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The Duffy Antigen/Receptor for Chemokines (DARC) and Prostate-Cancer Risk among Jamaican Men

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Abstract

As an evolutionary response to prevent malaria infection, most Africans do not express the Duffy Antigen/Receptor for Chemokines (DARC) on their red blood cells. Results from experimental studies suggest that DARC expression inhibits prostate-tumor growth. We tested the hypothesis that men of African descent who lack DARC expression are at increased risk of prostate cancer. A case–control study involving 81 age-matched pairs was conducted in Jamaica. Participants were interviewed to collect data, and they donated blood for determination of DARC expression. Logistic regression was used to estimate associations with prostate cancer and aggressive disease. Little or no association was observed between erythrocyte DARC expression and prostate cancer or between DARC expression and aggressive disease. These associations changed little when adjusting for other potential confounders. Our results do not support an effect of erythrocyte DARC expression on the risk or progression of prostate cancer in men of African descent.

Keywords

Prostate cancer; DARC; African descent; Epidemiology; Chemokine receptors; Erythrocytes

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Introduction

Prostate cancer is the most common cancer diagnosed among American men and the second leading cause of cancer death, with an estimated 192,280 new cases and 27,360 deaths expected in the United States in 2009 [1]. Despite the tremendous amount of epidemiologic research devoted to prostate cancer, there are few established risk factors—age, positive family history, and African American race [2,3]. African American men are 60% more likely to be diagnosed with prostate cancer and 2–3 times more likely to die from the disease than are Caucasian men [2,4], yet the reasons for these race differences remain largely unknown. The highest incidence rates of prostate cancer are not in African Americans, but rather in Jamaican men of African descent (304 per 100,000 per year) [5]. One suggested reason for this susceptibility is the lack of expression of the Duffy Antigen/Receptor for Chemokines (DARC) on erythrocytes (red blood cells) in men of African descent [6].

The DARC is an abundant receptor that is expressed on erythrocytes and vascular endothelial cells. During infection by *Plasmodium vivax*, the DARC is required for entry of the malarial parasite into the erythrocyte. In West Africa, an overwhelming majority of the population, through evolutionary selection, no longer express this protein on the surface of erythrocytes as protection against malarial infection [7]. In populations of African descent, the proportion of individuals who lack expression of DARC varies from ~70% in African Americans to 95% or more in Africans [6]. Recent research suggests that there may be a trade-off between infection and carcinogenesis. The DARC binds to a wide range of small proteins, called chemokines. Of relevance to prostate cancer, a subset of these chemokines of the CXC class has been shown to regulate prostate-cancer progression. The DARC functions by binding CXC chemokines that possess an amino-terminal sequence (Glu-Leu-Arg), referred to as the ELR motif [8]. Other studies have demonstrated that this motif is responsible for angiogenic properties of CXC chemokines [9,10]. ELR-positive CXC chemokines have been shown to be prevalent in prostate cancer cells, and elevated levels have been found in tumors of individuals with prostate cancer [11]. Using transgenic mouse models, Shen et al. [6] demonstrated that the DARC might work to remove the angiogenic chemokines from the prostate, thus reducing the risk for a more aggressive cancer. This led to the hypothesis that lack of erythroid DARC, as occurs in the vast majority of men of African descent, may facilitate prostate tumor growth by removing a clearance mechanism for angiogenic chemokines [12]. Subsequent studies have shown that endothelial DARC may also contribute to reduced angiogenesis[1] [13,14].

To examine whether expression of DARC on erythrocytes was related to prostate-cancer risk in men of African descent, we conducted a case–control study of Jamaican men. We hypothesized that men who do not express erythroid DARC would be more likely than men with DARC to develop prostate cancer and/or, more specifically, to develop a more aggressive form of the disease. If confirmed, this hypothesis would help to explain the relatively high incidence and mortality rates of prostate cancer in African Americans.

Methods

A case–control study was conducted over an 8-week period at Kingston Public Hospital (KPH) in Kingston, Jamaica. Eighty-one (81) prostate-cancer cases were recruited at the Radiotherapy Department (Radiation Oncology) of KPH with 81 cancer-free controls also recruited within the hospital and individually matched to cases by 5-year age intervals. Both

[1]Angiogenesis: The formation of new blood vessels growing into tumors which feed the tumor and provide oxygen needed for continued growth.

cases and controls were consented using the comprehensive oral method approved by the University of Michigan Health Sciences Institutional Review Board.

