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

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The comment submitted by Berulava and Horsthemke (1) on our recent work regarding the biological effects on gene expression of the *FTO* single nucleotide polymorphism (SNP) rs8050136 (2) is welcomed and achieves one of the study's goals—to inspire follow-up studies on our observation that these SNPs may affect *RBL2* expression but not *FTO* itself. The approach taken by Berulava and Horsthemke is to quantify allelic-specific transcript levels in cells that are heterozygous for a given indicator variant in the mRNA of the gene of interest (in this case, rs3929 in the 3′ untranslated region of *RBL2*). They measure the ratio of allele-specific transcripts for *RBL2* in three *FTO* SNP (rs8050136) heterozygotous individuals (AC) and two homozygous individuals (AA) and report that skewing of *RBL2* allelic expression was detected but not significant.

The presented data fail to replicate the observation published in our original study. There are many possible reasons besides a type I error in our original article for the failure to replicate. Our observations were made in lymphocytes, whereas Berulava and Horsthemke use blood samples, which contain a mixture of different cell types such as lymphocytes, monocytes, and neutrophils. The component proportions of these subsets may vary from individual to individual and within individuals over time due to changes in environment, such as infection and inflammation. Because cell types differ in their available pool of transcription factors influencing expression, these differences may mask subtle effects of transcriptional control elements.

Our study evaluated gene expression and SNP genotype in 854 individuals and was well powered to detect modest effects; however, Berulava and Horsthemke report data from only six individuals (two AA, three AC, and one CC) by examining allele-specific transcript levels (whereas we report on total transcript levels). Our results suggested that about 3% of the variance in *RBL2* transcript levels was attributable to the rs8051036 SNP. Just as in our study, Berulava and Horsthemke are also making a statistical argument. Thus, our primary concern is type II error in their very small experiment. As with any replication attempt, we need to know what the power to detect such a modest effect, using this alternative mode of testing, is in only six observations.

Additionally, it is most likely that our observed signal is due to linkage disequilibrium with another functional regulatory variant. Given the very substantial allele frequency differences between Europeans (f[A] = 0.45) versus Mexican Americans (f[A] = 0.23) for our focal allele, there also are likely to be differences in linkage disequilibrium values with other variants across populations. This could easily further attenuate the observed signal across these studies.

As discussed, in our study, we note that human transcriptional control is highly complex and involves many factors, combined with an additional layer of temporal control. It is possible that the dominant effects of the rs8050136 SNP may occur in tissues other than lymphocytes and at different times during development and aging in humans. Sex and age were both significant covariates in our genetic association model. These factors may explain why, in a large cohort with a range of ages and mixture of sexes, we were able to observe a statistically significant relationship between rs8050136 and *RBL2* gene expression that may not be apparent in a smaller sample.

We thank Berulava and Horsthemke for following up on our work. However, at this point, we still think the preponderance of the statistical evidence lies in the direction of a correlation of sequence variation marked by the focal SNP on *RBL2* transcription levels, at least in lymphocytes. We await a truly parallel replication test to answer the question more definitively.

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REFERENCES

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