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IGF2R polymorphisms and risk of esophageal and gastric adenocarcinomas

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The *mannose-6-phosphate/insulin-like growth factor 2 receptor* (*M6P/IGF2R*) encodes a protein that plays a critical role in tumor suppression, in part by modulating bioavailability of a potent mitogen, insulin-like growth factor-2 (IGF2). We tested the hypothesis that the common nonsynonymous genetic variants in *M6P/IGF2R* c.901C > G (Leu > Val) in exon 6 and c.5002G > A (Gly > Arg) in exon 34 are associated with risk of esophageal and gastric cancers. Study participants in this population-based study comprise 197 controls and 182 cases, including 105 with esophageal-gastric cardia adenocarcinoma (EGA), 57 with noncardia gastric adenocarcinoma and 20 with esophageal squamous (ES) cell carcinoma. Among white males, odds ratios (ORs) were elevated in relation to carrying at least 1 c.901C > G allele for EGA [OR = 1.9; 95% confidence intervals (CIs) = 1.0–3.6] and noncardia gastric cancer (OR = 2.5; 95% CI = 1.2–5.5), but not ES. Exploratory subgroup analyses suggested that associations between EGA and this variant were stronger among irregular or nonusers of nonsteroidal anti-inflammatory drugs (NSAIDs) (OR = 2.3; 95% CI = 1.2–4.2) and cigarette smokers (OR = 2.1; 95% CI = 1.0–4.2). An association between carrying the c.5002G > A genotype and EGA was not evident. These findings suggest that nonsynonymous polymorphisms in *M6P/IGF2R* may contribute to the risks of EGA and noncardia adenocarcinomas. Larger studies are required to confirm these findings.

Key words: genetic association; neoplasm; esophagus; gastric-cardia; gastric; insulin-like growth factors

Esophageal cancer is the eighth most common cause of cancer-related mortality in the United States, with a 5-year survival rate of 14%.[1] This cancer exhibits considerable gender, geographic, temporal and racial variation in incidence. Men comprise more than 80% of cases. Whereas squamous cell carcinoma of the esophagus squamous (ES) remains the predominant histologic subtype globally, in the United States and other Western countries, esophageal adenocarcinoma (EA) has been the predominant subtype since the mid-1990s.[2] The incidence of EA has increased >500% in the last 3 decades, an increase exceeding all other cancers.[3] Whereas, ES is 6-times more common in African American men than in white men, the incidence of EA is 4 times higher in white men than in African American men.[2]

Reasons for the sharp increase in incidence of EA and the closely linked gastric cardia adenocarcinoma (GCA) in Western countries and the preponderance among white men are unclear. However, the time trend parallels an increase in the prevalence of obesity in these countries.[4,5] Several lines of evidence suggest that most of these cancers arise in the setting of Barrett's esophagus, a metaplastic condition in which the native squamous cell epithelium in the distal esophagus is replaced by specialized columnar cells in response to chronic gastroesophageal reflux (GERD). Indeed both GERD and obesity are strongly associated with esophageal and gastric cardia adenocarcinomas (EGA) in population-based case-control and cohort studies.[6,7] However, the prevalence of obesity/overweight or GERD does not vary substantially between African Americans and whites, or between men and women, and therefore fails to explain the racial or gender differences in EGA.

The *Mannose-6-phosphate/Insulin-like Growth Factor Receptor-2 (M6P/IGF2R)*; hereafter referred to as *IGF2R*, encodes a 300 kDa Type 1 transmembrane glycoprotein that has been recently identified as a tumor suppressor.[8] This glycoprotein has binding sites for M6P-bearing proteins and IGF2,[8] the latter a potent mitogenic growth factor that when elevated in circulation, has been linked to increased risk of obesity, higher birth weight[9] and several adenocarcinomas. Extracellular binding of IGF2 to IGF2R results in internalization of IGF2 and its transport to the lysosomes where it is degraded, and this is the primary mechanism by which IGF2R modulates the bioavailability of IGF2.

