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Study of association between common variation in the *IGF2* gene and indices of obesity and body size in middle-age men and women.

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Abstract

Context: The Insulin-like Growth Factor 2 gene (*IGF2*) plays a key role in growth and is a candidate for association with obesity. Previous studies have reported that polymorphisms in *IGF2* are associated with body weight and body mass index (BMI), but the results have been inconsistent. The primary aim of this study was to confirm the association with BMI, and secondarily to study the associations with other indices of body size. **Methods:** In a sample of 2797 women and 2203 men aged 39-79 participating in the Norfolk arm of the European Prospective Investigation of Cancer (EPIC), we genotyped three SNPs in the *IGF2* gene that were previously associated with BMI (6815 *A/T*, 1156 *T/C (G/A)*, 820 *G/A* (*ApaI*)). **Results:** No significant associations were observed between these SNPs and BMI. However, all three SNPs were significantly associated with height (p=0.03 to 0.001). In a backwards elimination regression analysis, two SNPs 1156 *T/C (G/A)* and 820 *G/A* remained independently associated with height (p=0.003 and p=0.038 respectively). Haplotype analysis of these two SNPs showed that carriers of the GA haplotype were shorter than carriers of each of the other three haplotypes (p<0.001 for all comparisons). **Conclusion:** We did not confirm the previously reported associations between *IGF2* polymorphisms and BMI. However our results suggest that common variation in the *IGF2* gene may be associated with adult height. *IGF2* could be considered as a candidate gene for future research on mechanisms for the association between height and chronic diseases such as cancer, diabetes and coronary heart disease.

The Insulin-like growth factor 2 gene (*IGF2*) is exclusively paternally expressed, and maternally imprinted, and plays a key role in fetal growth and development (1, 2). Mice with null mutations in the *Igf2* gene exhibit decreased fetal growth (3). In humans *IGF2* under-expression caused by methylation defects results in the low birth weight Silver-Russell syndrome (4), and conversely, loss-of-imprinting leading to *IGF2* over-expression results in the Beckwith-Wiedemann somatic overgrowth syndrome (5).

In postnatal life, *Igf2* expression in mice is repressed in all tissues except the brain, and deregulation of *Igf2* expression results in postnatal overgrowth (6). In pigs a quantitative trait locus for muscle growth and leanness has been identified in the *IGF2* region (7). In humans, *IGF2* is biallelically expressed in the liver in postnatal life, and the syndromes of *IGF2* under- and over-expression exhibit postnatal growth failure and growth acceleration respectively (4, 5).

Common allelic variants in *IGF2* have been associated with body weight and BMI in large population-based studies. In a study of 2,500 UK middle-aged men, the *IGF2* ApaI (820 *G/A*) minor allele homozygotes had higher serum IGF-II concentrations and lower BMI and body weight than wild-type homozygotes (8). A subsequent study in the same population investigated 11 other polymorphic markers in *IGF2*, and found that two further *IGF2* SNPs were independently associated with BMI (9): minor allele homozygotes at 6815 A/T also had lower BMI, while minor allele homozygotes at 1156 T/C had higher BMI than wild-type homozygotes (9). By contrast, a separate study of 500 healthy men and women found that ApaI 820 G/A minor allele homozygotes had higher fat mass than wild-type homozygotes (10).

Given these inconsistent results, we selected the three *IGF2* SNPs previously reported to be independently associated with BMI (9) and aimed to confirm this

association in a UK population of 5000 middle-age men and women. Our secondary aim was to investigate the associations with body fat and also height, as some studies had identified associations with height but these were not highlighted in those reports (8, 10, 18-20).

Research design and methods

Population

We analysed data from the Norfolk arm of the European Prospective Investigation of Cancer (EPIC) prospective cohort study. This study has been described elsewhere (11, 12), but, in brief, is a prospective population study of 25,639 men and women aged between 39 and 79 years, resident in Norfolk, UK. Participants were recruited from age-sex registers of general practices in Norfolk as part of the 10-country collaborative EPIC study designed to investigate dietary and other determinants of cancer. Additional data were obtained in EPIC-Norfolk to enable the assessment of determinants of other diseases. The sub-cohort used for this study is a random sample of 5000 of the participants who were free of disease at baseline (cancer, coronary heart disease and diabetes), who had completed arrayed DNA samples, complete food frequency questionnaires, and HbA1C and BMI measured at two clinical assessments separated by an average of 3 years (13) (see **Table 1** for clinical characteristics). All participants gave informed consent.

