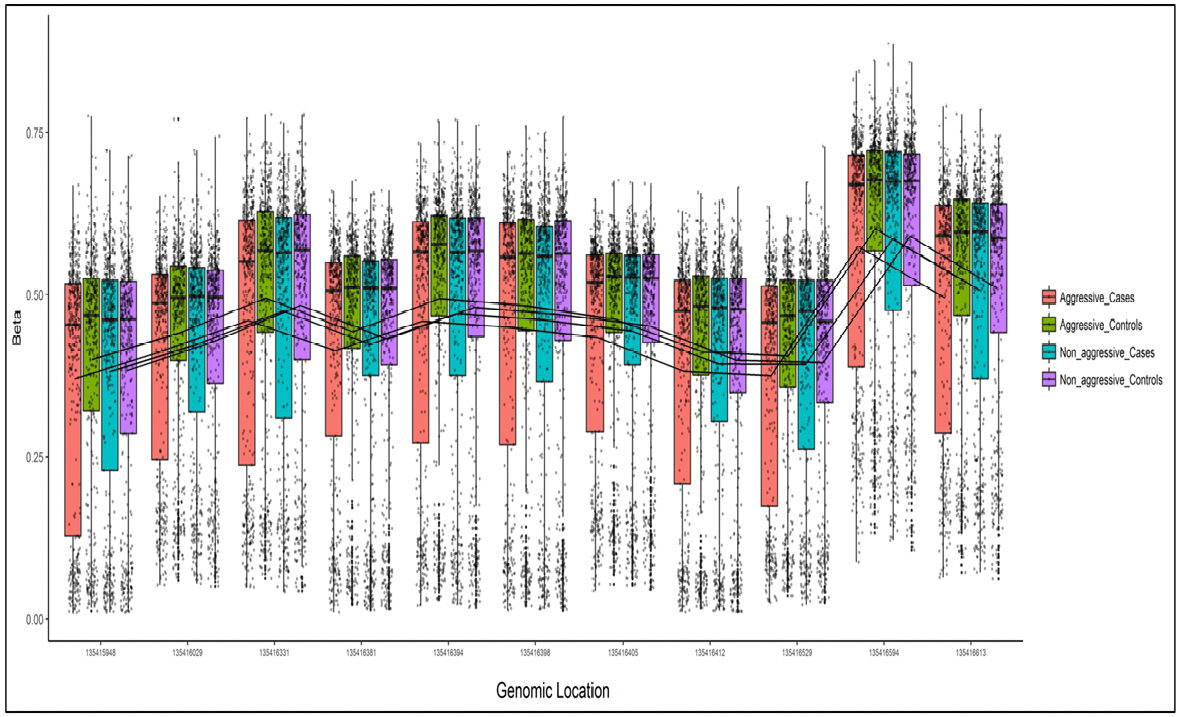

Figure 2 .

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