IGF2R inactivation has been associated with risk of poorly differentiated breast[10] and lung tumors.[11] Recurrent loss of heterozygosity at the *IGF2R* locus and mutations in the remaining allele appear to be early events in breast cancer, squamous cell lung cancer, hepatocellular carcinoma, non-Hodgkin lymphoma, ovarian cancer and renal cell carcinoma.[10,11] Chromosomal loss in a region that includes the *IGF2R* gene has also been linked with androgen insensitive prostate cancer.[8] These findings point to the involvement of *IGF2R* as a tumor suppressor in the development of cancer, but the relation of genetic variants in *IGF2R* to EGA risk has not been evaluated.

More than 1,200 single nucleotide polymorphisms (SNPs) have been identified in *IGF2R* to date. Six are in the coding region and 3 of these (c.6206 A > G, Asn2020Ser; c.901C > G, Leu252Val and c.5002G > A Gly1619Arg) are nonsynonymous.[12] Two of these, c.901C > G, Leu252Val and c.5002G > A Gly1619Arg, have a minor allele frequency that is more than 5% among Caucasians. Herein we report on analysis of the association between EGA risk and these 2 polymorphisms in *IGF2R*, utilizing resources from a multicenter population-based, case-control study of EGA across the United States.

Material and methods

Study participants

Methods for the identification of study participants have been described previously.[13] Briefly, this case-control study was

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conducted in the state of Connecticut, a 15-county area of New Jersey and a 3-county area of the state of Washington. Eligible study participants were individuals newly diagnosed with EA, GCA, ES and noncardia gastric adenocarcinoma. In Connecticut, all individuals who spoke English and were aged 30–79 years diagnosed with primary invasive cancer of the esophagus or gastric cardia from February 1, 1993, to January 31, 1995, were invited to participate. Similar criteria were used to identify and enroll study participants in New Jersey, between April 1993 and November 1994, and in Washington, from March 1, 1993, to February 28, 1995. All EGA cases were eligible, whereas the ES and noncardia gastric cancers were frequency-matched to the expected age distribution of cases with EGA, based on the state of identification, and 5 year age-group, for comparison. In New Jersey, further frequency-matching was also done on race (white *vs*. other). Case eligibility was ascertained by 2 study pathologists using pathology reports and confirmed by hematoxylin and eosin stained slides. Disagreements were resolved by consensus.

For each study site, controls aged 30 to 64 years were identified using random digit dialing techniques. For those 65 years and older, Health Care Financing Administration rosters were used to identify population controls. Controls were frequency-matched to the expected distribution of cases by 5-year age groups and sex, and additionally on race in New Jersey.

Data collection

Trained interviewers administered structured questionnaires in person to 80% of eligible EGA cases, 74% ES cases and 74% of controls or their proxy respondents. The 60-min questionnaire included assessment of the following: demographic characteristics; tobacco use, beverage consumption including alcohol; medical history, including weight and height in the year before the reference date (defined as date of diagnosis for cases and date of identification for controls); medication use including over-the-counter medications such as aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs); occupational history and food intake based on a semiquantitative food-frequency questionnaire that included usual intake of fruit, vegetables and multivitamin supplements 3–5 years before diagnosis.

Up to 30 ml of peripheral blood, from which DNA was extracted, was obtained from a sample of index subjects in Washington and 9 of the 15 counties in New Jersey at the time of the in-person interview.[14] DNA samples were available for 197 controls and 162 cases (53 EA, 52 GCA and 57 other gastric adenocarcinomas, corresponding to 40% of controls, and 25%, 29% and 23% for EA, GCA and noncardia gastric cases, respectively). We also included 20 ES cases for comparison. Although the proportion included was not large, individuals for whom DNA samples were available did not differ substantially from those for whom samples were not available with respect to age, gender, race, GERD and cigarette smoking (data not shown).

Laboratory analyses

Lyophilized genomic DNA was resuspended using PureGene system reagents (Gentra, Minneapolis, MN) and subsequently plated into 2 384-well plates with 16 controls including 4 housekeeping genes. Primers for the c.5002 (rs629849) in exon 34 and c.901 (rs8191754) in exon 6 were designed by Applied Biosystem (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.5002G > 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′. Genotyping was undertaken without the knowledge of case status of participants. Ten randomly selected replicate samples from each of the 2 384-well plate were also included. Undetermined genotypes were excluded from analyses and constituted 4.2% and 5% of the total population for the c.901 C > G and c.5002 G > A variants, respectively.

