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Associations between birth and one year anthropometric measurements and *IGF2* and *IGF2R* genetic variants in African American and Caucasian American infants

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Abstract. Insulin-like growth factor 2 receptor (*IGF2R*) and insulin-like growth factor 2 (*IGF2*) genetic variants have been inconsistently associated with low birth weight and birth length in Caucasian and Asian infants, however few studies have included African Americans (AA). Generalized linear models and logistic regression models were used to examine associations between *IGF2R* single nucleotide polymorphisms (SNP) rs629849 and rs8191754, and *IGF2* SNP rs680 and infant anthropometric measurements, in a racially diverse birth cohort in Durham County, North Carolina. Caucasian American (CA) carriers of the *IGF2R* SNP rs629849 were heavier ($P = 0.02$) and longer ($P = 0.003$) at birth, however body size at age 1 yr was similar to that of AA. Birth length significantly differed between carriers and non-carriers of the *IGF2* rs680 variant in both AA ($P = 0.04$) and CA infants ($P = 0.03$). Both AA and CA carriers were 1 cm shorter at birth compared to non-carriers. We found no evidence for an association between rs8191754 and infant anthropometric measurements. Associations between SNPs and

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one year weight gain were only observed for rs680; CA infant carriers of rs680 variants weighed less than non-carriers at year one (P = 0.03); however, no associations were found in AA infants at year one. Larger studies using ancestral markers are required to disentangle these associations.

Keywords: Insulin-like growth factor 2 receptor, insulin-like growth factor 2, genetic variants, African Americans, race/ethnicity

1. Introduction

Birth weight is a general indicator of maternal impact on fetal development that strongly predicts overall post-natal development [1,2]. Epidemiological evidence has repeatedly demonstrated consistent associations between low birth weight (LBW) and chronic diseases including cardiovascular diseases, type 2 diabetes and some cancers, later in life [3–5]. LBW, caused by cigarette smoking or famine, is also associated with high early growth velocity, which may increase the risk of adult obesity and type 2 diabetes [6,7]. For reasons that are as yet unclear, LBW varies considerably by race/ethnicity; infants of African descent are born smaller than infants of European descent. Although genetic factors have been hypothesized to underlie these racial differences, the loci are still largely unknown [8]. Causative loci of LBW may serve as potential markers for susceptibility to chronic disease and conditions including obesity and type 2 diabetes.

The insulin-like growth factor 2 (*IGF2*) gene encodes a potent mitogen that is critical to fetal development [9]. In mice, the disruption of the *IGF2* gene causes a 60% growth deficiency throughout life when compared to wild type mice [10]. In human adults, higher IGF2 protein levels are associated with increased risk of obesity. Carrying the *IGF2* single nucleotide polymorphism (SNP) rs680, has been associated with higher IGF2 serum protein concentration and higher body mass index (BMI) in adult men, although lower birth weight in a Japanese population has also been reported [11–13]. Roth et al. [14] also found that Caucasians homozygous for the variant SNP rs680 had a higher fat mass. The mannose six phosphate/insulin-like growth factor 2 receptor (*IGF2R*) gene encodes a receptor that regulates bioavailability of IGF2 by binding the IGF2 free ligand, sequestering and trafficking it to the lysosomes for degradation, thereby modulating IGF2 availability and its mutagenic activity. Allelic variation in the *IGF2R* SNP rs629849 has been associated with a slower growth trajectory at 3 and 7 yr of age in a European population, whereas, among Japanese children, carrying the *IGF2R* SNP rs629849 was associated with LBW [13,15–17]. In the present study, we examined associations of genetic variants in the *IGF2* and *IGF2R* genes: *IGF2R*'s SNP rs629849, rs8191754 and *IGF2* SNP rs680, in relation to anthropometric measurements at birth and at age one, in African Americans (AA) and Caucasian Americans (CA).

