Genetic Polymorphisms in IL-10 Promoter Are Associated With Smoking and Prostate Cancer Risk in African Americans

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Abstract. *Background/Aim:* Even though prostate cancer (PCa) has good prognosis, there is a discrepancy in the risk among ethnic groups, with high morbidity in African American men. Single nucleotide polymorphisms (SNPs) in interleukin 10 (IL-10) have been associated with inflammation and cancer risk. We investigated the association of five SNPs in the IL-10 promoter with clinical features such as Gleason score and smoking. *Materials and Methods:* A total of 413 DNA samples were obtained from a nested case-control study of African American males who were genotyped for 5 SNPs utilizing pyrosequencing. Multiple and binary logistic regression models were applied to analyze the clinical and genotypic data. *Results:* rs12122923 and rs1800871 were associated with PCa risk. Smoking was also found to increase the risk of PCa by 1.6-fold. rs1800893 was found to be associated with lower grades for prostate cancer. *Conclusion:* IL-10 promoter polymorphisms might be a risk factor for PCa development in smoking subjects and PCa progression.

Prostate cancer (PCa) is one of the most commonly diagnosed forms of cancer among men in the United States. As per Surveillance Epidemiology and End Results Program (SEER), 180,890 new cases were estimated in 2016, of

which 26,120 resulted in death. Even though PCa has a good prognosis due to early detection, there is a disparity in the risk of developing PCa among ethnic groups, specifically, the prevalence and morbidity rates are higher in African-American men and men of African descent than in most other populations (1, 2). The well-established risk factors that contribute to PCa include age, ethnicity, family history, genetic predisposition, diet and environmental factors (3). However, the molecular mechanisms underlying the development and progression of this malignancy remain poorly understood, and if investigated, could possibly lead to closing the disproportionate gap in prognosis and mortality of PCa among African Americans.

New advances in molecular and genetic studies have demonstrated a causal relationship between chronic infection, chronic inflammation, and PCa (4, 5). The hallmarks of cancer, as described by Hanahan and Weinberg, 2011, include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis where inflammation purportedly expedites these cancer characteristics (6). Also, chronic inflammation has been hypothesized to be a cause of PCa where it can contribute to carcinogenesis during disease initiation and subsequent stages (7, 8).

Interleukin-10 (IL-10), which is an anti-inflammatory cytokine, is produced by TH2 cells and is known as a cytokine synthesis inhibitory factor that has a censorious effect in the regulation of inflammatory responses which may contribute to angiogenesis and survival of tumor cells (9). Particularly, it can inhibit the production of interleukin-2, interleukin-3, interferon γ , and granulocyte monocyte-colony stimulating factor by T helper cells. Differential production of this cytokine has been associated with single-nucleotide polymorphisms (SNPs) in the promoter region

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Table I. Characteristics of the study population.

	Cases (n=242)	Controls (n=177)
Age (Years)		
Mean (SD)	69.02 (9.67)	57.5 (11.32)
Smoking N (%)		
Yes	101 (41.7)	47 (26.6)
No	80 (33.06)	59 (33.3)
Missing	61 (25.24)	71(40.1)
Vitamin D N (%)		
Severe deficiency <10	28 (11.6)	8 (4.5)
Low [10, 25)	99 (40.9)	30 (16.9)
Optimal [25, 80)	30 (12.4)	8 (4.5)
Very high ≥80	2 (0.8)	0
Missing	83 (34.3)	131 (74.1)
Gleason score N (%)		
Grade I [1-4]	18 (7.4)	
Grade II [5-7]	114 (47.15)	
Grade III [8-10]	25 (10.33)	
Missing	85 (35.12)	

(10-12). The involvement of IL-10 polymorphisms in cancer development is controversial as this pleiotropic cytokine has both immune-suppressive and anti-angiogenic properties.

Variations in IL-10 have been associated with PCa risk in many studies (13-16) that showed a reduced level of IL-10, having been associated with the presence of IL10-1082A/-819T/-592A haplotype on the IL-10 gene promoter as well as increased risk of cancer in several populations (17). Therefore, we investigated the association of promoter variations in IL-10 with the incidence of PCa using a nested case-control study of men of African descent.