Statistical analyses

We tested deviation from Hardy-Weinberg equilibrium (HWE) of *IGF2R* c.5002 and *IGF2R* c.901 genotypes among the controls for the overall and site-specific analyses using χ^2 tests. Unconditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between carrying the *IGF2R* c.5002 or the c.901 allele, and risk of esophageal or gastric cancer by histologic subtype. We compared carriers of at least 1 of the minor alleles (at risk genotype) to individuals homozygous for the common allele (referent genotype). Age at reference date, sex, race and study site were included in all statistical models. Factors previously reported to be associated with these histologic subtypes were explored for effect modification using stratified analyses. These factors were then evaluated for confounding comparing logistic regression models with and without the variables of interest. Factors evaluated include household income, body mass index (BMI = kg/m^2), number of years of education, current and former cigarette smoking, intake of alcohol in grams (wine, beer and liquor), use of aspirin and other NSAIDs, frequency of intake of fruit and vegetables and multivitamin supplement use. SAS version 9.1 (SAS, Cary, NC) was used for all statistical analyses.

Results

Table I summarizes the distribution of sociodemographic characteristics, anthropometric measures, dietary habits and other risk factors for esophageal and gastric cancers among study participants with DNA available for these analyses. More than 95% of cases were white males born in the United States. To ensure that the study population for whom blood samples were available was not systematically different from the population in whom blood samples were not collected, we compared the strength of the association between each cancer subtype and factors previously associated with these cancers in the larger study, including socioeconomic status (SES) as measured by educational level,[13] BMI[15] and a history of cigarette smoking.[13] Associations with BMI and educational levels in this subsample were similar to those found in the larger study at each cancer site, although we were unable to detect an association between cigarette smoking and adenocarcinomas of the esophagus and gastric cardia.

There was no evidence that genotype frequencies from the *IGF2R* c.901 deviated from HWE ($p = 0.14$), although the evidence for the c.5002 was less clear ($p = 0.05$). Table II summarizes the prevalence of genotypes and adjusted ORs for the association between these genotypes and esophageal cancer risk, by histologic subtype. There was little evidence for an association between carrying the minor *IGF2R c.5002* A-allele and risks for GCA (OR = 1.21, 95% CI = 0.58–2.54), noncardia gastric cancer (OR = 0.98, 95% CI = 0.46–2.08) or EA (OR = 0.60, 95% CI = 0.25–1.44) or ES (OR = 1.80, 95% CI = 0.64–5.06). Restricting these analyses to white males did not substantially change these findings. We conducted additional stratified analyses in the combined category of EGA (Table III), to explore the association with *IGF2R c.5002*, as the origin of these junctional tumors is difficult to pinpoint, and also share risk factor profiles, as well as incidence trajectories. Although this combined case group allowed for increased statistical power, there was no apparent association when carrying this genotype was examined within categories of low or high BMI, past or current regular NSAID use or high or low daily intake of fruits or vegetables.

There was no evidence of an association between *IGF2R c.901* A > G and ES (OR = 1.06, 95% CI = 0.33–3.36), but a suggestive association was seen with noncardia gastric adenocarcinomas (OR = 1.80, 95% CI = 0.92–3.57). Restricting analyses to white males generally strengthened these associations (OR = 2.07 for GCA, 95% CI = 0.96–4.50; OR = 1.92 for EA, 95% CI = 1.03–3.59 and OR = 2.51; 95% CI = 1.16–5.46 for noncardia gastric cancer). Interestingly, the associations between this variant and

