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IGF2R genetic variants, circulating IGF2 concentrations and colon cancer risk in African Americans and Whites

Cathrine Hoyo[a,*], Susan K. Murphy[b], Joellen M. Schildkraut[a], Adriana C. Vidal[a], David Skaar[c], Robert C. Millikan[d], Joseph Galanko[e], Robert S. Sandler[e], Randy Jirtle[c] and Temitope Keku[e]
[a]*Department of Community and Family Medicine, and Program of Cancer Detection, Prevention and Control, Duke University Medical Center, NC, USA*
[b]*Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, and Department of Pathology, Duke University Medical Center, NC, USA*
[c]*Department of Radiation Oncology, Duke University Medical Center, NC, USA*
[d]*Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill, NC, USA*
[e]*Department of Medicine and Center for Gastrointestinal Biology and Disease, School of Medicine, University of North Carolina, Chapel Hill, NC, USA*

Abstract. The *Mannose 6 Phosphate/Insulin-like Growth Factor Receptor-2* (*IGF2R*) encodes a type-1 membrane protein that modulates availability of the potent mitogen, IGF2. We evaluated the associations between *IGF2R* non-synonymous genetic variants (c.5002G>A, Gly1619Arg(rs629849), and c.901C>G, Leu252Val(rs8191754)), circulating IGF2 levels, and colon cancer (CC) risk among African American and White participants enrolled in the North Carolina Colon Cancer Study (NCCCS). Generalized linear models were used to compare circulating levels of IGF2 among 298 African American and 518 White controls. Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association of *IGF2R* genetic variants and CC risk. Women homozygous for the *IGF2R* c.5002 G>A allele, had higher mean levels of circulating IGF2, 828 (SD = 321) ng/ml compared to non-carriers, 595 (SD = 217) ng/ml (p-value = 0.01). This pattern was not apparent in individuals homozygous for the *IGF2R* c.901 C>G variant. Whites homozygous for the *IGF2R* c.901 C>G variant trended towards a higher risk of CC, OR = 2.2 [95% CI(0.9–5.4)], whereas carrying the *IGF2R* c.5002 G>A variant was not associated with CC risk. Our findings support the hypothesis that being homozygous for the *IGF2R* c.5002 G>A modulates IGF2 circulating levels in a sex-specific manner, and while carrying the *IGF2R* c.901 C>G may increase cancer risk, the mechanism may not involve modulation of circulating IGF2.

Keywords: *IGF2R* polymorphism, colon cancer, IGF2 concentration

1. Introduction

Colon cancer is the third most commonly diagnosed cancer and the second leading cause of cancer death in the US [1,2]. Over 106,600 new cases are diagnosed each year and 55,170 individuals die from this disease in the U. S. alone [1]. The highest incidence and mortality are among African Americans and individuals residing in the southeastern seaboard of the United States [2]. Whereas reasons for higher mortality can be attributed to poorer access to screening resulting in later stage at diagnosis among African Americans compared to Whites [3], reasons for higher incidence are unclear. The interaction of genetic and environmental factors has been hypothesized to underlie racial differ-

*Corresponding author: Cathrine Hoyo, Department of Community and Family Medicine, Duke University School of Medicine, P.O. Box 104006, Durham, NC 27710, USA. E-mail: hoyo0001@mc.duke.edu.

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ences for some time [4], however, the causative genetic loci are still unknown. Genetic variation in genes encoding insulin and insulin-like growth factors (IGF) have been evaluated in relation to CC risk although the focus has been on IGF1 [4–10]. Few studies have evaluated IGF2, also a potent mitogenic growth factor, in circulation at higher physiologic concentrations than IGF1; IGF2 also has been associated with several malignancies [11].