Measurements

All participants completed a detailed health and lifestyle questionnaire, which included a question on birth weight. Anthropometric measurements were taken with participants dressed in light clothing and no shoes. Standing height was measured to the nearest 0.1 cm using a stadiometer, and weight was measured to the nearest 100 g using Salter scales (Salter Brecknell Weighing Products, Fairmont, Minnesota). BMI was calculated as weight (kg)/height $(m)^2$. At follow-up only, sitting height was additionally measured, and leg length was calculated as the difference between overall height and sitting height, plus the height of the measuring stool used for the sitting

height measurements. Trunk length was calculated as the difference between leg length and total height. Also at follow-up, a Tanita TBF-531 bioimpedance analyzer was used to determine percent body fat, to the nearest 0.5%.

SNPs choice

In the Northwick Park Heart Study II (NPHSII), the authors conducted a systematic search for variants across the exons, the intron/exon boundaries and intron three of the *IGF2* gene, and identified 12 different markers (9). Four SNPs were associated with BMI, of which two were in strong linkage disequilibrium (LD) (pairwise coefficient = 0.87). We therefore selected three SNPs which were not in LD (see supplementary material): 6815 *A/T* (rs3842759) at nucleotide position 6815 in the GenBank sequence L15440, 1156 *T/C (G/A)* at nucleotide position 1156 in the GenBank sequence Y13633 and 820 *G/A* (*ApaI*, rs680) at nucleotide position 820 in the GenBank sequence X07868. 6815 *A/T* and 1156 *T/C (G/A)* are on intronic regions whereas 820 *G/A* (*ApaI*) is on exon 9.

Genotyping

All SNPs were genotyped using Custom TaqMan® assays (Applied Biosystems, Warrington, UK) on an ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems, Warrington, UK). Details for all genotyping primers, probes and PCR conditions are available upon request from the corresponding author. The three SNPs had a genotyping call rate above 93% and all of the duplicate controls used in the study set were fully concordant.

Statistical analysis

Each SNP was tested for Hardy–Weinberg equilibrium using the appropriate χ^2 test. We used linear regression to test the association between each genotype

(independent variable) and each anthropometric outcome (dependent variable) adjusting for age and sex. We tested the association under an additive (co-dominant) model by entering each genotype as a quantitative variable (wild/wild; wild/variant; variant/variant). Recessive models were also tested (i.e. variant/variant vs carrier of the wild allele). All analyses were performed with SAS version 8.2 (SAS Institute Inc, Cary, NC).

Haplotype analysis was undertaken using the SNPs which remained independently associated with height after a backwards elimination regression analysis. Because phase was unknown, assignment of haplotype probabilities was performed using the SNPHAP program (14, 15). We then used the *qhapipf* program in STATA to test whether the haplotypes explained more of the variance in height than the genotypes considered independently (16). Finally, tests for main haplotype effects were performed using a linear model, adjusted for age and gender, weighted by haplotype probability, and clustered by the individual identification to obtain robust standard errors (STATA regression command xi:regres).

Results

Genotype frequencies for the three SNPs are provided in **Table 2**. The two SNPs 6815 *A/T* and 820 *G/A ApaI* were in Hardy-Weinberg (H-W) equilibrium (p=0.12 and p=0.67 respectively). However, the frequencies for 1156 *T/C (G/A)* differed slightly from the expected Hardy-Weinberg proportions (p=0.015). The 1156 *T/C (G/A)* genotypes were confirmed on re-genotyping of the whole cohort. The genotype frequencies were similar in women (40%, 45% and 15% for the GG, GA and AA respectively) and in men (38%, 46% and 15%); however, the deviation was significant only in women (p=0.017 vs p=0.32 in men) and is likely explained by random sampling.

