Comment on: Jowett et al. (2010) Genetic Variation at the *FTO* Locus Influences *RBL2* Gene Expression. Diabetes;59:726–732

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Jowett et al. (1) have examined the association of single nucleotide polymorphism (SNP) rs8050136 in intron 1 of the *FTO* (fat mass– and obesity-associated) gene with levels of transcripts up to 5 Mb in either direction of this SNP in lymphocytes from 1,240 individuals. This is an important study because it has been a matter of debate through which gene the obesity-associated SNPs in intron 1 of the *FTO* gene affect body weight regulation. The authors did not find an association of these SNPs with *FTO* gene expression but found a strong association with transcript levels of the *RBL2* (retinoblastoma-like 2) gene located 270 kb upstream of *FTO*. We note that the findings have not been replicated in an independent cohort.

Studies on *cis*-regulatory effects on gene transcription in humans are hampered by the fact that the tested individuals unavoidably differ in genetic background, age, life events, and environment. These problems can be circumvented by determining the ratio of allelic transcript levels in heterozygous individuals so that each allele serves as an internal control for the other (2). For this approach, only a few subjects are needed.

Using fluorescence-tagged SNP analysis, we have determined allelic transcript levels of *RBL2* in blood samples from six individuals heterozygous for SNP rs3929 (C/G) in the 3′ untranslated region of the gene. Three of these individuals were also heterozygous for the *FTO* SNP rs8050136 (C/A), and three were homozygous (two AA and one CC). If *FTO* variants affected *RBL2* gene expression in *cis*, we would expect severely skewed *RBL2* allelic expression in the *FTO* heterozygous individuals, but not in the *FTO* homozygous individuals.

The forward primers used for amplification of genomic DNA (gDNA) and cDNA fragments spanning rs3929 were 5′-TGAGCTATGTGCATTTGCATT-3′ and 5′-CCTTATTAC GCCGTCTCCAA-3′, respectively. The reverse primer for both gDNA and cDNA was 5′-TCACCAAAATGTCCCCT CAT-3′. For primer extension, we used the reverse primer 5′-CCTCATGTTACTAACAGGCTGTAACT-3′ and the SNaPshot kit (Applied Biosystems, Foster City, CA). Allelic gDNA ratios were used to normalize the cDNA ratios. Means and SD were calculated with JMP7 (SAS, Cary, NC). For a more detailed description of the assay, see ref. 2.

In *FTO* heterozygous and homozygous individuals, we observed allelic *RBL2* transcript ratios of 0.88 ± 0.02 (mean ± SD) and 0.90 ± 0.02, respectively. Our results show that allelic expression of the *RBL2* gene is slightly skewed but independent of the *FTO* genotype. The skewing is probably due to a *cis*-regulatory *RBL2* SNP that is in linkage disequilibrium with rs3929.

In summary, we have used a direct experimental approach to investigate whether *RBL2* gene expression is regulated by SNPs in the *FTO* gene. Our findings do not support the claim of Jowett et al. that variants at *FTO* influence *RBL2* gene expression at large genetic distances.

Note added in proof: Recently, we found that the SNPs in intron 1 of the *FTO* gene affect allelic transcript levels of the *FTO* gene (3).

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