SHORT COMMUNICATION

Family-based association study of *IGF1* microsatellites and height, weight, and body mass index

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Height, weight, and body mass index (BMI) are partly heritable, known to be associated with chronic diseases, and are linked to circulating insulin-like growth factor I (IGF-I) concentrations. IGF-I concentrations are also partly heritable and thus genetic variation at *IGF1* could influence height, weight, BMI and the risk of developing chronic diseases. Our objective was to examine the association of genetic variation at *IGF1* with height, weight and BMI using a sample of premenopausal women. A family-based study design was used to investigate the association of three *IGF1* CA repeat variants at 5' (5'CA), intron 2 (In2CA) and 3' (3'CA) with these anthropometric measures. We analyzed the data for 827 families of different sizes and configurations, which included 1520 premenopausal women. Nominally significant associations ($P \leq 0.05$) were found for a rare 3' variant allele (3'CA-193) and BMI ($P=0.05$), and for the more common 3'CA-187 allele and weight ($P=0.04$). These associations did not remain significant when adjusted for multiple comparisons. Haplotype analysis did not support an association between these variants and anthropometric measures. This study does not support an association between *IGF1* and these anthropometric measures. Study limitations, including sample size and capturing genetic variation at *IGF1* with these markers, could mean associations were missed.

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Higher insulin-like growth factor I (IGF-I) concentrations have been associated with the risk of colorectal, prostate and premenopausal breast cancer,^{1,2} and with lower risk of cardiovascular disease.³ Twin studies suggest that circulating IGF-I concentrations are partly heritable, with estimates ranging from 38 to 63%.^{4,5} Several studies have shown an association between *IGF1* variants and circulating IGF-I concentrations,^{6,7} but there are no established associations between *IGF1* variants and chronic diseases.^{6,8–11} Anthropometric measures are associated with the same chronic diseases that are linked to IGF-I levels. Greater adult height is an established risk factor for colorectal and breast cancer.¹² Higher body mass index (BMI) is associated with an increased risk of cardiovascular disease,¹³ colorectal cancer and possibly reduced premenopausal breast cancer risk.¹² Furthermore, IGF-I concentrations are linked to height in childhood^{14,15} and show a non-linear association with BMI.¹⁶ Examining the associations between *IGF1* and anthropometric measures could have advantages over testing disease associations, as stronger associations might be seen with intermediate factors. Using a family-based study, we evaluated

the association of three *IGF1* CA repeats (at 5' (5'CA), intron 2 (In2CA) and 3' (3'CA)) with height, weight and BMI for premenopausal women.

The methods used are described here briefly, with details provided elsewhere.¹⁷ We included families from two Breast Cancer Family Registry sites: 310 families from the Ontario Familial Breast Cancer Registry and 517 from the Australian Breast Cancer Family Registry.¹⁸ Outcome measures were available for 1520 premenopausal women in families of various configurations and sibship sizes. These included 837 women (827 probands) with incident breast cancer and 683 unaffected sisters (Supplementary Table 1). Genotyping was performed at The Centre for Applied Genomics.¹⁹ The concordance of allele calls, based on 10% replicate samples, was 98.1–100 and 94.1–97.2%, respectively, in the Ontario Familial Breast Cancer Registry and Australian Breast Cancer Family Registry. Height and weight were self-reported.

The program FBAT 1.5²⁰ was used for testing genotype associations with height, weight and BMI, using an offset that minimizes the

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variance. Analyses (data not shown) using the empirical variance-covariance estimator to account for familial correlations in multi-sib families did not materially change the results. Multiple comparisons were accounted for by using the Benjamini-Hochberg procedure to produce a false discovery rate of 0.05.²¹ We report the results for breast cancer cases and unaffected individuals combined, as analyses excluding breast cancer cases did not materially change the results. There was little evidence for heterogeneity of associations across registries. Only three nominally significant interactions between variant alleles and registry were found from 69 statistical tests (height: 5'CA-19 ($P=0.03$); 3'CA-191 ($P=0.05$); and BMI: 3'CA-181 ($P=0.04$)). Combined analyses using both registries are therefore shown.

Primary analyses identified only nominally significant associations ($P \leq 0.05$): between 3'CA-187 ($P=0.04$) and weight, and between 3'CA-193 and BMI ($P=0.05$; Table 1). Although linkage disequilibrium (LD) between alleles of these variants never exceeded an R^2 of 0.21, we examined the associations with outcome for 5'CA-In2CA and In2CA-3'CA haplotypes, as these variant combinations had at least one allele pair with LD >0.10 . Three nominally significant associations were found: In2CA-3'CA/220-185 with height ($P=0.01$), and 5'CA-In2CA/22-214 with weight ($P=0.05$) and BMI ($P=0.02$). None of these results remained significant when adjusted for multiple

comparisons. Our findings do not support an association between genetic variation at *IGF1* and these anthropometric measures.

Consistent with our findings, several studies examining female White Caucasian populations did not find an association between 5'CA-19 and height²²⁻²⁴ or BMI,^{22,25} or between 5'CA-20 and height.²³ No association between 5'CA-19 and BMI was found for Hispanic or Chinese women (although among Chinese women 5'CA-17 was associated with lower BMI).^{25,26}

In a study from the Netherlands (Rotterdam), women homozygous for either 5'CA-19 or 5'CA-20 were significantly heavier than other homozygotes.²³ Another Netherlands (Amsterdam) study found that homozygous carriers of 5'CA-19, 5'CA-20 or 5'CA-19/20 heterozygotes had significantly higher BMI than women with other genotypes, for one of two cohorts examined.²⁷ We did not investigate these allele groupings specifically, but, inconsistent with these results, grouping 5'CA-19 and 20 alleles did not yield a significant association with weight or BMI when tested under a recessive model (data not shown). Our study may not, however, have been sufficiently powered to detect the small differences (3 kg) in weight found in the Rotterdam study ($n=2590$ women).