2. Materials and methods

2.1. Study participants

Pregnant women were enrolled as part of the Newborn Epigenetics Study, a prospective study of women and their offspring. Detailed methods for enrollment of study participants have been detailed elsewhere [18]. Briefly, pregnant women were recruited from prenatal clinics and obstetric facilities affiliated with Duke University and Durham Regional Hospitals. To be eligible, women had to be aged 18 yr and older, pregnant, and intend to use one of two Durham County obstetric care facilities: Duke University Obstetrics or Durham Regional Hospital. Those excluded were human immunodeficiency virus infected women and those who planned to have their offspring adopted. Of the 1101 approached participants, a total of 940 (85%) consented and were enrolled. Most enrollees (81%) were successfully followed to delivery (n = 757). The study protocol was approved by the Duke University Institutional Review Board. We genotyped 539 cases with complete baseline questionnaires and medical records data, which were comparable to those of the entire cohort not included in this analysis with respect to maternal age (P = 0.26), race (P = 0.60), education (P = 0.79) and infant gestational age at birth (P = 0.45).

2.2. Data collection

Participants completed a self-administered questionnaire at the time of enrollment that collected data on socio-demographic characteristics, reproductive factors and lifestyle factors such as cigarette smoking and

anthropometric measurements before and during pregnancy. Medical records were abstracted at delivery to obtain information on birth outcomes, including birth weight and length. Umbilical cord blood specimens were also collected at birth. A 1 yr survey was administered in person or via phone, or collected via mail approximately 1 yr after birth to collect anthropometric and dietary data of the child. Mothers self-reported weight and height of their child at 1 yr was validated by abstracting medical records measurements, and the correlation was r = 0.98, as previously described [19]. One yr weight gain was estimated as the difference between birth weight and weight at age 1 year. Weight-for-height percentile at age 1 yr was calculated using Statistical Analysis System (SAS) software.

2.3. Specimen handling and genotyping

Genomic deoxyribonucleic acid (DNA) was extracted from buffy coat of umbilical cord blood using the PureGene DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN). Genotyping of the genetic variants *IGF2R* SNP rs629849 (c.5002G>A) in exon 34, the *IGF2R* SNP rs8191754 (c.901G>C) in exon 6 and *IGF2* SNP rs680 (+3123/ApaI G>A) in exon 9, was performed using Taqman ® genotyping assays (Assay IDs: C__1096470_10, C__2200985_10, and RS680, respectively) from Applied Biosystems that run on 7900 HT fast real-time polymerase chain reaction (PCR) system. For the SNP rs629849 and the SNP rs680 variants the forward strands were used, while for the SNP rs8191754 variant we used the reverse strand. Due to the limited number of variant genotype for each SNP, genotype status was dichotomized into carrying the variant allele versus not carrying the variant allele for the respective polymorphism, in order to assess the influence of the variant genotype in the statistical models. Carriers were either heterozygous or homozygous for the variant allele. Non-carriers were homozygous for the common allele. Failure rate was <0.001% for the *IGF2R* rs629849 and the *IGF2* rs680, and approximately 0.01% for the *IGF2R* rs8191754.

2.4. Statistical analyses

We assessed the association between four anthropometric measurements (birth weight, birth length, 1 yr weight gain and age 1 weight-for-height percentile) and each genotype: *IGF2R* rs629849, *IGF2R* rs629849, and *IGF2* rs680 by comparing means and standard deviations of the outcome measurements by genotype carrier status, and utilizing both logistic regression and generalized linear models. Potential confounders were identified by reviewing the literature, e.g. whether factors known to influence birth or 1 yr anthropometric measures significantly differed in our study population. Linear regression models were utilized to examine the associations between birth and 1 yr measures and genotypes *IGF2R* rs629849, *IGF2R* rs8191754 and *IGF2* rs680 (carrying vs. not carrying the variant allele) and adjusting for the co-variates that were statistically significantly associated with birth and 1 yr measures: race, maternal education, gestational age, pre-pregnancy folic-acid use, maternal BMI, smoking status and baby gender. Birth weight was categorized into three levels: <2500 g, 2500–3999g, and ≥4000 g; birth length was dichotomized at the 80th percentile of the sample, and both 1 yr outcomes were dichotomized at the 85th percentile. An alpha level of 0.05 was defined as statistically significant. All statistical analyses were conducted in SAS v9.3 (SAS Institute Inc., Cary, NC).

