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# Comment on "A Common Genetic Variant Is Associated with Adult and Childhood Obesity"

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Herbert *et al.* (Reports, 14 April 2006, p. 279) reported an association between the *INSIG2* gene variant rs7566605 and obesity in four sample populations, under a recessive model. We attempted to replicate this result in 10,265 Caucasian individuals, combining family-based, case-control, and general population studies, but found no support for a major role of this variant in obesity.

Herbert *et al.* (1) identified a common DNA variant 10 kb upstream of the *INSIG2* gene associated with obesity in 9881 adults and children from different ethnic groups. Their study combined case-control, general population, and family studies. They concluded that variation in this gene, which had been found through whole-genome scans in families, may contribute to obesity under a recessive model. Association studies of genome screen results need to be confirmed by additional replication studies (2). We therefore genotyped the *INSIG2* genetic variant rs7566605 in 10,265 subjects of French Caucasian descent. Our first data set was recruited for studying childhood obesity and comprised 449 families (F1, 2426 subjects) (3) with at least one sibling over the 97th percentile of body mass index (BMI) and 145 obese unrelated French children (CC, BMI over the 97th percentile). The second set was recruited for studying adult obesity (4, 5) and combined 386 families (F2, 1765 subjects), 350 obese (MO, BMI > 30) and 230 nonobese (CO, BMI < 27 kg/m<sup>2</sup>) unrelated French adults. The third data set was composed of families with offspring from a general (6) French population, the Fleurbaix Laventie Ville Santé (FLVS) study (287 families, 619 individuals). Finally, from the Données Epidémiologiques sur le Syndrome d'Insulino-Résistance (DESIR) cohort, we genotyped a fourth data set of 4998 middle-aged

unrelated French individuals who were followed for a period of 9 years (7). We performed association studies in four groups, as described below. The distribution of BMI according to genotype for each study is shown in Table 1. The minor allele frequency varied from 30% to 35% in our study populations, which is similar to the frequency reported by Herbert *et al.* (1). The genotypes were in Hardy-Weinberg equilibrium in each of our study populations.

We conducted tests of association in the presence of linkage and tests of simple association. As shown in Table 2, we observed no overtransmission of the rs7566605 C allele to obese children (BMI > 97th percentile) or adults (BMI > 30) in the F1 and F2 family data ( $P = 0.84$  and  $P = 0.76$ , respectively) (8). Similar results were observed for more severely obese children (BMI > 99th percentile,  $P = 0.61$ ). Furthermore, no association with BMI, corrected for gender and age, was found in the FLVS sample [quantitative family-based association test (FBAT),  $P = 0.61$ ].

We performed association studies in four groups, described in Table 1, using the General estimating equation (GEE) method (9). This method allows logistic or linear regression analysis in clustered data (10), and thus pooling of related and unrelated individuals. The first case-control study was defined within the DESIR population (Table 1A). A second case-control study used adult cases and controls of the familial study F2 and the unrelated individuals from MO and CO (Table 1B). A third analysis was performed on the parents of the childhood obesity data set (F1) and parents of the FLVS study (Table 1C). The results of these three analyses of adult obesity were pooled in a meta-analysis. A BMI of 30 kg/m<sup>2</sup> was chosen as a cutoff to define cases and controls, as in (1). The fourth study was on all the children, from F1, FLVS, and CC (Table 1D). Because allele frequencies in this study are correlated with those

of the third case-control set, this study was not used in the meta-analysis.

As shown in Table 2, no significant association was observed between rs7566605 and obesity under a recessive model in any of the individual studies. When we combined the three independent analyses of adult obesity, we also detected no association with obesity [OR = 0.93 (0.77 to 1.12),  $P = 0.61$ ] under the recessive model. No association was found under additive and general models. Moreover, no effect on BMI was shown within the DESIR cohort in a linear mixed model ( $P = 0.32$ ) with up to four observations per individual, in children ( $P = 0.13$ ), or in parents ( $P = 0.68$ ) of the FLVS study (Table 2D).

Interestingly, the negative results found in the DESIR cohort are similar to those observed in the Nurses Health Study cohort in (1). Under the suggested recessive mode of inheritance, we would have 80% power to detect an increase of 0.6 baseline BMI units by genotype, the lowest effect shown in the KORA study (1) using Quanto software (11). The absence of replication in this French study population is therefore unlikely to be due to low power.