Previously, we reported a significant negative association of 3'CA-185 with BMI and a significant positive association with height.²² These results

Table 1 Association between alleles of *IGF1* polymorphisms and height, weight, and BMI

Loci/allele	Height				Weight				BMI			
	Allele	NIF	Z score	P-value	Allele	NIF	Z score	P-value	Allele	NIF	Z score	P-value
	frequency %				frequency %				frequency %			
5'												
11	0.5	11	0.53	0.60	0.5	11	0.68	0.50	0.5	11	0.54	0.59
17	1.6	32	−1.16	0.24	1.6	33	−0.46	0.64	1.6	33	0.07	0.94
18	6.3	126	−1.15	0.25	6.3	125	0.35	0.73	6.3	126	0.73	0.47
19	61.2	417	1.21	0.22	61.2	420	0.67	0.50	61.2	424	0.16	0.87
20	21.2	315	−0.65	0.52	21.2	317	−0.11	0.91	21.2	319	0.24	0.81
21	6.7	130	−0.30	0.77	6.7	132	−1.46	0.14	6.7	133	−1.36	0.17
22	2.2	45	0.85	0.39	2.2	46	−0.06	0.95	2.2	46	−0.43	0.67
Intron 2												
204	2.2	50	0.07	0.95	2.2	50	0.09	0.92	2.2	50	0.07	0.94
212	0.8	15	−0.07	0.94	0.8	16	−0.78	0.43	0.8	16	−1.02	0.31
214	3.0	59	0.12	0.90	3.0	59	−0.05	0.96	3.0	59	−0.12	0.90
216	38.3	426	0.68	0.50	38.3	424	0.55	0.58	38.3	428	0.08	0.94
218	21.9	336	0.91	0.36	21.9	331	0.37	0.71	21.9	333	0.18	0.85
220	16.8	256	−1.52	0.13	16.8	258	0.10	0.92	16.8	259	0.69	0.49
222	9.7	193	0.39	0.69	9.7	192	0.04	0.97	9.7	193	−0.01	1.00
224	6.2	141	−0.54	0.59	6.2	139	−1.18	0.24	6.2	141	−0.89	0.37
226	0.6	15	−1.52	0.13	0.6	15	0.34	0.73	0.6	15	1.18	0.24
3' end												
181	0.9	21	0.88	0.38	0.9	21	−1.25	0.21	0.9	21	−1.58	0.12
183	1.1	25	0.10	0.92	1.1	24	−0.04	0.97	1.1	24	−0.19	0.85
185	46.9	426	−0.95	0.34	46.9	428	−0.72	0.47	46.9	430	−0.36	0.72
187	28.8	373	1.50	0.13	28.8	372	2.02	0.04*	28.8	373	1.57	0.12
189	18.4	306	−0.52	0.60	18.4	301	−0.15	0.88	18.4	305	0.00	1.00
191	2.9	63	−1.05	0.29	2.9	64	−0.08	0.94	2.9	63	0.48	0.63
193	1.0	20	1.60	0.11	1.0	20	−1.59	0.11	1.0	20	−1.96	0.05*

Abbreviations: BMI, body mass index; NIF, number of informative families.

*Nominally significant ($P \leq 0.05$).

Results not reported when number of informative families is less than 10.

Minus signs indicate negative association; otherwise association is positive for the allele indicated.

were not replicated by this study, despite a larger sample size (426–430 families (Table 1) versus 163 unrelated individuals).

Three studies have examined the association of single-nucleotide polymorphisms of *IGF1* with height. Two candidate gene studies did not show convincing associations.^{28,29} A genome-wide association study from Korea reported a possible association between an *IGF1* single-nucleotide polymorphism and height, which did not achieve genome-wide significance. An association of the same single-nucleotide polymorphism with height for children with idiopathic short stature was reported by the same study.³⁰

Heterogeneity between registries did not seem to influence the results. Differences between important variables across registries (age, height, weight, BMI, Caucasian ancestry and allele frequencies) were small (Supplementary Table 1 and Table 1). A potentially more important limitation was that the LD between microsatellite repeats was too low to fully capture the genetic variation across *IGF1*. We would only be able to find an association if one or more of our repeats was in strong LD with a susceptibility locus or directly influenced the outcome. We know of no experimental results indicating that our repeat markers are functional or that there are functional polymorphisms in the same regions that might be in strong LD with one of the markers. Power might also have been limiting. Genome-wide association studies suggest that strongly associated common single-nucleotide polymorphisms explain, at most, about 1% of the variance for BMI and less for height.^{31,32} Our power estimates for 5'CA-19 (which, given our family configurations, cannot be calculated precisely using the standard software) were between 0.58 and 0.62 for detecting an association with a heritability of 1% and $\alpha=0.05$.

After adjusting for multiple comparisons, our results do not support an association between genetic variation at *IGF1* and height, weight or BMI. Study limitations, including sample size and capturing genetic variation at *IGF1* using these markers, could mean associations were missed.

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