3. Results

The characteristics of study participants and the offspring by birth and two early childhood anthropometric measurements are summarized (Table 1). Maternal age at enrollment ranged from 18–46 yr (mean 29 ± 6 yr). Most (65%) infants were born to older women (age >25 yr). Infants born to women who self-described themselves as AA comprised 51% of the study participants. Forty-three percent were CA and gave birth to heavier and longer infants compared to AA (BWT $P < 0.0001$; BL $P < 0.0001$). The majority of women (60%) who had some college education and/or attended graduate school also had larger infants than the 40% who only attended high school or less (BWT $P < 0.002$; BL $P \leq 0.0003$). Forty-nine percent of women had a BMI <25, 22% were overweight (BMI 25–30) and 29% were obese (BMI ≥30). Most reported no folic acid intake before pregnancy. Approximately one-fifth of the women smoked either throughout pregnancy or smoked during peri-conception (19%) where as 52% never smoked, and approximately 30% were adult smokers who quit sometime before pregnancy. As expected, women in the obese BMI category and those who never smoked gave birth to the largest babies compared to their respective category counterparts. Males and females were distributed almost

Table 1
Maternal and infant characteristics by birth and one year outcome measurements

	Birth outcomes				One year outcomes			
Maternal age at delivery	n	Birth weight (mean ± SD) (g) n = 523	n	Birth length (mean ± SD) (cm) n = 382	n	One year weight gain (mean ± SD) (g) n = 229	n	Weight-for-height percentile (mean ± SD) (g) n = 267
18–25	178	3078 ± 612	141	49 ± 2**	69	7069 ± 1817	90	60 ± 34
26–30	138	3121 ± 579	96	49 ± 3	50	6597 ± 1649	59	61 ± 33
31–34	95	3164 ± 623	67	49 ± 2	47	6656 ± 1381	48	59 ± 32
35+	112	3258 ± 737	78	49 ± 2	63	7003 ± 1236	70	57 ± 33
Race								
African-American	261	3031 ± 622*	198	48 ± 2*	90	7162 ± 1855**	130	62 ± 33
Caucasian American	222	3265 ± 635	154	49 ± 3	122	6653 ± 1339	112	56 ± 34
Asian/North American/other	29	3348 ± 542	22	49 ± 3	14	6662 ± 960	20	61 ±27
Maternal education								
≤High school	204	3044 ± 645*	152	48 ± 3*	65	6977 ± 2047	96	64 ± 32**
Some college/ College grad/ grad School	312	3217 ± 624	225	49 ± 2	163	6815 ± 1316	168	56 ± 33
Marital status								
Married/with partner	325	3158 ± 657	226	49 ± 3	160	6820 ±1453	161	57 ± 33
Never married/ divorced/ widowed	192	3132 ± 604	152	49 ± 3	68	6958 ± 1784	103	62 ± 34
Gestational age								
<35 weeks	37	1977 ± 695*	4	48 ± 3	19	7124 ± 1335	22	52 ± 34
≥35 weeks	486	3232 ± 537	378	49 ± 3	210	6839 ± 1572	245	60 ± 33
Pre-pregnancy folic acid use								
None	274	3085 ± 628**	200	49 ± 2*	111	6959 ± 1693**	133	60 ± 33
<1000 μg/d	93	3203 ± 634	69	49 ± 2	40	6577 ± 1447	48	56 ± 36
1000–1200 μg/d	89	3272 ± 606	65	50 ± 3	51	6630 ± 1415	52	53 ± 34
>=1200 μg/d	59	3158 ± 715	43	49 ± 3	26	7334 ± 1263	31	69 ± 25
Maternal body mass index								
<25	231	3158 ± 635*	172	49 ± 3*	120	6706 ± 1320**	131	55 ±33**
25 − < 30	99	2986 ± 700	70	48 ± 3	42	7058 ± 1855	48	57 ± 34
≥30	135	3235 ± 599	96	49 ± 2	52	7204 ± 1628	59	69 ± 32
Smoking status								
Never smoked	277	3203 ± 613*	197	49 ± 3*	132	6691 ± 1415**	141	57 ± 33
Smoked during periconception	99	2938 ± 691	78	48 ± 2	33	7063 ± 2234	48	55 ± 34
Quit smoking before periconception	147	3170 ± 617	107	49 ± 3	64	7115 ± 1374	78	65± 33
Gestational or type II diabetes								
No	456	3134 ± 631**	364	49 ± 3**	212	6826 ± 1502**	245	59 ± 32**
Gestational diabetes	7	2954 ± 497	5	48 ± 1	4	7078 ± 918	6	62 ± 39
Type II diabetes	24	3275 ± 651	15	48 ± 2	9	6567 ± 1400	10	51 ± 35
Infant gender								
Male	266	3186 ± 629**	195	49 ± 2*	125	6955 ± 1540	142	59 ± 34
Female	257	3099 ± 642	187	49 ± 3	104	6753 ± 1569	125	59 ± 32