The *Mannose 6 Phosphate/Insulin-like Growth Factor 2 Receptor* (*IGF2R*) encodes a ∼300 kDa multifunctional type 1 membrane protein recently identified as a tumor suppressor [12,13]. Although IGF2R regulates transport of lysosomal proteases and activates latent transforming growth factor β1, a potent growth inhibitor [14], this receptor also sequesters free IGF2 ligand, internalizing it for intracellular trafficking to the lysosomes for degradation [13–18], thereby modulating IGF2 availability and its mitogenic activity. In the mouse, loss of IGF2R function results in disproportionate embryonic overgrowth and perinatal death [19,20]. In human cells, loss of IGF2R function has been associated with the development of cancers of the breast, lung, liver and ovary [21,22].

More than 1,700 genetic variants of *IGR2R* have been reported to the NCBI database to date. In functional studies, Killian and Jirtle et al. [13] described six of these SNPs in the coding region of *IGF2R*, of which three are non-synonymous [c.6206 A>G, Asn2020Ser; c.901C>G, Leu252Val and c.5002G>A Gly1619Arg]. The effects of these genetic polymorphism on IGF2R function are only now beginning to be elucidated in physiological studies [15,23]. Despite their functional significance, these genetic variants are generally not interrogated in Genome-wide Association Studies (GWAS), because the commonly used Affymetrix 100K [24] and the Illumina Hap300 [25] chips do not include these rare alleles. Herein we evaluate two of the three non-synonymous *IGFR2* variants, c.901 C>G and c.5002 G>A, that have a minor allele population frequency of more than 5%, their association with higher IGF2 circulating levels, and colon cancer risk in African Americans and Whites.

2. Methods

2.1. Study participants

Study participants were recruited as part of the North Carolina Colon Cancer Study (NCCCS) and detailed accrual methods have been described in earlier reports [26]. Briefly, the NCCCS is a population-based, case-control study based in 33 contiguous rural and urban counties in central North Carolina comprising 39% African Americans (294 cases and 437 controls) and 61% Whites (349 of them cases and 611 controls). Cases of histologically confirmed invasive adenocarcinoma of the colon aged 40–85 diagnosed between July 1, 1996 and June 30, 2000 were identified using the rapid case ascertainment system administered by the North Carolina Cancer Registry. Because the sampling frame for < 65 year age group was the drivers' license roster, cases aged < 65 years had to have a North Carolina's driver's license or state identification cards. To oversample African Americans and women, and also maintain analytic flexibility, a randomized recruitment strategy [27,28] was employed; adjusted for in statistical analyses using a sampling fraction determined *a priori*. Controls under age 65 were sampled from the North Carolina's Division of Motor Vehicles driver's license list, matched by race and sex and frequency-matched by 5-year age group. Controls aged 65 years and older were frequency-matched to cases using the Medicare-eligible beneficiaries' roster. We were able to contact 86% of cases identified through the registry, and (84%), agreed to participate, an overall response rate of 72%, higher in Whites (76%) than African Americans (62%). Ninety-percent of controls identified were successfully contacted and of these, 62%, agreed to participate. Individuals who refused to participate or could not be reached were slightly younger than those who participated. The study was approved by the IRB at the University of North Carolina and all patients gave informed consent.

2.2. Data collection

Questionnaire data were collected using a standardized questionnaire by trained interviewers in person as previously described [26]. Information solicited included socio-demographic characteristics such as age, sex, marital status, education and household income; anthropometric measurements, including estimated weight one year prior to the interview and weight at interview, family history of cancer and personal medical history, as well as lifestyle factors that included physical activity, occupational history, smoking habits, and caloric intake. Weight, circumference of the waist and hip, and height were also measured at the time of interview. Information on nutrition was obtained using a version of the Block semi-quantitative ques-

tionnaire [29,30] modified to include some regional foods common in NC with a one year reference to fully account for seasonality. The questionnaire has been validated [31]. Information on non-steroidal anti-inflammatory drugs (NSAID) use was based on an affirmative response to a battery of questions about frequency of intake of several pharmaceutical products by name.