Materials and Methods

Study population. The study population consisted of 242 PCa patients and 177 controls that were recruited at Howard University Hospital Urology department and cancer center screening programs. PCa patients were recruited from Washington DC area through the Division of Urology and PCa screening clinic at the Howard University Cancer Center, while the controls were recruited from volunteers enrolled among individuals undergoing regular physical examinations. The following characteristics were collected from all subjects in the study: All subjects were of African descent as defined by Mason *et al* (5). Clinical characteristics that included Gleason grade, smoking history, and vitamin D deficiency were obtained from medical records (Table I). Individuals diagnosed with benign prostatic hyperplasia (BPH) were not considered in this analysis because studies showed that BPH does not increase or contribute to the risk of prostate cancer (4). PCa cases were diagnosed by transrectal ultrasound-guided biopsy using a standard saturation technique (18). Biopsy cores were reviewed by members of the Department of Pathology in the College of Medicine at Howard University. PCa cases were classified according to well-

Table II. IL-10 promoter region SNP information.

SNP	Chromosomal location	Chromosomal position	Gene location
rs1800871	1	206773289	promoter
rs1800893	1	206773822	promoter
rs12122923	1	206778052	promoter
rs10494879	1	206778859	promoter

established parameters of the Gleason Scoring System (19-22) (Table I). The study was approved by IRB and informed consent form was obtained from all participants.

SNPs selection. Interleukin-10 SNPs were obtained from the NCBI dbSNP (National Center for Biotechnology Information) database (23), and prioritized using the SNP prioritization algorithm which was developed at the Howard University-National Human Genome Center (NHGC) and by Haploview® (Haploview 3.32) (24). Based on power and sample size calculations, SNPs were chosen with minor allele frequencies (MAF) greater than 10%. The frequencies were compared in European-Americans and Yoruba populations using chi-square analysis of significance to identify SNPs with differential allele frequencies (Δ MAF). Greater consideration was given to SNPs found in exonic regions that resulted in non-synonymous (missense) amino acid changes. Also, we considered SNPs located in splice junctions, promoter and 3' untranslated regions (UTRs). Based on their differential race frequencies, the following four SNPs within the IL-10 promoter region were retained for this study: rs12122923, rs1800871, rs10494879 and rs18000893 (Table II).

Genotyping. Genomic DNA was isolated from whole blood samples using the Gentra Puregene Blood Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. DNA was used for genotyping SNPs utilizing pyrosequencing. Custom primer sequences for each SNP were designed using published sequences data and then were optimized for PCR amplification using Platinum *Taq* DNA Polymerase High Fidelity kit (Thermo Fisher Scientific, Waltham, MA, USA). Pyrosequencing was performed in an automated Pyrosequencing instrument, PSQ96 (Pyrosequencing AB, Uppsala, Sweden). Biotinylated single-stranded DNA fragments were generated by mixing the PCR product with streptavidin-coated paramagnetic beads (Dynalbeads M280, Dynal, Norway). Pyrosequencing reactions were carried out at 25°C, with sequencing primer, DNA polymerase (exonuclease deficient), 40 mU apyrase, 4 µg of purified luciferase/ml, 15 mU of recombinant ATP sulfurylase, 0.1 M Tris-acetate (pH 7.75), 0.5 mM EDTA, 5 mM Mg-acetate, 0.1% BSA, 1 mM dithiothreitol, 10 uM adenosine 5'-phosphosulfate (APS), 0.4 mg of poly(vinylpyrrolidone)/ml and 100 µg of D-luciferin/ml. Pyrosequencing software (Pyrosequencing, AB) was used to determine and analyze single peaks in the pyrogram.

Statistical analysis. The statistical analyses were performed using the SAS/STAT® software, version 9.1 (SAS Institute Inc., Cary, NC, USA) (23). Allele frequencies in controls were tested for the Hardy-Weinberg equilibrium using Chi-square analysis or Fisher's exact test

Table III. Distribution of IL-10 genotypes in cases and controls.