TABLE I – DISTRIBUTION OF CHARACTERISTICS OF ESOPHAGEAL AND GASTRIC CANCER CASES AND CONTROLS

Characteristic	Controls (n = 197)		Gastric cardia adenocarcinoma (n = 52)		Esophageal adenocarcinoma (n = 53)		Esophageal squamous cell carcinoma (n = 20)		Other gastric adenocarcinomas (n = 57)	
	n	%	n	%	n	%	n	%	n	%
Age in years										
<50	25	13	5	10	8	15	2	10	6	11
50–59	41	21	14	27	10	19	2	10	10	18
60–69	71	36	15	29	19	36	9	45	17	30
70+	60	30	18	35	16	30	7	35	24	42
Sex										
Male	169	86	45	87	45	85	19	95	49	86
Female	28	14	7	13	8	15	1	5	8	14
Ethnicity										
White	187	95	50	96	52	98	15	75	48	84
Black	6	3	1	2	0	0	2	10	4	7
Native American, Asian, Other	4	3	3	2	1	2	3	15	4	7
Birth country										
Not United States	17	9	5	10	3	6	3	15	8	14
United States	180	91	47	90	50	94	17	85	49	86
Highest level of schooling										
High school graduate or less	79	40	29	56	24	45	14	70	36	54
Vocational, some college	49	25	14	27	15	28	1	5	11	20
College, graduate, profess.	69	35	9	18	14	27	5	25	9	16
BMI adult										
<25	89	46	10	19	23	43	13	65	17	30
25–29.9	91	47	35	67	22	42	4	20	29	52
≥30	15	8	7	13	8	15	3	15	10	18
Pack year cigarette smoking										
Any	132	67	41	79	41	77	18	90	38	67
None	65	33	11	21	12	23	2	10	19	33
Beer per week										
Any	126	64	32	63	29	55	17	85	33	58
None	71	36	19	37	24	45	3	15	24	42
Wine per week										
Any	81	42	18	35	14	26	8	40	18	32
None	113	58	34	65	39	74	12	60	39	68
Liquor per week										
Any	118	60	30	59	32	60	17	85	33	58
None	79	40	21	41	21	40	3	15	24	42
Aspirin										
Current or former user	64	33	18	35	21	40	6	30	15	26
Nonuser	128	67	33	65	31	60	14	70	42	74
Other NSAIDs										
Current or former user	24	12	12	24	3	6	0	0	5	9
Nonuser	172	88	39	76	50	94	20	100	51	91
Daily multivitamin use										
Yes	67	34	18	35	19	36	5	25	16	29
No	13	66	34	65	34	64	15	75	40	71
Number of fruit and vegetables per day										
≤2	141	72	34	67	33	62	16	89	35	63
>2	56	28	17	33	20	38	2	11	21	38

TABLE II – ADJUSTED[1] ODDS RATIOS FOR THE ASSOCIATION BETWEEN *M6P/IGF2R* GENOTYPES *c.5002* AND *c.910* AND RISK OF ESOPHAGEAL AND GASTRIC CANCER, BY HISTOLOGIC TYPE

M6P/IGF2R G > A, rs629849/c.5002	All participants				White males only			
	GG (*n*)	AG (*n*)	AA (*n*)	OR (95% CI) for AA/AG *vs.* GG	GG (*n*)	AG (*n*)	AA (*n*)	OR (95% CI) for AA/AG *vs.* GG
Controls	150	35	3	1.00 Referent	122	27	3	1.00 Referent
Gastric cardia adenocarcinoma	39	11	1	1.21 (0.58–2.54)	33	10	0	1.27 (0.56–2.88)
Esophogeal adenocarcinoma	46	6	1	0.60 (0.25–1.44)	37	6	1	0.77 (0.31–1.89)
Esophageal + gastric cardia adenocarcinomas	85	17	2	0.88 (0.48–1.63)	70	18	1	1.00 (0.51–1.94)
Squamous cell carcinoma	13	5	1	1.80 (0.64–5.06)	9	4	1	2.03 (0.63–6.60)
Other gastric adenocarcinomas	44	10	1	0.98 (0.46–2.08)	32	8	0	0.98 (0.41–2.35)
M6P/IGF2R C > G, rs8191754/c.901	CC (*n*)	CG (*n*)	GG (*n*)	OR (95% CI) for GG/CG *vs.* CC	CC (*n*)	CG (*n*)	GG (*n*)	OR (95% CI) for GG/CG *vs.* CC
Controls	154	32	5	1.00 Referents	128	24	3	1.00 Referents
Gastric cardia adenocarcinoma	37	11	3	1.58 (0.77–3.22)	30	11	2	2.07 (0.96–4.50)
Esophogeal adenocarcinoma	39	13	1	1.50 (0.74–3.04)	32	11	1	1.78 (0.81–3.90)
Esophageal + gastric cardia adenocarcinomas	76	24	4	1.53 (0.87–2.70)	62	22	3	1.92 (1.03–3.59)
Squamous cell carcinoma	16	4	0	1.06 (0.33–3.36)	12	2	0	0.74 (0.15–3.54)
Other gastric adenocarcinomas	39	16	1	1.82 (0.92–3.57)	26	14	0	2.51 (1.16–5.46)

[1]Adjusted for age, sex and site of recruitment.