In conclusion, in a data set as large as that of Herbert *et al.* (1), we found no effect of the *INSIG2* intronic variant on the risk of adult obesity or childhood obesity either in case-control or family-based designs. Our design combined extreme cases and general populations, which allowed testing of the effect both on a continuous trait and/or on morbid obesity. Furthermore, combining our results with the published case-control odds ratios (1) in a meta-analysis results in a global nonsignificant OR under a recessive model [1.10 (0.98 to 1.23),  $P = 0.10$ ]. Although a major contribution of *INSIG2* rs7566605 to the genetic risk of obesity in the West European population is unlikely, it remains possible that *INSIG2* contributes to BMI variation in other ethnic groups.

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**Table 1.** BMI (kg/m<sup>2</sup>) distribution according to rs7566605 genotypes in French case-control data sets. Study populations: **(A)** DESIR cohort. Cases: adults having obese status (BMI ≥ 30 kg/m<sup>2</sup>) at least once in the four clinical examinations (over 9 years). Controls: nonobese adults (BMI < 30 kg/m<sup>2</sup>) in any of the four clinical examinations. **(B)** Adult obesity. Cases: obese adults (BMI ≥ 30 kg/m<sup>2</sup>) from families F2, recruited for one overweight (BMI > 27 kg/m<sup>2</sup>), one morbidly obese (BMI > 40 kg/m<sup>2</sup>), and unrelated morbid obese individuals (sibships and unrelated). Controls: nonobese individuals (BMI < 30 kg/m<sup>2</sup>) from the adult obesity familial set

and additional controls (BMI < 27) recruited within the same study. **(C)** Parents of F1 and FLVS. Cases: obese parents (BMI ≥ 30 kg/m<sup>2</sup>) of the obese children of the F1 childhood obesity families and parents of the FLVS families. Controls: nonobese parents (BMI < 30 kg/m<sup>2</sup>) of the obese children of the F1 childhood obesity families and parents of the FLVS families. **(D)** Childhood obesity. Cases: obese children (BMI percentile ≥ 97th) from the childhood obesity sample (sibships of F1 and unrelated individuals). Controls: nonobese (BMI percentile < 90th) from the childhood obesity sample and from the FLVS child sample (sibships).

A. DESIR sample	Mean*	SD	SE	Proportion of individuals with: BMI < 18.5; 18.5 ≤ BMI < 25; 25 ≤ BMI < 30; 30 ≤ BMI < 35; 35 ≤ BMI < 40; BMI ≥ 40
<i>Females</i>				
GG	24.09	4.13	0.12	0.0368; 0.6424; 0.2284; 0.0719; 0.0171; 0.0034
CG	24.00	4.16	0.13	0.0365; 0.6475; 0.2200; 0.0826; 0.0086; 0.0048
CC	23.87	4.00	0.25	0.0502; 0.6641; 0.1969; 0.0811; 0.0077; 0.0000
<i>Males</i>				
GG	25.43	3.34	0.1	0.0093; 0.4875; 0.4123; 0.0817; 0.0084; 0.0009
CG	25.54	3.41	0.1	0.0046; 0.4745; 0.4279; 0.0803; 0.0109; 0.0018
CC	25.35	3.47	0.21	0.0038; 0.5267; 0.3893; 0.0611; 0.0115; 0.0076
<b>B. Adult obesity</b>	<b>Mean</b>	<b>SD</b>	<b>SE†</b>	
<i>Females</i>				
GG	39.31	12.17	0.59	0.0093; 0.1422; 0.0862; 0.1538; 0.0839; 0.5245
CG	37.38	11.21	0.51	0.0083; 0.1801; 0.1077; 0.1118; 0.1284; 0.4638
CC	38.05	11.26	0.98	0.0076; 0.1450; 0.1145; 0.1374; 0.0916; 0.5038
<i>Males</i>				
GG	34.46	10.97	0.75	0.0000; 0.1963; 0.2420; 0.1918; 0.0868; 0.2831
CG	35.44	11.43	0.72	0.0000; 0.2112; 0.1633; 0.1952; 0.0837; 0.3466
CC	34.26	10.52	1.38	0.0169; 0.1525; 0.2712; 0.1695; 0.0678; 0.3220
<b>C. Parents of F1 and FLVS</b>	<b>Mean</b>	<b>SD</b>	<b>SE</b>	
<i>Females</i>				
GG	28.86	7.62	0.50	0.0044; 0.3772; 0.2412; 0.1667; 0.1272; 0.0833
CG	29.00	8.64	0.49	0.0083; 0.3734; 0.2697; 0.1577; 0.1037; 0.0871
CC	29.50	7.78	1.18	0.0000; 0.3256; 0.2791; 0.2093; 0.0698; 0.1163
<i>Males</i>				
GG	28.21	5.37	0.33	0.0078; 0.2335; 0.5019; 0.1566; 0.0700; 0.0311
CG	28.22	5.12	0.35	0.0047; 0.2736; 0.4292; 0.1934; 0.0755; 0.0236
CC	29.18	5.51	0.85	0.0000; 0.2143; 0.4524; 0.1429; 0.1429; 0.0476
<b>D. Childhood obesity‡</b>	<b>Mean</b>	<b>SD</b>	<b>SE†</b>	<b>Proportion of individuals with BMI in percentiles: BMI &lt; 0.05; 0.05 ≤ BMI &lt; 0.58; 0.58 ≤ BMI &lt; 0.87; 0.87 ≤ BMI &lt; 0.97; 0.97 ≤ BMI &lt; 0.99; BMI ≥ 0.99</b>
<i>Females</i>				
GG	2.49	2.43	0.12	0.0106; 0.1941; 0.1223; 0.0372; 0.0878; 0.5479
CG	2.60	2.25	0.13	0.0153; 0.1713; 0.1437; 0.0367; 0.0489; 0.5841
CC	2.45	2.38	0.26	0.0172; 0.1897; 0.1207; 0.0172; 0.1207; 0.5345
<i>Males</i>				
GG	2.40	2.27	0.13	0.0295 ; 0.1967; 0.1246; 0.0426; 0.0721; 0.5344
CG	2.21	2.35	0.13	0.0261 ; 0.2313; 0.1466; 0.0293; 0.0684; 0.4984
CC	2.67	2.17	0.26	0.0147 ; 0.1912; 0.1324; 0.0147; 0.0293; 0.6176