Case participants were of African descent, diagnosed with prostate cancer, and over 40 years of age at the time of diagnosis. Cases were confirmed by medical record of diagnosis using results of prostate specific antigen (PSA), digital rectal exam, and prostate biopsy reports. Information abstracted from the medical record on all cases included PSA at time of diagnosis, Gleason score, clinical stage, and results from a bone or CT scan, if performed. Aggressive disease was defined as a tumor Gleason grade at diagnosis ≥7, regional or distant stage (T3 or T4), or PSA at diagnosis ≥20 ng/ml [15]. Other definitions of aggressive disease were also used in our analyses, but they produced similar results. Control participants were aged 40 years and older, of African descent, and without a history of prostate cancer. Controls were identified through a pre-screening questionnaire, which determined prostate cancer history, race and age by self-report. Because many controls were recruited from the phlebotomy laboratory, a large proportion (40–45%) of these participants were diabetic.

All participants completed an interviewer-administered questionnaire, which collected information on sociodemographic factors, prostate symptoms and urinary function, sexual behavior and sexually transmitted diseases, diet, smoking and alcohol consumption, and family history of prostate cancer in first- or second-degree relatives. Body mass index (in kg/m^2) was calculated using height and weight measured at the time of interview. Participants were also asked to donate a 5 ml blood sample, which was conventionally frozen at KPH. The samples were then shipped on dry ice to the University of Cincinnati and stored at −80°C.

Laboratory Methods

Deoxyribonucleic acid (DNA) was extracted from the blood samples using the Qiagen® kit. Polymerase chain reaction (PCR) was conducted on each DNA sample to determine DARC (Fy) status. For PCR, the primers were selected and the electrophoretic methods were carried out according to Olsson et al. [16]; however, the gene amplification settings were taken from Hessner et al. [17]. Four reactions per sample were run to determine Fy genotype[2]: hypothetical silent, Fy, Fya, and Fyb. A negative reaction was determined to be the presence of only the 419-bp DNA fragment, while a positive reaction was the presence of the 711-bp DNA fragment, with or without the 419-bp fragment [16]. Existence of the DARC (Fy) protein was defined as presence of either the Fya or the Fyb, regardless of the presence of the hypothetical silent or the Fy. Lack of DARC expression (the “exposure” in this study) was defined as having only the hypothetical silent or the Fy banding [16]. To establish reliability of the measurement of DARC status, blood samples from a Nigerian and a Caucasian-American were analyzed, as controls of negative expression and positive expression, respectively.

Statistical Analysis

All statistical analyses were carried out using SAS® version 9.2 (Cary, NC). Conditional logistic regression models were fitted to 81 age-matched pairs to estimate the effects of DARC expression (negative vs. positive) and other factors on prostate cancer. Models were fitted by adding covariates to the model sequentially to assess possible confounding. Unconditional logistic regression was used to examine the association between DARC expression and aggressive disease by comparing aggressive cases to controls, adjusting for

[2]Fy genotype: The Fya and Fyb genotypes represent the two main antigens which differ by a single amino acid (G42D) and define the four major erythrocyte Duffy phenotypes.

age (the matching variable) and other potential confounders. Potential confounders included a family history of prostate cancer, a history of sexually transmitted infection, a history of an enlarged prostate, smoking status, alcohol consumption, BMI, education, and meat consumption [2–4,18]. Odds ratios (OR) and 95% confidence intervals (CI), derived from the fitted logistic models, were used to measure associations with prostate cancer.

Results

An equal proportion (22%) of prostate cancer cases and controls expressed DARC (Table 1). DARC expression was more common for men under 65 years of age than for men 65 years of age and older. Clinical characteristics of prostate-cancer cases are described in Table 2. Fifty-one percent of the cases had a PSA at the time of diagnosis that was 20 ng/ml or greater, and 24% of the cases had a Gleason grade of 8 or greater. The distribution of clinical stage at diagnosis was 36% T1, 32% T2, 14% T3, and 18% T4 (metastatic). Sixty (74%) of the 81 cases were classified as having aggressive disease.

Table 3 presents the numbers and proportions of cases and controls falling into each covariate category as well as the age-adjusted odds ratios and 95% confidence intervals (CI) for the associations with prostate cancer. A positive family history was strongly associated with prostate cancer (OR = 11; 95% CI = 2.5, 45). Having an enlarged prostate was also strongly associated with prostate cancer (OR = 10; 95% CI = 3.1, 33). Having a college or vocational education versus a primary-school education was positively associated with prostate cancer (OR = 2.7; 95% CI = 0.94, 8.2), though a monotonic association was not observed across all categories of education. Weaker associations with prostate cancer were also observed for fat intake, consumption of meat (white or red), and BMI. Ever smokers in this study were less likely than never smokers to have prostate cancer (OR = 0.42; 95% CI = 0.18, 0.96). No consistent associations were observed between prostate cancer and either history of sexually transmitted diseases or alcohol consumption.

The estimated effects of DARC on prostate cancer, controlling for selected combinations of potential confounders in addition to age (Models 1–7), are shown in Table 4. Adjusting only for age, we observed no association between DARC expression and prostate cancer. Adjusting for selected combinations of other potential confounders, including family history of prostate cancer, did not appreciably change these results.