*f-test $P \leq 0.05$
**f-test $P > 0.05$

equally among body sizes at both birth and at age 1 yr, and most (93%) were born at gestational age of ≥35 wk. Full term infants' mean birth weight was 3232 ± 537 g, with the exception of those born at gestational age ≤35 wk, who weighed approximately 1977 ± 695 g. Mean length at birth was 48.8 ± 2.6 cm. At 1 yr of age, mean weight was 10,023 ± 1595 g, and mean recumbent length was 78.1 ± 8.6 cm. 1-year-old AA infants gained more weight over the course of the first year compared to CA infants (AA: 7161 ± 1855 g; C: 6653 ± 1339 g, $P = 0.06$); however, the difference was not statistically significant. Maternal BMI was associated with both one yr anthropometric measurements (1 yr gain $P = 0.11$, WTPCT $P = 0.02$). Maternal race/ethnicity, pre-pregnancy folic acid use, and smoking status also differed with respect to 1 yr weight gain, ($P = 0.06$, $P = 0.15$, $P = 0.15$, respectively), however differences were not statistically significant.

3.1. Birth outcomes by genotype

We ran generalized regression models to analyze the relationship between *IGF2R* and *IGF2* SNPs and birth anthropometric measurements, adjusting for race, maternal education, gestational age, baby gender and maternal BMI. Infants who carry at least one *IGF2R* rs629849 variant were statistically significantly heavier and longer at birth, compared to non-carriers (ß = 168.3, SE = 85.4, $P = 0.05$) (Table 2). Stratifying by race/ethnicity revealed that the differences in weight ($P = 0.02$) and length ($P = 0.003$) at birth persisted only in CA infant carriers. However, the interaction term for the association between race and carrying the *IGF2R* rs629849 variant was not statistically significant ($P = 0.21$) (Table 2). In logistic regression models, adjusted for the same variables, infants who carried at least one *IGF2R* rs629849 variant were twice as likely to weigh ≥ 4000g when compared to non-carriers born with normal birth weight (2500–4000g) (OR = 2.2, 95% CI = 1.00–5.12) (data not shown). We found no evidence for an association between the *IGF2R* rs8191754 genotype and birth or 1 yr outcomes.