IL-10 SNPs	Genotype	Frequency (%)	Cases	Controls	OR (95%CI)	p-Value
rs1800871	CC	37.7	84 (34.7)	74 (41.8)	1	
	TC	48.9	122 (50.4)	83 (46.9)	1.29 [0.85, 1.97]	0.227
	TT	13.4	36 (14.9)	20 (11.3)	1.59 [0.85, 3.01]	0.151
rs1800893	CC	36.5	86 (35.5)	67 (37.8)	1	
	TC	46.5	119 (49.2)	76 (42.9)	1.22 [0.79, 1.87]	0.365
	TT	17.0	37 (15.3)	34 (19.3)	0.84 [0.48, 1.49]	0.567
rs12122923	CC	83.5	164 (65.9)	85 (34.1)	1	
	TC	14.8	24 (46.2)	28 (53.8)	0.44 [0.24, 0.81]	0.009
	TT	1.7	3 (42.9)	4 (57.1)	0.39 [0.07, 1.8]	0.22
rs10494879	GG	96.4	234 (96.7)	170 (96.4)	1	
	GA	1.9	4 (1.65)	4 (2.2)	0.73 [0.17, 3.11]	0.655
	AA	1.7	4 (1.65)	3 (1.7)	0.96 [0.21, 4.97]	0.967
Adjusted for smoking						
rs12122923	CC		164 (56.9)	85 (34.1)	1	
	TC		24 (46.2)	28 (53.8)	0.44 [0.23, 0.83]	0.0110
	TT		3 (42.9)	4 (57.1)	1.66 [1.01, 2.72]	0.1853

when appropriate. The association between genotype, specific phenotype, and case/control status was investigated *via* logistic regression. Odds ratios (OR) of relative risk with 95% confidence intervals (CI) were calculated and adjusted for age as it is a possible confounder. Two-sided p -values ≤ 0.05 were considered statistically significant. For each SNP, tests using the genotypic, dominant, recessive, co-dominant, over-dominant, and log-additive genetic models were performed. Additionally, the Bonferroni test was performed to correct for multiple comparison testing and subsequent adjustments were made accordingly. Frequencies were estimated using the implementation of the EM algorithm coded into the haplostats package to test the combinatorial effects of the SNPs and haplotypes.

Results

Study population features and IL-10 SNPs distribution. For this study, 242 cases and 177 controls from the African American population were recruited. Their characteristics are depicted in Table II. The cases were generally older than controls. Indeed, the cases had a mean age of 69.02 years while controls mean age was 57.5 years. Vitamin D deficiency, whether severe or moderate was more pronounced in cases than controls. After correcting for the Bonferroni test, the p -value was 0.0125. There was also a significant difference in smokers within the cohort (41.7% in cases *vs.* 26.6% in controls). Within the PCa cases, there were 18 (7.4%), 114 (47.15%) and 25 (10.33%) grade I, II and III Gleason score, respectively (Table II). The 4 genotyped IL-10 promoter SNPs distribution in cases and control populations are reported in Table III.

Analysis of the SNPs associations with PCa. Each of these SNPs associations with PCa risks was examined with regard to genotype. rs12122923 was found to be associated with an

approximately 50% decreased risk [$p < 0.009$, OR=0.44 (0.24-0.81)] for PCa in heterozygotes genotype of TC (14.8%) (Table III). After adjusting for smoking, the SNP remained significant [24%, $p=0.0110$, OR=0.44 (0.23-0.83)] (Table III). The association between rs12122923 and PCa risk was further analyzed to determine the most likely model of inheritance (Table IV). CC genotype (86.8%) in the dominant model of inheritance showed an association with PCa risk ($p=0.0374$) providing a decreased risk [OR=0.58 (0.34-0.97)] for developing Pca (Table IV). CC genotype for rs12122923 showed significant association which does not agree with the dominant model for that SNP, but the fact that there were few T Homozygotes may confirm the dominant model as more reliable. When adjusted by age, only SNP rs12122923 showed an association with increased PCa risk in the dominant model [T/C-T/T, OR=1.78 (1.01-3.11)] (Table V).

The associations between the SNPs and PCa risk were further analyzed once adjusted for smoking status. SNP rs12122923 remained associated with smoking in the dominant model of inheritance [$p < 0.0056$, OR=0.43 (0.23-0.78)] (Table V). Additionally, adjusting for smoking status revealed an association between PCa risk and rs1800871 by the Log-additive model of inheritance ($p < 0.03901$). This association shows an increased risk of PCa development by 1.5-fold [OR=1.49 (1.02-2.18)] (Table VI).