A comparison of aggressive cases only with all controls yielded results that were inconsistent with our hypothesis. We found the incidence of aggressive prostate cancer to be slightly *lower* in DARC-negative men than in DARC-positive men (age-adjusted OR = 0.79; 95% CI = 0.36, 1.7). Adjusting for other potential confounders did little to change this finding; the estimated odds ratio remained slightly less than 1 (results not shown).

Discussion

Our results suggest that lack of DARC expression does not increase the risk of prostate cancer or aggressive disease in Jamaican men as we hypothesized. The DARC is expressed primarily on two cell types, erythrocytes and vascular endothelial cells, and it functions by binding a group of CXC chemokines that possess the ELR motif [8]. These ELR-positive CXC chemokines have been shown to have angiogenic properties related to endothelial cells [9,10]. Results of preclinical studies suggested that the DARC might work to remove angiogenic chemokines from the prostate, thus reducing the risk of more aggressive cancer. Shen et al. [6] demonstrated that tumors from mice without DARC expression had greater amounts of angiogenic chemokines, increased tumor vessel density, and greatly augmented prostate-tumor growth. However, one notable limitation of that investigation was that the

mice used in that study lacked DARC expression on both erythrocytes and vascular endothelial cells, which is unlike humans of African descent. It is well established that humans who do not express DARC on erythrocytes (e.g., men of African descent) retain DARC expression on vascular endothelial cells [19]. While the role of endothelial DARC has not been clearly delineated, more recent studies suggest that it also has anti-angiogenic properties via an unknown mechanism [13,14]. This raises the possibility that erythroid and endothelial DARC may have similar and perhaps overlapping functions. As such, it is possible that the cause of the increased tumor growth observed in DARC-knockout mice [6] was due to the lack of DARC expression on both erythrocytes and endothelial cells. In contrast, men of African descent retain endothelial expression of the DARC, and this may be sufficient to limit tumor angiogenesis.

There are limitations to our study that necessitate some caution in the interpretation of the findings. The relatively small sample size reduced the precision of effect estimation, as demonstrated by the wide confidence intervals, and increased likelihood of chance findings. Furthermore, we did not attempt clinical verification of control status (via PSA testing and/or physical examination). Control selection was based on subjects' self reports that they were free of prostate cancer. Consequently, it is possible that some cases of prostate cancer were included in the control group, leading to misclassification. It is unlikely, however, that such vmisclassification could explain our null finding (crude OR = 1.0). Suppose, for example, that 15% of the controls actually had prostate cancer and that all of those misclassified controls were DARC-negative, creating the greatest downward bias. Deleting those controls from the analysis would result in a corrected crude OR estimate of only 1.2—a very weak association that still gives little support to our hypothesis.

Another methodologic limitation is possible selection bias in this hospital-based case–control study, possibly due to the predominance of diabetics in the control group. If diabetes was inversely associated with DARC expression in the source population, our selection of controls would have produced a downward bias in the estimation of the DARC effect. Thus, this source of bias might explain our negative findings. Unfortunately, we cannot estimate the DARC-diabetes association in our study because we did not collect information on diabetes status.

Conclusions

Our results do not support an effect of erythrocyte DARC expression on the risk or progression of prostate cancer in Jamaican men of African descent. To the best of our knowledge, ours is the first study to examine this association in humans. Given the small sample size and possible bias, however, we cannot conclude at this time that DARC expression is neither a risk factor for the disease nor a prognostic factor for disease progression. Furthermore, it is not clear how generalizable our results are to other populations. Thus, new research is needed to establish the possible etiologic or prognostic role of erythroid and/or endothelial DARC expression in the natural history of prostate cancer.

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Table 1

Number of cases and controls, by DARC status (positive vs. negative) and age: Jamaican men, 2007

Age	Cases			Controls		
	DARC+	DARC−	Total	DARC+	DARC−	Total
50–54	1	1	2	1	1	2
55–59	0	5	5	0	5	5
60–64	5	5	10	1	9	10
65–69	2	14	16	5	11	16
70–74	8	20	28	5	23	28
75–79	2	10	12	4	8	12
80+	0	8	8	2	6	8
Total	18	63	81	18	63	81

Table 2

Number and percent of prostate-cancer cases by category of selected clinical characteristics

Characteristic	Category	Number	Percent
Pre-diagnostic prostate specific antigen (PSA) level (ng/ml)			
	<4	6	7
	4 to <20	36	44
	20 to <50	22	27
	50 to <100	7	9
	100+	10	13
	Total	81	100
Gleason grade			
	5 (low)	1	2
	6	32	42
	7	24	32
	8	12	16
	9	5	6
	10 (high)	2	2
	Total	76	100
Clinical stage[a]			
	T1	29	36
	T2	26	32
	T3	11	14
	T4	15	18
	Total	81	100
Aggressive disease[b]			
	Yes	60	74
	No	21	26
	Total	81	100