Unadjusted birth weight and length means by *IGF2R* rs629849, *IGF2R* rs8191754 and *IGF2* rs680 genotype status are shown in table 3. Two percent of CAs were homozygous carriers of the *IGF2R* rs629849 variant, while 4% of AA were carriers, but none were homozygous. Infants who carry at least one *IGF2R* rs629849 variant allele weighed significantly more (mean birth weight = 3392 ± 538 g), and were longer at birth, (mean length = 50 ± 2.1 cm), compared to non-carriers (mean birth weight = 3105 ± 642 g, $P = 0.0004$; mean birth length = 49 ± 2.6 cm, $P = 0.0001$).

Birth length significantly differed between carriers and non-carriers of the *IGF2* rs680 genotype in both AA and CA infants. AA infant carriers of at least one variant allele of this polymorphism were ~one cm longer at birth, compared to non-carriers ($P = 0.04$); while CA carriers were one cm shorter at birth compared to CA non-carriers $P = 0.03$). A gene dose-response relationship between carrying the *IGF2* rs680 polymorphism and birth weight, although not statistically significant, is suggested for AA, since homozygous carriers for the *IGF2* rs680 were heavier compared to non-carriers and heterozygous carriers (Table 3).

Table 2
Adjusted regression coefficients and for the association between genotype and birth anthropometric measurements and interaction term *P* values for the race/genotype

	IGF2R rs629849		*IGF2* rs680	
Birth weight	ß-coefficient ± SE	*P* value	ß-coefficient ± SE	*P* value
Model 1	168.3 ± 85.4	0.05	67.1 ± 61.6	0.28
Model 2 interaction term	—	0.21	—	0.09
P value for race/genotype				
Birth length				
Model 3	0.8 ± 0.4	0.05	0.04 ± 0.3	0.90
Model 4 interaction term	—	0.90	—	0.01
P -value for race/genotype				

IGF2R = Insulin-like growth factor 2 receptor gene; *IGF2* = Insulin-like growth factor 2 gene.
Model 1: Adjusted for race, maternal education, gestational age, pre-pregnancy folic-acid use, maternal body mass index, and smoking status.
Model 2: Race/genotype interaction term, adjusted for race, maternal education, gestational age, maternal body mass index.
Model 3: Adjusted for race, maternal education, pre-pregnancy folic-acid use, maternal body mass index, smoking status, and baby gender.
Model 4: Race/genotype interaction term adjusted for race, maternal education, baby gender.

Table 3
Birth outcomes by genotype for all participants, African American and Caucasian American infants