TABLE III – ADJUSTED[1] ODDS RATIOS FOR THE ASSOCIATION BETWEEN *M6P/IGF2R c.901* GENOTYPES AND GASTRIC CARDIA AND ESOPHAGEAL ADENOCARCINOMAS BY POTENTIAL EFFECT MODIFIERS IN ALL AND IN WHITE MEN

Characteristic	All participants (105 cases, 197 controls)	White males only (87 cases, 160 controls)
	OR and 95% CI for GG/CG v./CC in *M6P/IGF2R* c.901	OR and 95% CI for GG/CG v./CC in *M6P/IGF2R* c.901
Age in years		
<60 years	1.44 (0.56–3.72)	2.30 (0.76–6.90)
60+ years	1.57 (0.78–3.17)	1.82 (0.85–3.92)
Body mass index (kg/m^2)		
<25	2.40 (1.08–5.36)	1.94 (0.67–5.57)
≥25	1.03 (0.46–2.32)	2.02 (0.90–4.52)
Cigarette smoking		
Ever	1.73 (0.90–3.32)	2.08 (1.02–4.22)
Never	1.09 (0.34–3.54)	1.60 (0.33–7.63)
Aspirin use		
Current or former user	1.30 (0.48–3.48)	2.00 (0.66–6.01)
Non-user	1.57 (0.78–3.16)	1.88 (0.87–4.09)
NSAIDS use		
Current or former user	0.50 (0.11–2.35)	0.30 (0.03–3.62)
Non-user	1.86 (1.01–3.43)	2.28 (1.18–4.42)
Daily multivitamin use		
Yes	1.63 (0.55–4.85)	2.25 (0.84–6.01)
No	1.53 (0.58–4.02)	2.00 (0.85–4.75)
Number of fruit and vegetables per day		
≤2 servings	1.90 (0.95–3.80)	1.95 (0.91–4.17)
>2 servings	0.96 (0.37–2.55)	2.07 (0.65–6.60)

[1]Adjusted for age, sex and site of recruitment.

risks of ES, GCA, EA and noncardia gastric cancer were weaker for esophageal tumors and stronger and statistically significant for gastric cardia and gastric ones.

Exploratory stratified analyses in Table III also suggest that the associations between carrying the *IGF2R c.901* G-allele and EGA varied with past exposures to NSAID use and cigarette smoking. Associations were stronger in those reporting no regular use of NSAIDs in the past or at the time of interview (OR = 1.86, 95% CI = 1.01–3.43), and these associations were strongest in white males (OR = 2.28, 95% CI = 1.18–4.42). These associations were not apparent in regular NSAID users. An association between this variant and EGA was also suggested in those reporting a history of cigarette smoking. However, interactions were not statistically significant, and adjusting for potential confounders did not substantially change these associations. Separate analyses for EA and GCA also did not alter these findings.

Discussion

This is the first epidemiologic study to evaluate associations between nonsynonymous variants of the *IGF2R* tumor suppressor gene and esophageal and gastric cancer. Several years ago, Killian *et al.*[16] identified 9 novel polymorphisms, 6 of them in the coding region of the *IGF2R* with 3 being nonsynonymous. We analyzed the 2 polymorphic variants with minor allele frequencies more than 5%, with the working hypothesis that these variants altered this tumor suppressor. We found no evidence of an association between carrying the *IGF2R* c.5002 G > A minor allele and any subtype of esophageal or gastric cancer regardless of the subpopulation evaluated. However, an association was seen between carrying at least 1 *IGF2R* c.901C > G allele and EGA and with noncardia gastric adenocarcinoma among white males with a history of cigarette smoking but infrequent use of NSAIDs.