2.3. Collection and processing of blood specimens

Blood samples were collected from consenting participants at the time of the interview. All blood specimens were received by the laboratory within 24 hours, where buffy coat and plasma were separated, aliquoted and stored at $-80°$C. Eighty six percent of cases ($n = 529$) and 83% of controls ($n = 836$) who agreed to participate also agreed to provide peripheral blood samples for DNA and protein analyses. Participants who gave blood were more likely to be male, white, and to have never smoked compared to those who did not donate blood samples. To evaluate whether individuals who provided blood samples systematically differed from those who declined, we compared anthropometric measurements, NSAID use and stage at diagnosis of cases and found blood donors and non-donors to be comparable (data not shown).

2.4. Enzyme linked immunosorbent assays (ELISA) for IGF2

Plasma IGF2 was measured after acid-ethanol extraction to remove IGFBPs using reagents from Diagnostic Systems Laboratory (Webster, Texas), according to manufacturer's protocol. These analyses were measured in duplicates on stored plasma samples and laboratory personnel were blinded to the case or control status of samples. IGF2 levels were measured in 805 and 522 blood samples of the controls and cases, respectively. Each assay batch included positive controls, and masked samples derived from pooled plasma specimens labeled in a manner identical to that of study samples. Assay results were evaluated for implausible values and samples with questionable values were repeated. The intra-assay coefficient of variation was 1.5%, and the inter-assay coefficient of variation was less than 12%.

2.5. Genotyping

Genomic DNA was extracted from buffy coat using the PureGene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN). Genotyping was done using the 5' exonuclease (Taqman) assay. Primers for the *IGF2R* c.5002 G>A (rs629849) and c.901 C>G (rs8191754) variants were designed by Applied Biosystems (Foster City, CA). For c.901 C>G, the forward primer was 5'-CTA AGG GTA CTG TGA TTA TCA CTC-3' and the reverse primer was 5'-GAA AGT CAG GTC CTT GCT GGA G-3'. For the c.5002 G>A, the forward primer was 5'-GAA ATT GAT GGT CCT GAC TTG CG-3' and the reverse primer was 5'-GCA CTG GAG ATG CAC TTC TCC-3'. For quality control, a randomly selected 10% of samples were run in duplicate and genotyping was conducted without the knowledge of case status. Undetermined genotypes were excluded from analyses and constituted 1% and 0.7% of the total population for the *IGF2R* c.901 C>G and *IGF2R* c.5002 G>A variants, respectively. The average genotype concordance rate for duplicate samples was $> 98\%$.

2.6. Statistical analyses

We used chi-square tests to evaluate deviation from Hardy-Weinberg equilibrium (HWE) of *IGF2R* c.5002 G>A and *IGF2R* c.901 C>G genotypes among controls for Whites and African Americas separately. We used t-tests and analysis of variance, to evaluate mean differences in IGF2 levels between individuals who carried one or two of the minor alleles ('CG' or 'GG' for *IGF2R* c.901 C>G; 'GA' or 'AA' for *IGFR2* c.5002 G>A) and those homozygous for the common allele ('CC' or 'GG', for *IGF2R* c.901 C>G and *IGF2R* c.5002 G>A, respectively), adjusting for sex, age and race, since participants varied by these characteristics. Unconditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between carrying these genotypes and risk of colon cancer risk, again comparing carriers of at least one of the minor alleles to non-carriers, adjusting for potential confounding factors. Matching variables age, sex and race were included in all models estimating main effects as was the sampling fraction [26–28]. Effect modification by body mass index (BMI $=$ kg/m^2), cigarette smokers (ever, vs. never), caloric intake (1,800 or more vs. less than 1,800 calories per day), circumference of the waist (< 102 cm vs. $\geqslant 102$cm), use of NSAIDS (NSAID use for $\geqslant 15$ months vs. < 15 months in preceding diagnosis for cases and interview for controls), was evaluated using stratified analyses and cross-product terms of the genotype and potential effect modifier. We also assessed potential confounding by socioeconomic factors including household in-

Table 1
Distribution of characteristics of cases and controls in the North Carolina Colon Cancer Study