Characteristics	All participants (n = 539)			African Americans (n = 266)			Caucasians (n = 225)		
	Birth weight (g)								
IGF2R rs629849	n	Mean ± SD	*P* value	n	Mean ± SD	*P* value	n	Mean ± SD	*P* value
Non-carrier	453	3105 ± 642	Referent	250	3024 ± 628	Referent	170	3212 ± 654	Referent
Heterozygous carrier	66	3399 ± 548	0.0001	11	3195 ± 441	0.37	48	3452 ± 554	0.01
Homozygous carrier	4	3283 ± 302	0.33	–	–	–	4	3283 ± 302	0.68
Any carrier	70	3392 ± 538	0.0004	11	3195 ± 441	0.37	52	3439 ± 539	0.02
IGF2R rs8191754									
Non-carrier	402	3135 ± 646	Referent	205	3021 ± 615	Referent	170	3275 ± 656	Referent
Heterozygous carrier	106	3163 ± 601	0.68	47	3063 ± 639	0.69	47	3216 ± 573	0.54
Homozygous carrier	9	3134 ± 691	0.99	6	3041 ± 849	0.96	3	3320 ± 172	0.72
Any carrier	115	3160 ± 605	0.71	53	3060 ± 656	0.69	50	3222 ± 557	0.60
IGF2 rs680									
Non-carrier	357	3121 ± 656	Referent	213	3006 ± 620	Referent	121	3296 ± 692	Referent
Heterozygous carrier	139	3198 ± 585	0.21	41	3125 ± 655	0.28	84	3232 ± 555	0.48
Homozygous carrier	26	3146 ± 639	0.85	7	3260 ± 424	0.17	17	3208 ± 598	0.58
Any carrier	165	3189 ± 592	0.25	48	3145 ± 625	0.16	101	3228 ± 560	0.43
	Birth length (cm)								
IGF2R rs629849									
Non-carrier	329	49 ± 2.6	Referent	187	48 ± 2.4	Referent	117	48 ± 2.6	Referent
Heterozygous carrier	49	50 ± 2.2	0.0001	11	49 ± 2.5	0.53	33	51 ± 2.0	0.002
Homozygous carrier	4	50 ± 0.5	0.20	–	–	–	4	50 ± 0.5	0.38
Any carrier	53	50 ± 2.1	0.0001	11	49 ± 2.5	0.53	37	50 ± 1.9	0.003
IGF2R rs8191754									
Non-carrier	284	49 ± 2.6	Referent	151	48 ± 2.4	Referent	114	49 ± 2.6	Referent
Heterozygous carrier	84	49 ± 2.6	1.00	39	48 ± 2.3	0.84	35	50 ± 2.5	0.82
Homozygous carrier	8	49 ± 2.8	0.82	5	48 ± 3.6	0.92	3	50 ± 0.0	0.69
Any carrier	92	49 ± 2.6	0.94	44	48 ± 2.4	0.89	38	50 ± 2.4	0.75
IGF2 rs680									
Non-carrier	264	49 ± 2.6	Referent	162	48 ± 2.5	Referent	82	50 ± 2.5	Referent
Heterozygous carrier	99	49 ± 2.3	0.50	30	49 ± 2.0	0.03	61	49 ± 2.3	0.04
Homozygous carrier	18	49 ± 3.0	0.95	6	49 ± 1.5	0.61	11	49 ± 3.8	0.16
Any carrier	117	49 ± 2.4	0.55	36	49 ±1.9	0.04	72	49 ± 2.5	0.03

IGF2R = Insulin-like growth factor 2 receptor gene; *IGF2* is insulin-like growth factor 2 gene.

3.2. One year anthropometric measures by genotype

Differences in weight for age and length for height at age approximately 1 yr in AA and CA are summarized in table 4. We found no differences between weight-for-height percentile measurements and *IGF2R* rs629849, *IGF2R* rs8191754 and *IGF2* rs680 polymorphisms. However, carriers of the variant allele of the *IGF2* rs680 gained less weight over the course of 1 yr compared to non-carriers ($P = 0.01$), after adjusting for maternal education and gender of infant. Following race-restricted analyses, statistical significance was retained only in CA infants ($P = 0.03$). The interaction term for *IGF2* rs680 genotype and race was $P = 0.01$. The minor allele frequencies for rs680 were in All = 018, AA = 0.1, CA = 0.26; for

Table 4
Age one outcomes by genotype for all participants, African American and Caucasian infants

