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 ≥ 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 ≥ ~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.