When associations between the SNPs and Gleason score were analyzed, the heterozygous genotype (TC) for rs1800893 was found to be associated with lower grades for prostate cancer ($p < 0.0423$, OR=0.24) (Table VII, co-dominant model). The SNP remained associated when adjusted by age ($p < 0.053$, OR=0.25) for the heterozygous genotype (TC) (Table VII, co-dominant model adjusted by age). This association was further analyzed using the

Table IV. Genetic association between rs12122923 and PCa.

Model	Genotype	Cases (%)	Controls (%)	OR (95% CI)	p-Value
Co-dominant	CC	210 (86.8)	140 (79.1)	1.0	0.11
	TC	29 (12)	33 (18.6)	0.59 [0.34, 1.01]	
	TT	3 (1.2)	4 (2.3)	0.5 [0.11, 2.27]	
Dominant	CC	210 (86.8)	140 (79.1)	1.0	0.0374
	TC, TT	32 (13.2)	37 (20.9)	0.58 [0.34, 0.97]	
Recessive	CC, TC	239 (98.8)	140 (79.1)	1.0	0.424
	TT	3 (1.2)	4 (2.3)	0.54 [0.12, 2.46]	
Over-dominant	CC, TT	213 (88)	144 (81.4)	1.0	0.0594
	TC	29 (12)	33 (18.6)	0.59 [0.35, 1.02]	
Log-additive	0, 1, 2	242 (57.8)	177 (42.2)	0.62 [0.39, 0.98]	0.0402

Table V. Genetic association between rs12122923 and PCa (n=442, adjusted by age).

Model	Genotype	Cases	Controls	OR (95% CI)	p-Value
Codominant	C/C	221 (86.7%)	148 (79.1%)	1.00	0.13
	T/C	31 (12.2%)	35 (18.7%)	1.74 (0.97-3.13)	
	T/T	3 (1.2%)	4 (2.1%)	2.14 (0.41-11.21)	
Dominant	C/C	221 (86.7%)	148 (79.1%)	1.00	0.046
	T/C-T/T	34 (13.3%)	39 (20.9%)	1.78 (1.01-3.11)	
Recessive	C/C-T/C	252 (98.8%)	183 (97.9%)	1.00	0.43
	T/T	3 (1.2%)	4 (2.1%)	1.96 (0.38-10.24)	
Overdominant	C/C-T/T	224 (87.8%)	152 (81.3%)	1.00	0.071
	T/C	31 (12.2%)	35 (18.7%)	1.72 (0.96-3.08)	
Log-additive	–	–	–	1.65 (1.01-2.69)	0.047

dominant model, which showed a similar outcome to the co-dominant model ($p<0.0548$) providing a 74% decreased risk (OR=0.26) and was associated with a lower grade of prostate cancer. When adjusting for age, the association by the dominant model of inheritance did not remain significant ($p<0.0672$) (Table VII, dominant model, adjusted by age).

SNPs haplotype analysis. Haplotype analysis of the four IL-10 SNPs was evaluated for PCa risk. Pairwise linkage disequilibrium showed an association between all four SNPs; therefore, haplotypes from all four SNPs were analyzed to determine if there was consistency with the individual SNP analyses that were performed. The findings were similar to the individual SNP analyses: compared to the reference haplotype TCCG, only the heterozygous CTTG allele (which included combinations of rs1800871, rs1800893, and rs12122923) was associated with decreased risk of PCa (OR=0.26, $p<0.02$). Odds of PCa decreased by 74% when comparing CTTG to TCCG (Table VII, model 2). In analyzing haplotypic combinations between rs1800893, rs12122923, and rs10494879, only TTG ($p<0.05$) showed an association with PCa risk (Table VIII, model 4). Both rs1800871 and rs12122923 were found associated with a

decreased risk of PCa, and since both are in linkage disequilibrium, we cannot determine which one is associated with the disease. Further investigation is needed to determine which of both is a tag SNP and whether it is a representative of the haplotype (25).

To determine the strength of association between the number of copies of the risk allele and Prostate cancer, we used the likelihood ratio test to compare the null allele and ordinal SNP as a factor. All studied SNPs showed a nonlinear relationship between the number of copies of the risk allele and prostate cancer based on a non-significant p -value (data not shown).