Characteristics	All Participants (n = 539)			African Americans (n = 266)			Caucasian Americans (n = 225)		
	1 year weight gain (g)								
IGF2R rs629849	n	Mean ± SD	*P* value	n	Mean ± SD	*P* value	n	Mean ± SD	*P* value
Non-carrier	191	6916 ± 1589	Referent	85	7169 ± 1859	Referent	92	6696 ± 1352	Referent
Heterozygous carrier	35	6658 ± 1323	0.31	5	7033 ± 1987	0.89	27	6591 ± 1278	0.71
Homozygous carrier	3	5871 ± 1733	0.41	–	–	–	3	5871 ± 1733	0.50
Any carrier	28	6596 ± 1348	0.25	5	7033 ± 1987	0.89	30	6519 ± 1311	0.53
IGF2R rs8191754									
Non-carrier	170	6902 ± 1616	Referent	72	7194 ± 1907	Referent	88	6683 ± 1365	Referent
Heterozygous carrier	52	6754 ± 1327	0.51	15	7001 ± 1639	0.69	30	6606 ± 1287	0.78
Homozygous carrier	5	6201 ± 1395	0.33	2	5968 ± 1107	0.35	3	6357 ± 1785	0.78
Any carrier	57	6706 ± 1329	0.41	17	6880 ± 1595	0.53	33	6583 ± 1306	0.72
IGF2 rs680									
Non-carrier	144	7071 ± 1669	Referent	72	7261 ± 1921	Referent	64	6900 ± 1416	Referent
Heterozygous carrier	69	6481 ± 1322	0.01	14	6642 ± 1621	0.22	47	6361 ± 1266	0.04
Homozygous carrier	16	6640 ± 1027	0.31	4	7191 ± 1373	0.93	11	6460 ± 925	0.20
Any carrier	85	6511 ± 1268	0.01	18	6764 ± 1548	0.31	58	6380 ± 1202	0.03
	Weight-for-height percentile								
IGF2R rs629849									
Non-carrier	225	60 ± 32	Referent	122	61 ± 33	Referent	83	57 ± 32	Referent
Heterozygous carrier	38	56 ± 38	0.61	8	71 ± 38	0.50	25	50 ± 39	0.45
Homozygous carrier	4	66 ± 41	0.79	-	–	–	4	66 ± 41	0.70
Any carrier	42	57 ± 38	0.67	8	71 ± 38	0.50	29	52 ± 39	0.54
IGF2R rs8191754									
Non-carrier	197	59 ± 34	Referent	98	63 ± 33	Referent	83	56 ± 34	Referent
Heterozygous carrier	61	60 ± 29	0.73	28	58 ± 30	0.53	24	57 ± 31	0.87
Homozygous carrier	5	55 ± 48	0.87	2	47 ± 66	0.51	3	61 ± 49	0.88
Any carrier	66	60 ± 31	0.81	30	58 ± 32	0.47	27	57 ± 32	0.83
IGF2 rs680									
Non-carrier	182	59 ± 34	Referent	107	61 ± 34	Referent	60	55 ± 34	Referent
Heterozygous carrier	72	60 ± 31	0.90	18	70 ± 27	0.30	45	56 ± 34	0.85
Homozygous carrier	13	57 ± 31	0.83	5	56 ± 34	0.76	7	56 ± 33	0.92
Any carrier	85	59 ± 31	0.97	23	67 ± 28	0.45	52	56 ± 34	0.85

IGF2R = Insulin-like growth factor 2 receptor gene; *IGF2* = Insulin-like growth factor 2 gene.

rs629849: All = 0.08, AA = 0.02, CA = 0.13; and for rs8191754 All = 0.13, AA = 0.12, CA = 0.12. The genotype frequencies of the *IGF2R* gene variants were in Hardy-Weinberg equilibrium (HWE) (http://www.ncbi.nlm.nih.gov/projects/SNP) for both CA (rs629849: $P = 0.51$; rs8191754: $P = 0.80$) and AA (rs629849: $P = 0.73$; rs8191754: $P = 0.12$). The *IGF2* rs680 variant deviated from HWE in AAs ($P = 0.01$), but not in CAs ($P = 0.60$).

4. Discussion

In these analyses, we examined the association between common genetic variants in the *IGF2* and *IGF2R* genes and phenotypic outcomes that included body size at birth and at age 1 yr. We found that CA, but not AA, infant carriers of at least one *IGF2R* rs629849 variant were significantly larger at birth than

non-carriers. These birth outcomes did not vary by genotype in AA infants, presumably because there were no AA homozygous for the *IGF2R* rs629849 variant. Carriers and non-carriers of the *IGF2R* rs629849 variant had comparable anthropometric measurements at age 1 yr. Evidence for differences in carriers and non-carriers of the *IGF2R* rs8191754 and *IGF2* rs680 variants were less consistent. In general, AA infants were born smaller but gained an average of half a kg more weight during the first year of life, compared to CA infants, regardless of genotype (mean birth weight AA: 3031 g, CA: 3265 g and age 1 yr mean weight gain AA: 7162 g, C: 6653g).

