Assessing regulatory potential and functional consequences of Type 2 diabetes-associated variants on 9p21.

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Variants within a 9.5 kb linkage disequilibrium block on chromosome 9p21, ~125 kb upstream of the heavily studied genes *CDKN2A/2B*, have been associated with type 2 diabetes (T2D) and decreased beta cell function in multiple studies and populations. Another gene on the opposite strand, *ANRIL*, is also a potential candidate for driving the T2D association, but its noncoding RNA is much less well characterized. Independent studies in mouse indicate that overexpression of *CDKN2A* (p16) inhibits beta cell proliferation. Taken together, these observations suggest the following hypothesis: (1) the associated region in humans harbors regulatory elements, (2) these elements regulate *CDKN2A*, and (3) the causal variant(s) increase expression in islets. We have cloned the entire T2D-associated LD block, as well as two smaller evolutionarily constrained sequences residing within this block, from risk and non-risk haplotypes present at $\geq$ 5% frequency in FUSION samples and tested them for enhancer activity in a minimal promoter luciferase assay in both beta cell-like (INS-1E) and non-beta cell (HeLa) lines. In preliminary experiments, we have observed a decrease in luciferase activity with these sequences. We are testing these elements for silencer activity and for specific enhancer/silencer activity of the *CDKN2A* promoter and cloning additional haplotypes present at a frequency of $\geq$ ~1% for testing. In addition, we are analyzing effects of the risk-associated variants on allele-specific and overall expression levels of *CDKN2A/B* and the putative noncoding transcript *ANRIL* in fibroblasts, peripheral blood lymphocytes, monocytes, adipose, and pancreatic islets. Though challenging, we expect these studies of allele-specific gene regulation in 9p21 will reveal possible mechanisms for T2D susceptibility.