Discussion

While the risk factors for PCa are well known, the molecular mechanisms and the genetics underlying the disease are still poorly understood. One such risk factor for PCa is ethnicity, in which African-American males are not only more likely to develop PCa (26), but there is also a 2.4-times higher risk of death from the disease (27). Chronic inflammation is also considered to play a role in the initiation and progression of the disease (7). The cytokine IL-10 acts to regulate an

Table VI. Genetic association between SNPs and PCa when adjusted for smoking.

IL-10 SNPs	Genotype	Cases (%)	Controls (%)	OR (95% CI)	p-Value
rs12122923					
Co-dominant	CC	155 (85.6)	77 (72.6)	1.0	0.0206
	TC	23 (12.7)	25 (23.6)	0.44 [0.23]	
	TT	3 (1.7)	4 (3.8)	0.35 [0.08, 1.64]	
Dominant	CC	155 (85.6)	77 (72.6)	1.0	0.0056
	TC, TT	26 (14.4)	29 (27.4)	0.43 [0.23, 0.78]	
Recessive	CC, TC	178 (98.3)	102 (96.2)	1.0	0.254
	TT	3 (1.7)	4 (3.8)	0.41 [0.09, 1.91]	
Over-dominant	CC, TT	158 (87.3)	81 (76.4)	1.0	0.0143
	TC	23 (12.7)	25 (23.6)	0.45 [0.24, 0.85]	
Log-additive	0,1,2	181 (63.1)	106 (36.9)	0.49 [0.29, 0.83]	0.0066
rs1800871					
Dominant	CC	62 (34.3)	46 (43.4)	1.0	0.095
	TC, TT	119 (65.7)	60 (56.6)	1.53 [0.93, 2.51]	
Recessive	CC, TC	155 (85.6)	98 (92.5)	1	0.0908
	TT	26 (14.4)	8 (7.5)	2.0 [0.86, 4.6]	
Log-additive	0,1,2	181 (63.1)	106 (36.9)	1.49 [1.02, 2.18]	0.03901

inflammatory response, and polymorphisms within this gene have been found to affect levels of cytokine expression (28) and associate with PCa risk and severity (13).

Herein, we analyzed the relationship between IL-10 promoter SNPs and PCa risk and severity specifically in African American men, where two SNPs, rs12122923, and rs1800871 found associated with decreased severity. Hovart *et al.* (14) showed similar results where PCa patients with IL-10-1082 AA genotype had a higher risk of developing PCa with GS >7 in Eastern Croatians (14) and North Indians (29). A genome-wide association study in Turkish and Korean cohorts showed a genome-wide significant association between rs1899871 and Behçet's disease (30). This disease and other immune-mediated diseases were found to be associated with increased malignancy risks. However, the relationship between Behçet's disease (BD) and cancer remains unclear (31). Further analysis of rs1800871 association with immune-mediated disease and cancers is needed to confirm its role in both outcomes.

This study also showed an association between 12122923 and PCa regardless of smoking status, in which the TC genotype provides a decreased risk of around 50%. When the associations were adjusted for smoking, we found an increased risk of PCa development by 1.6-fold. SNP rs1800871 was also found to result in a 1.5-fold increase in the risk of PCa development in the presence of the T allele (Table VI). A study authored by Sam S. Oh *et al.* showed similar findings, where rs1800871 was found to be associated with increased risk of many types of tobacco-related cancers (32).

Gleason score may be used as a measurement for the tumor severity by assigning a numerical value to the biopsy sample (21, 22). Of the four SNPs, only rs1800893 was associated with low Gleason score in which the TC genotype decreases the risk

Table VII. Association between rs1800893 and Gleason score.