Findings that CA carriers of the *IGF2R* rs629849 variant were heavier at birth are consistent with those of others; however, our data demonstrated statistical significance and present evidence for a possible race-specific effect [17]. Our results are also in agreement with current understanding that carrying the variant 'A' allele of *IGF2R* rs629849 SNP may result in decreased soluble *IGF2R*, thus increasing IGF2 circulating levels, a known mitogen [8]. Our findings suggest that at least in CA, the *IGF2R* rs629849 and the *IGF2* rs680 polymorphisms may influence birth length and weight. Consistent to a Greek cohort and as reported by the only other study with AA mothers and infants, we did not find any association with birth nor with 1 yr anthropometric measures and *IGF2R* rs8191754 genetic variants [15,16]. Our findings, however, contrast with reports by Kaku et al. [13] in a Japanese population, where the *IGF2R* rs8191754 genotype was associated with heavier birth weight. Those investigators also observed that carriers of *IGF2* rs680 variants had lower weight at birth, an association not found in our analyses, nor in analyses reported by others [15,20,21]. However, we found that the *IGF2* rs680 polymorphism was associated with longer length at birth in AA but not in CA infants. Although the difference may have been too small to be of clinical significance, it is possible that unmeasured loss of imprinting of the *IGF2* gene may account for some of the observations. Those studies, together with our findings suggest that there may be racial/ethnic differences in genetic factors modulating bioavailability of IGF2, which may influence growth and birth outcomes; these differences could possibly be the product of functional rare variants in linkage disequilibrium with these polymorphisms, as suggested by Souren et al. [21]. In this light, our inability to find associations with 1 yr weight and weight gain would be consistent with the hypothesis that larger infants at birth would not require catch-up growth in the first months of life, as is the case for infants who suffer fetal growth restriction [22,23]. Nevertheless, as previously suggested, longer growth follow-ups at yr 3 and 7 will be required to examine the phenotypic outcomes of these polymorphisms [17].

The mechanisms by which these variants could influence IGF2 protein and fetal growth are unclear. IGF2R binds and internalizes IGF2, thus maintaining appropriate levels of IGF2 locally and in circulation in many tissues, including *in utero*. Some published data suggest that *IGF2R* rs629849 variants have no effect on function and that this polymorphism is in strong linkage disequilibrium with a yet unidentified causative mutation. Moreover, single nucleotide polymorphisms with suggested linkage disequilibrium with rs629849 are in intronic sequences and may affect intron-exon splice sites, and thus IGF2R protein function [24]. Furthermore, using a bioinformatic splice-site prediction tool, there are indications that the 'A' variant of rs629849 may alter splice-site choice for IGF2R pre-mRNA [25]. In brief, intronic SNPs, related unknown mutations or, alternatively, other non-synonymous SNPs in other regions of the protein could exist in the different ethnic populations thus far studied, which would affect IGF2R function and the associations described herein.

Findings reported here should be interpreted in the context of the study limitations. The *IGF2R* rs629849 variant allele is rare in AA and our sample size was small. Small sample size notwithstanding, studies of rare variants are important for risk stratification purposes and targeted interventions. Although we found a strong positive association between the *IGF2* rs680 polymorphism and birth length in AA, the distribution of this variant in our population deviated from HWE. This HWE deviation could be due in part to the small sample size of AA carrying this polymorphism (n = 36). In addition, infants' race was self-reported mother's race. It will be important for future studies to use ancestral markers. Despite these limitations, our analyses strongly suggest associations between *IGF2R* and *IGF2* genetic variants and infants' size at birth and at one yr of age, which maybe race-specific.

In conclusion, we present evidence for associations between the *IGF2R* rs629849 polymorphism and infant size at birth in CA. However, larger studies will be required to clarify these associations.

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