Table 3 presents the regression coefficients for each anthropometric outcome with genotype considered as a quantitative trait (co-dominant model). The three SNPs were not associated with any of the obesity indices, but all three SNPs were associated with height. The minor alleles of 6815 *A/T* and 820 *G/A* were additively associated with shorter height (-0.32 cm per T allele carried, p=0.027 and -0.37 cm per A allele carried, p=0.009 respectively), while the minor allele of 1156 *T/C (G/A)* was associated with taller height (+0.40 cm per A allele carried p=0.001). The effect sizes were very similar with trunk length and leg length for all SNPs. Birth weight and body fat percent were only available for 2235 and 3750 individuals respectively and no significant association was observed with genotype for either of these outcomes.

Adjusted means for BMI and height by genotype are displayed separately in men and women in **Table 4** and **Table 5** respectively. No association with BMI was disclosed. The pattern of association with height was similar in men and women for

the 1156 *T/C (G/A)* and the 820 *G/A* SNPs, but was stronger in women than in men for 1156 *T/C (G/A)* (p=0.002 vs p=0.37 under the co-dominant model).

In a backwards elimination multiple regression analysis, 1156 *T/C (G/A)* and 820 *G/A* (*ApaI*) remained independently and significantly associated with height (p=0.003 and p=0.037 respectively).

The frequencies for the haplotypes corresponding to those two SNPs, and the age and sex adjusted mean height according to the haplotypes are presented in **Figure 1**. The overall association between haplotypes and height was highly significant (p=0.0002). The haplotypes explained more of the variability in height than when the SNPs were considered independently (p=0.0391). Mean height of individuals with the haplotype GA (frequency of 18.6%) was significantly lower than that of any other haplotype (p<0.001 with AG, AA and GG). Among the remaining haplotypes, only a weak difference was observed between AG and GG (p=0.075).

Discussion

This study of around 5,000 middle age women and men from a population-based cohort provides no evidence for an association between *IGF2* common polymorphisms and indices of obesity. However, all three SNPs were associated with height, and similar trends were seen in men and women separately. Our results suggest a role of the *IGF2* gene in adult height, which may be consistent with the action of IGF-II protein as a growth factor.

It was previously reported in the NPHSII Study of men only, that the three *IGF2* polymorphisms we selected were independently associated with BMI (8, 9, 17). However, our study in a larger number of individuals including both men and women, did not support that observation. Another study in a Caucasian population showed that the *IGF2 ApaI* polymorphism was related to fat mass, but the association was in the opposite direction to that in the NPHSII (10). Furthermore O'Dell et al. did not find an association between the *ApaI* polymorphism and body weight in their sample of young adults from NPHSII (8), in contrast to their older population. They concluded that the effect on BMI might occur only later in life, possibly as a consequence of interaction with environmental factors related to age (8). Since these factors are likely to vary between populations, gene-environmental interactions could explain the discrepancies observed between the studies. However, in our population with a wide age range of men and women (39 to 79 years), we did not observe any significant interaction between the effects of age and genotypes. Therefore, the associations identified in the original study between the 3 SNPs and BMI could be false-positives;

however, discrepant findings between studies could also arise from differences in subject ascertainment or population structure.

However, consistent with our current findings those previous studies also found associations between *IGF2* polymorphisms and adult height, although these findings were not highlighted in those reports. First, in the NPHSII population a trend for an association between the 820 *G/A* (*ApaI*) and height was found. Consistent with our findings GG homozygotes tended to be taller than AA (8): the size of the effect (0.7 cm difference; p=0.10) was comparable to our study but the sample size of that study was smaller (8). In the same population a further *IGF2* SNP at 1252 *T/C AluI*, which is in positive LD with 820 *G/A* (*ApaI*), was significantly associated with height (p=0.0019) (18), as were two *IGF2*, *INS* and *TH* gene region haplotypes (19). Furthermore, in a Hertfordshire UK population 820 *G/A* (*ApaI*) GG homozygotes tended to be both heavier and taller than AA homozygotes (20). Finally, in the Baltimore Longitudinal Study of Aging, women with the 820 G/A (ApaI) A/A genotype were shorter than G/G carriers ($p<0.05$), and a similar trend was observed in men (10).