\*Mean is given for the BMI at first examination. †Standard errors are estimated through the GEE procedure implemented in the Stata 5.0 software (command xtgee). ‡For the childhood case-control study, the BMI distribution is shown by percentiles from a general population.

**Table 2.** Results of family-based and case-control analyses. Meta-analyses were performed on the French study populations alone and together with the study populations reported by Herbert *et al.* Tr, transmitted alleles; Non-TR, nontransmitted alleles.

Study	Design	Informative families	Tr	Non-Tr	Test	Recessive model	Additive model
<b>A. Test of association and linkage</b>							
Child obesity F1	Family	54	152	153	FBAT	Z = 0.20 P = 0.84	Z = 0.13 P = 0.89
FLVS	Family	31	—	—	Quantitative FBAT	Z = 0.52 P = 0.61	Z = 0.22 P = 0.82
Adult obesity F2	Family	47	62	72	FBAT	Z = -0.29 P = 0.76	Z = 0.44 P = 0.62
Study	Design	Genotyped	Obese	Controls	Statistic	Recessive model	General model*
<b>B. Independent case-control studies on adult obesity</b>							
DESIR	Case-control	4998	905	4093	Logistic regression	OR = 0.86 [0.68–1.11] P = 0.25	$\chi^2_{2df} = 1.35$ P = 0.49
Adult obesity	Case-control	1572	1076	496	Logistic regression†	OR = 0.93 [0.66–1.29] P = 0.67	$\chi^2_{2df} = 1.37$ P = 0.50
Parents (F1 and FLVS)	Case-control	1023	329	694	Logistic regression	OR = 1.18 [0.69–2.03] P = 0.61	$\chi^2_{2df} = 1.16$ P = 0.58
French population meta-analysis					Mantel-Haenszel	OR = 0.93 [0.77–1.12] P = 0.61	Fixed effects
Overall meta-analysis					Mantel-Haenszel	OR = 1.10 [0.98–1.23] P = 0.10	Fixed effects
<b>C. Case-control study on childhood obesity</b>							
Children	Case-control	1531	912	532	Logistic regression†	OR = 1.11 [0.72–1.69] P = 0.67	$\chi^2_{2df} = 1.14$ P = 0.56
<b>D. Test of association with the quantitative trait BMI</b>							
DESIR	Cohort	4998	—	—	Linear regression‡	$\beta = -0.17 [-0.5–0.17]$ P = 0.32	$\chi^2_{2df} = 2.14$ P = 0.34
FLVS parents	Cohort	342	—	—	Linear regression	$\beta = 0.36 [-1.39–2.12]$ P = 0.68	P = 0.72
FLVS children	Family	1138	—	—	Linear regression†	$\beta = 0.27 [-0.08–0.62]$ P = 0.13	$\chi^2_{2df} = 3.42$ P = 0.18

\*The general model included two variables for the single-nucleotide polymorphism genotype. The first one (0,1,2) is the number of C alleles, and the second reports whether the genotype is heterozygous (0) or not (1). †GEEs (corrected for age and sex). Familial correlation was accounted for by using a sandwich estimator of the variance and exchangeable correlation. ‡Mixed model (corrected for age and sex) on four time points, every 3 years.