	White men ($n = 466$)		White women ($n = 371$)		Black men ($n = 263$)		Black women ($n = 294$)	
Characteristic	Case	Control	Case	Control	Case	Control	Case	Control
Age Group	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n(%)	n (%)
< 65	75 (44)	111 (37)	56 (41)	81 (35)	64 (55)	65 (45)	67 (55)	62 (36)
$\geqslant 65$	94 (56)	186 (63)	81 (59)	153 (65)	53 (45)	81 (55)	55 (45)	110(64)
Education Level								
$<$ HS grad	50 (30)	67 (23)	32 (23)	46 (20)	58 (50)	59 (40)	49 (40)	74 (43)
HS grad, college	74 (44)	137 (46)	79 (58)	136 (52)	50 (43)	70 (48)	64 (53)	78 (45)
College grad or more	44 (26)	92 (31)	26 (19)	52 (22)	9 (8)	17 (12)	8 (7)	20 (12)
NSAID use in past year								
Yes	81 (48)	175 (59)	48 (35)	122 (52)	46 (39)	67 (46)	35 (29)	92 (53)
No	88 (52)	122 (41)	89 (65)	112 (48)	71 (61)	79 (54)	86 (71)	80 (47)
BMI 1 year ago								
18.5–25 (Normal)	29 (17)	76 (26)	54 (41)	80 (36)	25 (22)	31 (23)	9 (8)	28 (18)
25–30 (Overweight)	79 (48)	143 (49)	46 (35)	77 (34)	49 (42)	63 (46)	47 (42)	48 (30)
30+ (Obese)	58 (35)	70 (24)	31 (24)	68 (30)	42 (36)	43 (31)	56 (50)	84 (52)
Regular Multivitamin User								
Yes	64 (39)	141 (51)	74 (56)	136 (61)	36 (32)	43 (31)	37 (32)	73 (45)
No	100 (61)	134 (49)	59 (44)	86 (39)	78 (68)	98 (69)	77 (68)	90 (55)
Smoking Status								
Current	21 (13)	55 (19)	20 (15)	28 (12)	30 (26)	42 (29)	20 (17)	30 (17)
Former	111 (66)	158 (53)	50 (36)	81 (35)	51 (44)	62 (42)	30 (25)	46 (27)
Never	35 (21)	83 (28)	67 (49)	125 (53)	35 (30)	42 (29)	70 (58)	96 (56)
Alcohol Use								
Yes	74 (45)	138 (48)	47 (35)	70 (31)	38 (33)	39 (27)	12 (10)	19 (11)
No	90 (55)	148 (52)	88 (65)	157 (69)	77 (67)	105 (73)	104 (90)	147 (89)
Calories per day								
$\leqslant 1{,}800$	44 (27)	102 (35)	88 (64)	175 (75)	40 (37)	71 (50)	72 (62)	123(74)
$> 1{,}800$	120 (73)	192 (65)	49 (36)	58 (25)	68 (63)	70 (50)	44 (38)	43 (26)

come and education, as well as lifestyle factors, including daily average caloric intake, cigarette smoking and frequency of alcohol intake. These factors did not change the β-coefficient for the association between genotypes evaluated and colon cancer by $\geqslant 10\%$, and were thus not retained in final models. Statistical models were developed for African Americans and Whites separately and then combined. We used SAS version 9.1 (SAS, Cary, NC, USA) for all statistical analyses.

3. Results

Characteristics of study participants have been previously described in several reports [26,32,33]. Table 1 summarizes the distribution of characteristics of study participants. Although minor gender variations were noted, in general, when compared to controls, cases were older, reported higher daily average caloric intake, had a higher BMI one year prior to interview, a larger waist circumference at interview, and were less likely to report intake of NSAIDs. However, cases did not significantly differ from controls with respect to education, alcohol consumption and a history of cigarette smoking.