In the present study, participants were exclusively European, living in the same geographical area in the east of England. Although this is an ethnically homogeneous population, we cannot completely exclude the existence of underlying genetic heterogeneity.

The mechanisms by which the *IGF2* gene could be associated with adult height remain to be elucidated. IGFs are peptides that regulate growth, differentiation and regeneration of cells (21). In particular, placental-specific IGF-II is a major modulator

of placental and fetal growth (22, 23). Common variants in the *IGF2* gene have been associated with the Beckwith-Wiedemann fetal overgrowth syndrome, and such children are noted to be tall (24, 25). However, if the effect observed here originated in fetal life, one would expect an association with birth weight that we did not find. The absence of this association could be explained by a lack of power in our study, as the information on birth weight was missing for more than half of the population. Furthermore, a standardized measure of birth length would have been a much better outcome to test this hypothesis than reported birth weight. Alternatively, *IGF2* may impact postnatal growth in infancy, childhood or adolescence. Children with constitutionally tall stature had significantly higher growth velocity and higher IGF-II levels than children with normal height (26). That study suggested that IGF-II might be responsible for overgrowth of children with constitutionally tall stature, having an increase in activity on target tissues, particularly at the level of cartilaginous and bone tissue (26).

In conclusion, we did not confirm previous results of the association between *IGF2* polymorphisms and obesity indexes in adults in our study. Others SNPs in the *IGF2* region could be involved and our limited choice of SNPs does not allow us to exclude a possible role of common *IGF2* variation in body weight regulation in adults. However, our results suggest that common *IGF2* SNPs may regulate adult height. More studies are needed, especially in populations of healthy infants, children and adolescents, to investigate the timing of this *IGF2* effect on postnatal growth velocity and height. *IGF2* should be considered as a candidate gene to explain the associations between adult height and chronic diseases such as cancer, diabetes and coronary heart disease (27-29).

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Table 1: Population description, the EPIC5000 study

	Women N=2797	Men N=2203
Age (y)	56.5 ± 9.2	57.9 ± 9.3
Weight (kg)	66.9 ± 10.9	80.2 ± 10.6
Height (cm)	161.5 ± 6.2	174.5 ± 6.4
BMI (kg/m^2)	25.7 ± 4.0	26.3 ± 3.1
Body Fat percent (%) *	38.2 ± 7.6	22.9 ± 5.1
Birth Weight (kg) †	3.3 ± 0.71	3.5 ± 0.76

Results are mean ± sd

* N = 1997 in women and 1725 in men

† N = 1442 in women and 778 in men

Table 2: Genotype frequencies and Hardy-Weinberg (HW) equilibrium test

SNP	N	11	12	22	p-value for HW
6815 A/T (1=A; 2=T)	4771	57.5%	36.1%	6.4%	0.12
1156 T/C (G/A) (1=G; 2=A)	4879	39.9%	45.2%	15.0%	0.015
820 G/A (ApaI) (1=G; 2=A)	4634	51.7%	40.6%	7.7%	0.67

Table 3: Regression coefficients ± SE for each anthropometric outcome regressed on the three genotypes considered as quantitative traits (number of variant allele carried, corresponds to a co-dominant model).