Model	N	OR	p-Value
Co-dominant			
CC	17	1	-
TC	23	0.24	0.0423
TT	3	0.62	0.7194
Dominant			
CC	17	1	-
TC, TT	26	0.26	0.0548
Co-dominant adjusted by age			
CC	17	1	-
TC	23	0.25	0.053
TT	3	0.69	0.794
Dominant adjusted by age			
CC	17	1	-
TC, TT	26	0.27	0.0672

by 75%, indicating these individuals have a greater chance of developing less severe tumors in the presence of this SNP. The finding that multiple individual SNPs were associated with PCa risk and/or severity gives cause to consider the possibility that certain haplotypes within the IL-10 promoter would also associate with PCa risk. SNP array can become handy to map disease loci and determine the susceptibility genes that are in linkage disequilibrium with IL-10. Furthermore, we conducted a bioinformatics analysis of SNP-related data from Pca patients provided in the TCGA database. The results revealed that one mutant in IL10 was significantly associated with prostate cancer, specifically, the IL10 R125C mutation, where its oncogenic function is considered unknown.

Table VIII. Associations between SNPs' haplotypes and PCa.

	Model 1			Model 2		
	Coef. (Std)	OR	p-Value	Coef. (Std)	OR	p-Value
Intercepts	0.53 (0.23)	1.7	0.024	-6.89 (0.88)	0.0	0
Haplotypes						
TCCG (ref)		1		1	1	
CCCG	-0.095 (0.21)	0.9	0.656	0.276 (0.25)	1.32	0.270
CCTG	-0.64 (0.42)	0.53	0.125	0.25 (0.44)	1.29	0.566
CTCG	-0.183 (0.18)	0.83	0.309	0.038 (0.205)	1.04	0.852
CTTG	-0.65 (0.42)	0.52	0.126	-1.35 (0.58)	0.26	0.021
TTCG	0.72 (0.51)	2.06	0.162	0.997 (0.56)	2.71	0.074
Rare <3%	-0.118 (0.35)	0.89	0.734	0.38 (0.38)	1.47	0.321
Age				0.111 (0.012)	1.12	0.0
loglike	-279.74 (8df)	-220.14 (7df)				
	Model 3		Model 4			
	Coef. (Std)	p-Value	Coef. (Std)	p-Value		
Intercepts	0.44 (1.64)	0.008	-6.4 (0.8)	0.00		
CCG (ref)						
CTG	-0.51 (0.4)	0.202	0.04 (0.488)	0.925		
TCG	-0.03 (0.15)	0.82	0.04 (0.17)	0.794		
TTG	-0.53 (0.4)	0.197	-1.12 (0.57)	0.05		
Rare <0.03	-0.13 (0.34)	0.707	0.24 (0.37)	0.517		
Age			0.106 (0.01)	0.00		

Models 1 and 2 involve rs1800871 and rs1800893, rs12122923 and rs10494879. Models 3 and 4 involve rs1800893, rs12122923 and rs10494879.

Two haplotypes were associated with the risk of developing PCa. The CTTG haplotype at all four SNP loci resulted in a 74% decreased risk while the haplotype TTG for rs1800893, rs12122923, and rs1049479 was associated with increased PCa risk. A meta-analysis conducted by Zhang *et al.* showed that one of our SNPs (rs1800871) is part of a common haplotype in the promoter for IL-10 that is associated with increased cancer risk in Caucasian and in non-Caucasians (33). Determining an association between these individual SNPs and haplotypes to the development and severity of PCa implies that IL-10 may play a role in the biology of PCa. Dysregulation of cytokines may be linked to the incidence and clinical course of malignant diseases with the development of acute or chronic inflammatory reactions at tumor sites (34). IL-10 is a multifunctional cytokine with both immunosuppressive and anti-angiogenic functions, consequently resulting in both tumor-promoting and tumor-inhibiting properties (35). Results from this study suggest that IL-10 promoter polymorphisms might be a risk factor for PCa development in subjects who smoke and may contribute to high-grade cancers which may impact PCa progression in African American patients. A study conducted in Indians with PCa showed a strong association between SNPs and haplotypes in interleukin 10 and the aggressiveness of Pca.

The association increased when lifestyle habits including smoking were considered in the study (36). Determining IL-10 genotypes and haplotypes along with smoking history may help establish screening and prevention programs based on a stratification of the disease risk in the high risk African American male population.

Conflicts of Interest

The Authors have no conflicts of interest to disclose.

Authors' Contributions

MA, TM, GD, and HB contributed to the design and implementation of the research; AI, MK, YK, BW, LR, and MA participated in data acquisition and analysis; VA performed the statistical analysis; MA and HB wrote the manuscript.

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