To gain insights into the functional significance of *IGF2R* genetic variants, we evaluated whether individuals who carry the *IGF2R* c.5002 G>A and *IGF2R* c.901 C>G variants had higher average circulating levels of the IGF2 ligand among otherwise healthy controls (Table 2). We found that individuals homozygous for the *IGF2R* c.5002 G>A variant had higher mean levels of circulating IGF2, 593 (SD = 204) ng/ml compared to non-carriers, 563 (SD = 206) ng/ml (p-value = 0.06). These differences in mean IGF2 levels were most pronounced among women (p-value = 0.01). Notably, a gene-dose response trend was apparent in both African Americans and Whites (p for trend = 0.003). Adjusting for age, sex and race did not alter these findings.

We also found that individuals carrying at least one *IGF2R* c.901 C>G allele had higher mean circulating IGF2 levels, 587 (SD = 205) ng/ml) when compared to individuals who did not carry the genetic variant, 563 (SD = 205) ng/ml (p-value = 0.15). However, mean IGF2 concentrations were higher in individuals heterozygous for the *IGF2R* c.901 C>G allele, 592 (SD = 208) ng/ml) than those homozygous for this allele 520 (SD = 146) ng/ml (p-value = 0.09). These analyses were repeated in Whites and African Americans

Table 2
Means and standard deviations of plasma IGF2 levels (ng/ml) in $n = 805$ controls carrying the *IGF2R* c.901 and C.5002 variants

Characteristic	IGF2R c.901C>G, rs8191754		IGF2R c.5002G>A, rs629849		
Variant	Mean (sd)	p-value	Variant	Mean (sd)	p-value
All[1]					
CC ($n = 610$)	563 (205)		GG ($n = 661$)	563 (206)	
CG ($n = 182$)	592 (208)	0.09	AG ($n = 129$)	586 (193)	0.25
GG ($n = 13$)	520 (146)	0.46	AA ($n = 10$)	684 (313)	0.06
CG and GG ($n = 195$)	587 (205)	0.15	AG and AA ($n = 139$)	593 (204)	0.12
Men[2]					
CC ($n = 314$)	537 (192)		GG ($n = 336$)	532 (188)	
CG ($n = 102$)	552 (173)	0.48	AG ($n = 84$)	573 (180)	0.07
GG ($n = 9$)	521 (162)	0.92	AA ($n = 4$)	469 (139)	0.50
CG and GG ($n = 111$)	551 (171)	0.52	AG and AA ($n = 88$)	569 (179)	0.10
Women[2]					
CC ($n = 296$)	590 (214)		GG ($n = 325$)	595 (217)	
CG ($n = 80$)	642 (237)	0.06	AG ($n = 45$)	608 (215)	0.71
GG ($n = 4$)	496 (117)	0.39	AA ($n = 6$)	828 (321)	0.01
CG and GG ($n = 84$)	635 (235)	0.09	AG and AA ($n = 51$)	634 (237)	0.24
White[3]					
CC ($n = 381$)	587 (216)		GG ($n = 387$)	589 (219)	
CG ($n = 119$)	618 (218)	0.18	AG ($n = 111$)	595 (199)	0.80
GG ($n = 9$)	515 (166)	0.32	AA ($n = 8$)	687 (352)	0.21
CG and GG ($n = 128$)	611 (216)	0.29	AG and AA ($n = 119$)	602 (212)	0.59
Afr. American[3]					
CC ($n = 229$)	522 (177)		GG ($n = 274$)	526 (179)	
CG ($n = 63$)	542 (180)	0.42	AG ($n = 18$)	525 (139)	0.98
GG ($n = 4$)	533 (106)	0.90	AA ($n = 2$)	672 (115)	0.24
CG and GG ($n = 67$)	542 (176)	0.42	AG and AA ($n = 20$)	540 (141)	0.74

[1]Adjusted for age, sex and race; [2]Ajusted for age and race; [3]Ajusted for age and sex.