The finding of no association between the *IGF2R* c.5002 A-allele and risk of esophageal or gastric cancers was unexpected because this polymorphism is located within extracellular domain 11 of the M6P/IGF2R where IGF2 binding occurs. Based on this location, carrying this variant was expected to alter IGF2 binding affinity,[11,12] thereby increasing the bioavailability of IGF2. Individuals carrying the c.5002 A-variant have been reported to be at higher risk for lung cancer[11] whereas no association was found in a recent study of osteosarcoma.[17] Moreover, the racial distribution of this genetic variant parallels that of EA in that it is very low (<2%) in Africans in whom EA is rare, it is found in moderately low frequency in African Americans (13%) in whom these adenocarcinomas occur in slightly higher frequency, and its highest frequency is among Americans of European descent (36%) in whom the incidence of EA is also highest. However, a recent study suggests that the c.5002 A-allele does not significantly alter binding of IGF2 relative to the c.5002 G-allele nor does it alter protein trafficking or stability, although effects on IGF2R dimerization or the stability and binding of IGF2 to the soluble form of the IGF2R were not evaluted.[18] It is also possible that our inability to find associations may have been due to small sample sizes, the suboptimal call rate or because the variant was not clearly in HWE ($p = 0.05$).

The etiologic significance of finding an association between c.901 and esophageal and gastric cancer is presently unclear. The amino acid variant c.901Leu252Val is a conservative substitution with respect to hydrophobicity, but may lead to protein destabilization due to replacement of the isobutyl group with an isopropyl side chain.[19] It is located in repeat domain 3 and is involved in binding M6P moieties on other proteins and this position has been strictly conserved throughout mammalian evolution.[16] Because of this important function, altered affinity for M6P-bearing ligands may lead to changes in protein trafficking and secretion,[16] causing a shift in the protein milieu, within cells and tissues, including that of IGF2. The IGF2R is also responsible for binding and activating the latent form of TGFβ1,[20,21] a potent growth inhibitor. It is possible that this role may be impeded by the c.901 variant allele presumably enhancing cancer risk. Alternatively, the risk of developing esophageal and gastric cancer may involve decreased capacity for IGF2 binding due to steric hindrance mediated through binding of IGF2R to other lysosomal enzymes,[22] thereby increasing bioavailability of this mitogenic growth factor. Indeed recent data from a cohort study suggest that esophageal and noncardia adenocarcinoma risk may be determined, in part, *in utero*[23] and birth weight has been positively associated with carrying the c.901 variant in at least 1 allele.[22] Nonetheless, the associations found are consistent with the tumor suppressive effect of IGF2R and with the association reported in relation to high birth weight[22] presumably reflecting increases in bioavailable IGF2 *in utero*.

A limitation of this study was the small sample sizes and low *p*-value for the HWE test that may have contributed to our inability to detect associations between the c.5002 variant and the subtypes of esophageal and gastric cancers. However, this SNP is also not in HWE in whites in the HapMap and SNP500Cancer databases, confirmed by a recent report.[18] The small sample size also limited our ability to evaluate gene-environment effects on risk, even when we combined EA with GCA. Although other studies have also combined EA and GCA, tumors arising in these 2 locations may not always share the same etiologic mechanisms. An additional limitation often found in case-control studies of rapidly fatal diseases is the low response rate, which raises a possibility of selection (survival) bias as an alternative explanation for our findings. However, genotype frequencies in our control population were similar to publically available estimates (NCBI.nih.gov) of the prevalence of the c.901 variant in a white population. Hence, the inability to participate due to disease severity and death may not have differed by genotype. Furthermore, survival bias is an unlikely explanation because these SNPs are not known to be related to survival.

In summary, we found evidence of an association between esophageal, gastric cardia and noncardia gastric adenocarcinomas and carrying the *IGF2R c.901* G allele, particularly among white men reporting cigarette smoking and none or irregular use of NSAIDs. No association was found between these tumors and the *IGF2R c.5002* genotype. Because of a limited sample size and low call rates for these genotypes, our findings should be considered preliminary.

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