[a] *T1* tumor present but not palpable or detectable with imaging, *T2* tumor can be palpated on examination but has not spread beyond the prostate capsule, *T3* tumor has spread through the prostate capsule, *T4* tumor has metastasized

[b] Aggressive disease defined as Gleason grade 7–10, Stage T3–T4, or PSA >20ng/ml

Table 3

Number (and %) of cases and controls and the estimated effect (odds ratio and 95% CI) of selected variables on prostate cancer, by category of each variable: results of conditional logistic regression analyses

Variable category	No. (%) of cases	No. (%) of controls	Odds ratio[a] (95% CI)
DARC expression			
Yes	18 (22)	18 (22)	1
No	63 (78)	63 (78)	1.0 (0.48, 2.1)
Family history of prostate cancer in a first-degree relative			
Yes	22 (27)	3 (4)	11 (2.5, 45)
No	59 (73)	78 (96)	1
Education			
None	6 (7)	5 (6)	1.4 (0.42, 4.9)
Primary school	53 (66)	63 (78)	1
Secondary school	10 (12)	8 (10)	1.6 (0.55, 5.0)
College or vocational	12 (15)	5 (6)	2.7 (0.94, 8.2)
Eats meat regularly			
Yes	79 (97)	76 (94)	2.5 (0.49, 13)
No	2 (3)	5 (6)	1
Ever smoked at least 5 packs of cigarettes			
Yes	55 (68)	66 (81)	0.42 (0.18, 0.96)
No	26 (32)	15 (19)	1
History of sexual transmitted infection (gonorrhea, herpes, syphilis, or other)			
Yes	43 (53)	50 (62)	0.70 (0.38, 1.3)
No	38 (47)	31 (38)	1
Fat intake			
Low	45 (56)	52 (64)	1
Medium	22 (27)	12 (15)	2.4 (0.98, 5.9)
High	14 (17)	17 (21)	0.92 (0.40, 2.1)
Average alcohol consumption (drinks per week)			
0–1	11 (15)	10 (14)	1
2–5	23 (30)	18 (25)	1.6 (0.65, 3.9)
6–15	14 (18)	14 (20)	1.3 (0.46, 3.4)
15+	28 (37)	29 (41)	1.2 (0.52, 2.7)
Body mass index (BMI)			
Normal (<25)	39 (48)	52 (64)	1
Overweight (25–30)	33 (41)	23 (28)	1.9 (0.94, 3.7)
Obese (>30)	9 (11)	6 (8)	1.9 (0.60, 5.8)
Enlarged prostate			
Yes	32 (60)	5 (6)	10 (3.1, 33)
No	49 (40)	76 (94)	1

[a] Estimated odds ratio (and 95% confidence interval), adjusting for age (the matching variable)

Table 4

Estimated effects (odds ratio and 95% CI) of DARC status (negative vs. positive) and selected covariates on the incidence of prostate cancer among Jamaican men, by choice of covariates in the model (models 1–7): results of conditional logistic regression analyses

Covariate	Model						
	1	2	3	4	5	6	7
Lacks DARC	1.1 (0.52, 2.5)	1.1 (0.50, 2.4)	1.2 (0.44, 3.1)	1.1 (0.42, 3.1)	1.1 (0.41, 3.1)	1.1 (0.41, 3.1)	1.2 (0.32, 4.2)
Family history of prostate cancer	11 (2.5, 45)	10 (2.4, 44)	13 (2.4, 67)	10 (2.0, 52)	10 (1.9, 52)	10 (2.0, 55)	9.7 (1.6, 59)
History of sexually transmitted infection		0.71 (0.32, 1.6)	1.2 (0.46, 3.1)	1.2 (0.46, 3.1)	1.1 (0.42, 3.0)	1.1 (0.41, 3.0)	1.4 (0.43, 4.6)
Enlarged prostate			12 (3.0, 48)	12 (2.8, 50)	12 (2.8, 49)	11 (2.7, 48)	12 (2.4, 60)
Ever smoked				0.57 (0.20, 1.6)	0.58 (0.20, 1.6)	0.61 (0.21, 1.8)	0.58 (0.17, 2.1)
Eats meat					1.5 (0.24, 9.0)	1.5 (0.24, 9.2)	1.4 (0.22, 8.5)
BMI ≥30 vs.<30						1.4 (0.30, 6.4)	1.6 (0.26, 9.9)
Less education[a]							3.8 (0.61, 23)

All odds-ratio estimates are adjusted for age (the matching variable) and other covariates in the model

[a]Primary-school education vs. secondary-school or more education