Table 3
Adjusted[1] odds ratios and 95% CIs for the association between carrying the *IGF2R* c.901 and c.5002 variants in African Americans and Whites

	IGF2R c.901C >G, rs8191754				IGF2R c.5002G >A, rs629849		
Genotype	Cases	Controls	OR (95% CI), p-value	Genotype	Cases	Controls	OR (95% CI), p-value
All	n (%)	n (%)			n (%)	n (%)	
CC	400 (74)	640 (76)		GG	457 (85)	691 (83)	
CG	123 (23)	187 (22)	1.1 (0.8, 1.4), ($p = 0.61$)	AG	79 (15)	133 (16)	1.0 (0.7, 1.3), ($p = 0.80$)
GG	14 (3)	13 (2)	1.9 (0.9, 4.1), ($p = 0.10$)	AA	3 (0.6)	11 (1)	0.5 (0.1, 1.8), ($p = 0.27$)
CG and GG	137 (26)	200 (24)	1.1 (0.9, 1.4), ($p = 0.37$)	AG and AA	82 (15)	144 (17)	0.9 (0.7, 1.3), ($p = 0.62$)
White[2]							
CC	226 (75)	393 (75)		GG	238 (78)	397 (76)	
CG	66 (22)	121 (23)	1.0 (0.7, 1.3), ($p = 0.80$)	AG	64 (21)	114 (22)	1.0 (0.7, 1.4), ($p = 0.79$)
GG	11 (4)	9 (2)	2.2 (0.9, 5.4), ($p = 0.09$)	AA	3 (1)	9 (2)	0.6 (0.2, 2.3), ($p = 0.47$)
CG and GG	77 (26)	130 (25)	1.0 (0.7, 1.4), ($p = 0.81$)	AG and AA	67 (22)	123 (24)	0.9 (0.7, 1.3), ($p = 0.67$)
African American[2]							
CC	174 (74)	247 (78)		GG	219 (94)	294 (93)	
CG	57 (24)	66 (21)	1.3 (0.8, 1.9), ($p = 0.27$)	AG	15 (6)	19 (6)	1.0 (0.5, 2.0), ($p = 0.91$)
GG	3 (1)	4 (1)	1.4 (0.3, 6.6), ($p = 0.64$)	AA	0 (0)	2 (1)	$-$ ($p = 0.98$)
CG and GG	60 (25)	70 (22)	1.3 (0.9, 1.9), ($p = 0.24$)	AG and AA	15 (6)	21 (7)	0.9 (0.4, 1.8), ($p = 0.70$)

[1]Adjusted for age, sex and race; [2]Adjusted for age and sex.

separately and also in men and women. The patterns persisted although the difference was most pronounced in women (p-value = 0.06). We found no evidence for higher IGF2 levels in individuals who carry both *IGF2R* c.901 C>G and the *IGF2R* c.5002 G>A genetic variants.

Table 3 shows the distribution of genetic variants *IGF2R* c.901 C>G and *IGF2R* c.5002 G>A, and the ORs and 95% CIs for the associations between carrying the variant alleles and risk of colon cancer among all participants and separately by race/ethnicity. There was no evidence that genotype frequencies in the *IGF2R*

c.901 C>G deviated from Hardy-Weinberg Equilibrium (HWE) among white or African American controls. Genotype frequencies among controls for the *IGF2R* c.901 CG and GG were 21% and 1% respectively in African Americans, and 23% and 2% respectively in Whites. Control participants genotype frequencies for the *IGF2R* c.5002 AG and AA were 22% and 2% respectively in Whites, and 6% and 1% in African Americans. While *IGF2R* c.5002 G>A genotype frequencies for White controls did not deviate from HWE (p-value>0.49), African American women controls deviate from HWE (p-value = 0.02). These frequencies are consistent with those reported in public databases, HapMap and NCBI databases.