	6815 A/T Number of allele T carried N=4770		1156 T/C (G/A) Number of allele A carried N=4879		820 G/A (ApaI) Number of allele A carried N=4634	
	β ± SE	p	β ± SE	p	β ± SE	p
Weight (kg)	-0.34 ± 0.25	0.19	0.33 ± 0.22	0.12	-0.17 ± 0.25	0.51
Body Fat percent (%)[†]	-0.14 ± 0.18	0.42	-0.10 ± 0.15	0.53	0.10 ± 0.18	0.58
BMI (kg/m^2)	-0.02 ± 0.09	0.78	0.01 ± 0.07	0.89	0.07 ± 0.08	0.41
Height (cm)	-0.32 ± 0.14	0.027	0.40 ± 0.13	0.001	-0.37 ± 0.14	0.009
Leg length (cm)	-0.15 ± 0.10	0.13	0.21 ± 0.08	0.013	-0.18 ± 0.10	0.058
Trunk length (cm)	-0.16 ± 0.08	0.039	0.19 ± 0.07	0.003	-0.19 ± 0.07	0.01
Birth Weight (kg)[‡]	0.03 ± 0.02	0.30	0.02 ± 0.02	0.27	0.03 ± 0.02	0.29

Linear regression models including age and sex as covariates.

[†] N = 3584, 3673 and 3493 respectively for the 3 SNPs

[‡] N = 2134, 2185 and 2088 respectively for the 3 SNPs

Table 4: Age adjusted means (sem) of BMI (kg/m^2) according to the three SNP genotypes in men and women.

SNP	Gender		11	12	22	p-value Recessive model	p-value Co-dominant model
6815 A/T (1=A; 2=T)	Men	*N*	*1229*	*765*	*117*		
		mean (sem)	26.36 (0.09)	26.23 (0.11)	26.58 (0.29)	0.36	0.90
	Women	*N*	*1514*	*958*	*188*		
		mean (sem)	25.71 (0.10)	25.63 (0.13)	25.71 (0.29)	0.92	0.77
1156 T/C (G/A) (1=G; 2=A)	Men	*N*	*829*	*1000*	*330*		
		mean (sem)	26.31 (0.11)	26.30 (0.10)	26.39 (0.17)	0.63	0.74
	Women	*N*	*1091*	*1217*	*412*		
		mean (sem)	25.6 (0.12)	25.83 (0.11)	25.41 (0.20)	0.14	0.82
820 G/A (ApaI) (1=G; 2=A)	Men	*N*	*1079*	*827*	*149*		
		mean (sem)	26.28 (0.09)	26.37 (0.11)	26.14 (0.25)	0.50	0.96
	Women	*N*	*1316*	*1054*	*209*		
		mean (sem)	25.62 (0.11)	25.61 (0.12)	26.01 (0.28)	0.17	0.39

Table 5: Age adjusted means (sem) of height (cm) according to the three SNP genotypes in men and women.

SNP	Gender		11	12	22	p-value Recessive model	p-value Co-dominant model
6815 A/T (1=A; 2=T)	Men	*N*	*1229*	*765*	*117*	0.65	0.23
		mean (sem)	174.7 (0.18)	174.2 (0.23)	174.8 (0.58)		
	Women	*N*	*1514*	*958*	*188*	0.13	0.058
		mean (sem)	161.7 (0.15)	161.4 (0.19)	160.9 (0.43)		
1156 T/C (G/A) (1=G; 2=A)	Men	*N*	*829*	*1000*	*330*	0.33	0.37
		mean (sem)	174.3 (0.22)	174.6 (0.20)	174.8 (0.35)		
	Women	*N*	*1091*	*1217*	*412*	0.0005	0.002
		mean (sem)	161.3 (0.18)	161.4 (0.17)	162.5 (0.29)		
820 G/A (ApaI) (1=G; 2=A)	Men	*N*	*1079*	*827*	*149*	0.80	0.068
		mean (sem)	174.8 (0.19)	174.2 (0.22)	174.4 (0.52)		
	Women	*N*	*1316*	*1054*	*209*	0.086	0.07
		mean (sem)	161.8 (0.16)	161.5 (0.18)	160.9 (0.41)		

Figure 1: Association of *IGF2* haplotypes for 1156T/C (G/A) and 820G/A (ApaI) with adult height. Tests for main haplotype effects used the Stata regression command xi:regres weighted by the haplotype assignment probability and clustered by the individual identification to obtain robust standard errors. The test of haplotype information against independent genotype information was tested using the Stata command qhapipf and was statistically significant (p=0.039). Analyses were adjusted for age and gender.

***** p<0.0001 ** p=0.0008**