Letter to the Editor

Comment on: Marchand and Polychronakos (2007) Evaluation of Polymorphic Splicing in the Mechanism of the Association of the Insulin Gene with Diabetes: *Diabetes* 56:709–713

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This letter concerns quantitative modeling of allelic insulin gene splicing, transcription, and translation in complex traits. Marchand and Polychronakos (1) report an ex vivo study of allelic insulin gene pre-mRNA splicing in follow up to our demonstration (2) of the in silico and in vitro of effects of the −23*Hph*I (IVSI-6A/T) polymorphism on 5′-noncoding intron I retention. This retention occurs in insulin mRNA, and there are additional consequential effects on translation from which we proposed a new category of quantitative trait loci dubbed STEPs (splice translational efficiency polymorphisms) (3). Marchand and Polychronakos confirmed the existence of message with intron I retention in human fetal pancreata, and they also confirmed that this is mostly driven by A alleles. It was estimated that this fraction could not represent >3–4% of total insulin mRNA, in which case (using our measurement of sixfold greater translational efficiency from the intron I retention fraction), one would not predict >15–18% greater insulin production in AA individuals. The authors conclude that their findings suggest a minimal effect, if any at all, of allelic alternative splicing in the causal chain between insulin (*INS*) gene haplotypes and type 1 diabetes risk (1). However, more in-depth quantitative modeling of allelic insulin gene splicing, transcription, and translation will be required to prove this.

First, the studies were undertaken on fetal pancreata (and thymus). This is relevant to the early development of immunological tolerance, but it does not closely model the "stressed " β-cells of a pre-diabetic individual. It has been shown in normal mouse islets that some cells conduct more insulin gene transcription compared with others in response to glucose (4). In the observed model, the use of arithmetic means for the cell population obscures this reality. It is possible that the slow splicing of intron I (pronounced on IVSI-6A alleles) might lead to greatest intron I retention in fast (F) transcribing cells. By defining subscripts F and S (slow) and using designations I6 and I4 for retention and fully spliced isoforms, the mole fraction of I6 observed would be $I6_F/(I4_F + I4_S)$. In a healthy fetus, $I4_S$ would predominate, but the value $I6_F/I4_F$ could be several-fold higher (4). When all (or residual) β-cells become fast transcribing (due to β-cell loss, insulin resistance, or postprandial glucose spike), the proportion of $I6_F$ might increase several-fold. Thus, there are both spatial, temporal, and disease-associated reasons to consider nonlinear effects. Previous reports are of 15–30% lower mRNA expression from *INS* class III alleles, whereas the odds ratio for diabetes is two- to threefold lower compared with class I homozygosity. Again, nonlinear models may be pertinent in considering possible pancreatic effects.

Lastly, as for the two polymorphic codons of *APOE*, both individually affecting lipoprotein, type III dyslipidemia, coronary disease, and late-onset Alzheimer's disease risks (5), it is quite plausible that both *INS* VNTR and IVSI-6A/T loci (and even the 3′ noncoding SNP in perfect linkage disequilibrium) exert effects, possibly under different pathophysiological conditions, in contrast with the situation for rare severe gene mutations where a single change is usually responsible for the phenotype(s). In the quest to identify a causal mechanism, it will be challenging to examine *INS* alternative splicing products at the single cellular level in the β-cells of pre-diabetic, diabetic, pediatric or adult obese, or other subjects.

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