A trend towards a significant association between homozygous for the G-allele for the *IGF2R* c.901C>G and colon cancer risk in all participants, OR = 1.9 [95% CI(0.9–4.1)] was observed. This association appeared to be primarily among Whites, OR = 2.2 [95% CI(0.9–5.4)] and not African Americans, OR = 1.4 [95% CI(0.3–6.6)]. However, the number of individuals homozygous for this genotype in African Americans was small. This association did not vary by NSAID use, BMI, waist circumference, cigarette smoking and daily caloric intake, (data not shown). We found no evidence for an association between being homozygous for the *IGF2R* c.5002 G>A and colon cancer risk among Whites, OR = 0.9 [95% CI(0.7–1.3)], and African Americans, OR = 0.9 [95% CI(0.4–1.8)]. We also evaluated whether the accumulation of the two genetic variants increased colon cancer risk, and no such synergistic association was found, (data not shown).

4. Discussion

We present the first population-based, epidemiologic evidence that otherwise healthy individuals who carry the non-synonymous *IGF2R* c.901 C>G, Leu252Val or c.5002 G>A Gly1619Arg genetic variants have higher average IGF2 levels. Gene-dose response trends were most apparent in individuals homozygous for the *IGF2R* c.5002 G>A. We also observed a trend towards an increased risk of colon cancer in white *IGF2R* c.901 C>G carriers. The accumulation of the two at-risk genotypes did not alter the association of these genotypes with protein levels, nor with colon cancer risk. Despite the identification of the IGF2 chromosomal location as one of three colon cancer susceptibility loci [34,35], the non-synonymous *IGF2R* genetic variants evaluated here have not been interrogated in Genome-wide Association Studies (GWAS), because the commonly used Affymetrix 100K [24] and the Illumina Hap300 [25] chips do not include these genetic variants.

Our findings that carrying the *IGF2R* c.5002 G>A is associated with elevated IGF2 levels are consistent with previous studies showing that variant *IGF2R* c.5002 G>A disrupts IGF2 ligand binding functions within IGF2R domain 11 [36,37]. However, a more recent NMR study showed no association between this polymorphism and IGF2 binding [15], and crystallography data showed that Gly1619Arg does not lie within the IGF2 binding site [16]. This polymorphism though, could be affecting splice site choice and exon skipping either directly or through linkage disequilibrium (LD) with other SNPs located in intronic sequences [15]. A recent study found a threefold increased risk of advanced oral squamous cell carcinoma in individuals carrying the *IGF2R* c.5002 variant Gly1619Arg A-allele compared to the wild type or G-allele [38]. *IGF2R* variant haplotypes were also associated with increased pancreatic cancer risk [39]. IGF2 circulating levels were not reported in these studies. Zhao et al. [40], reported that very high levels of circulating IGF2 are found in advanced colorectal cancer, however Renehan et al. [41] found an association between serum IGF2 and early colon cancer stages.

IGF2R binds and internalizes IGF2 maintaining appropriate levels of IGF2 locally and in circulation. When this equilibrium is disrupted either by loss of IGF2R function [18–20] or IGF2 loss of imprinting [42, 43], increased growth occurs in human and murine tumors [44,45]. However, the exact mechanism and its association to *IGF2R* polymorphisms is still unknown. Evidence based on published data suggests that even if *IGF2R* c.5002 G>A has no effect on function, it is in strong LD with a causative mutation. However, informatics suggest that *IGF2R* c.5002 G>A may have a regulatory effect at the RNA level. *IGF2R* c.5002 G>A sits in possible binding sites for multiple miRNAs, with the G and A alleles creating sequences complementary to different miRNAs. In addition, splice site predictions shows that the A- allele may create a splice acceptor site, as well as eliminate an enhancer motif for Serine/Arginine (SR) protein binding [46]. Recent results have indicated that *IGF2R* exon 34, which contains c.5002 G>A, is affected by alternative splicing, and is also contained in an antisense transcript [47]. The role of *IGF2R* c.5002 G>A or linked polymorphisms in splice site choice, as well as antisense expression or function, have yet to be determined.

We observed a different population distribution for the *IGF2R* c.5002 G>A variant when we stratified by sex and race; HWE deviations were more common in women ($p = 0.003$) and in African Americans ($p = 0.02$). HWE deviations in black women could have been due, at least in part to small sample size of African Americans ($n = 20$) carrying the *IGF2R* c.5002 G>A allele, compared to Whites ($n = 119$); genotyping errors were uncommon. Furthermore, *IGF2R* c.5002 G>A is a rare allele in linkage disequilibrium (LD) with a potentially functional mutation. The level of LD is influenced by the rate of mutation and population structure and it is known to influence HWE [48]. However, the association of variant *IGF2R* c.5002 G>A and IGF2 levels in women is strong ($p = 0.01$) and unlikely to be affected by the deviation from HWE, although further studies with a larger sample of women are required.

Although we also found a correlation between higher levels of IGF2 and carriers of the *IGF2R* c.901 C>G variant in healthy women, the molecular mechanisms underlying the suggested association are unclear and may differ from those related to the *IGF2R* c.5002 G>A variant. The amino acid variant c.901 Leu252Val is located in repeat domain 3 and this domain is involved in binding M6P moieties on other proteins [12]. Thus, *IGF2R* c.901 C>G may also alter function by changing the affinity for M6P bearing ligands which in turn may alter protein trafficking, with a consequent increase in the IGF2 ligand [50,51]. In predominantly white populations, carrying the *IGF2R* c.901 C>G has been associated with another gastrointestinal malignancy [52] and a tall stature [53]. Tall stature has been associated with colon cancer risk [54], and elevated IGF2 levels [55]. In the current data set, colon cancer risk was of borderline significance in Whites homozygous for the c.901 GG variant, and intriguingly, four of the five women were colon cancer cases compared to seven of fifteen men.

Data from publically available databases including HapMap and SNP500, suggest that the relative population frequency of *IGF2R* c.901 C>G and *IGF2R* c.5002 G>A do not parallel the higher incidence of colon cancer in African Americans. The prevalence of *IGF2R* c.901 C>G is similar in Whites (25%) and African Americans (22%), yet the risk of colon cancer associated with carrying this allele differs markedly between the two groups. In contrast, *IGF2R* c.5002 G>A is rare in African Americans (13%) and common in Whites (36%). This may suggest that while these genetic variants may contribute to modulating IGF2 concentrations via IGF2R binding, other mechanisms including deregulation of genomic imprinting may also play a role. Relaxation of imprinting controls leading to biallelic expression of IGF2 may also increase circulating IGF2 ligand directly and this may be important since the population frequency of IGF2 loss of imprinting could be as high as 10% to 20% in the population [51]. IGF2 loss of imprinting has been associated with a five-fold increase in colon cancer risk [34,35]. When taken together, *IGF2R* c.5002 G>A and c.901 C>G polymorphisms may exert their effects in a multifaceted manner, among them, by modulating IGF2 in circulation.

A major strength of this study is that it is one of the largest population-based case-control studies with the largest number of African Americans included, enabling evaluation of genetic effects on both colon cancer risk and on IGF2 protein concentrations in African Americans and Whites. Although this is not a genome-wide study and we cannot exclude the influence of other genes or other genetic variants in the insulin growth factor or related pathways, we evaluated genetic variants known to be amino acid-changing and found positive associations between carrying either variant and IGF2 protein levels. Despite its size, a limitation of this study is that the sample size limited our ability to adequately evaluate race-specific associations of these relatively rare alleles, as well as gene-environment interaction effects on colon cancer risk within race/ethnic group-specific associations. Departure from HWE may have been due, at least in part, to small sample size and rare allele frequencies of variant *IGF2R* c.5002 G>A in African American women.

In summary, we provide the first epidemiologic evidence that women homozygous for the *IGF2R* c.5002 G>A genetic variant have, on average, higher circulating levels of the mitogen IGF2. IGF2 is associated with increased risk of developing colorectal neoplasia. Our data also suggest that carrying the non-synonymous *IGF2R* c.901 C>G may be associated with increased colon cancer risk, although this association may not be via modulation of the ligand. Future studies in a larger sample of individuals carrying these rare allele variants are required to evaluate IGF2 levels and CC risk associations.

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