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Preliminary report: No association between *TCF7L2* rs7903146 and euglycemic-clamp–derived insulin sensitivity in a mixed-age cohort

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Abstract

There are conflicting reports about the significance of *TCF7L2* single nucleotide polymorphism rs7903146, a single nucleotide polymorphism found to be associated with type 2 diabetes mellitus in several genomewide association studies, and insulin sensitivity. The association of rs7903146 and euglycemic-clamp–derived insulin sensitivity was tested in a cohort of children and their parents. Four hundred seventy whites (from 226 families) and 89 African Americans (from 48 families) were included in the analysis. No significant associations were seen between rs7903146 and insulin sensitivity. Adjusted genotype means were consistent across races and generational subgroups.
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1. Introduction

Studies have sought to determine the mechanisms underlying the well-established association between single nucleotide polymorphism (SNP) rs7903146 in *TCF7L2* and type 2 diabetes mellitus. Studies that have examined the association of this SNP with insulin sensitivity derived from the euglycemic clamp [1-3] or the intravenous glucose tolerance test [4-6] have produced inconsistent results. Single nucleotide polymorphism rs7903146 has been associated with insulin sensitivity in American whites and elderly Danish twins [3,5,6], but not in Hispanic Americans, Germans, African Americans, or Swedish men [1,2,4,6].

2. Methods

Subjects were drawn from a longitudinal study of cardiovascular risk factors in adolescents. Details of the recruitment have been published previously [7]. The study was approved by the Human Subjects Committee of the University of Minnesota. Briefly, in 1996, Minneapolis school children were randomly selected with stratification according to sex, race (African American and white), and systolic blood pressure. Informed consent was obtained from 401 children (probands) and their parents. Probands returned for a subsequent study visit at a mean age of 19 years, at which time parents and siblings of the probands also were recruited into the study and underwent many of the same measurements as the probands. Individuals with both rs7903146 genotypes and insulin sensitivity measurements from this visit were included in the analysis. Individuals who self-reported a diagnosis of diabetes or use of diabetes medication were excluded, as were individuals with a fasting glucose measurement greater than 126 mg/dL.

Insulin sensitivity was determined from the euglycemic clamp. Euglycemic clamp studies were conducted in the University of Minnesota Clinical Research Center after a 12-hour fast as previously described [7]. Plasma glucose was measured at baseline and every 5 minutes during the clamp. The insulin infusion was started at time 0 and continued at 1 mU/kg/min for 3 hours. An infusion of 20% glucose was started at time 0 and adjusted, based on plasma glucose levels, to maintain plasma glucose at 100 mg/dL. Insulin sensitivity was determined from the amount of glucose

The Human Subjects Committee of the University of Minnesota approved this study.

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Table 1
Adjusted[a] means and confidence intervals of insulin sensitivity by rs7903146 genotype

Population	Genotype means (95% CI)[b] of insulin sensitivity in mg/kg/min			*P* value[c]
All	CC = 10.9 (10.6, 11.3) n = 314	CT = 11.1 (10.4, 11.7) n = 205	TT = 11.1 (9.9, 12.2) n = 40	.85
Whites	CC = 11.1 (10.6, 11.5) n = 262	CT = 11.1 (10.5, 11.7) n = 175	TT = 11.8 (10.6, 13.0) n = 33	.48
African Americans	CC = 10.1 (9.2, 11.0) n = 52	CT/TT = 10.7 (9.4, 12.0) n = 37		.40[d]
Parents (ages 32-64, median = 48)	CC = 11.1 (10.4, 11.8) n = 117	CT = 11.4 (10.6, 12.3) n = 81	TT = 11.7 (9.6, 13.8) n = 14	.75
Offspring (ages 11-30, median = 18)	CC = 10.7 (10.3, 11.2) n = 197	CT = 11.0 (10.2, 11.7) n = 124	TT = 10.5 (9.2, 11.7) n = 26	.77

CI indicates confidence interval.
[a] All regression analyses adjusted for age and sex.
[b] 95% Confidence intervals presented in parentheses next to genotype means.
[c] For a 2-*df* test.
[d] CT and TT genotypes were combined for analysis because of small numbers, resulting in a 1-*df* test.

administered over the final 40 minutes of the euglycemic clamp and was expressed as glucose utilization per kilogram lean body mass per minute. Percentage of body fat and lean body mass or fat-free mass were calculated by dual-energy x-ray absorptiometry.

The SNP associations were calculated in a mixed linear regression model (SAS version 9.1; SAS, Cary, NC). A compound symmetry correlation structure was specified to account for expected correlation within families, and a sandwich estimator was used to calculate the variance. All analyses were adjusted for age and sex. A 2-*df* association test was used for SNP genotypes, except for association analyses in African Americans, where a 1-*df* test was used because of small numbers; in this case, individuals having CT or TT genotypes were pooled for analysis.

3. Results

After exclusions, there were 470 whites from 226 families and 89 African Americans from 48 families in the sample. Forty-seven percent of the families included 1 parent, 24% included 2 parents, and 29% included no parents. Thirty-eight percent of the families included 1 child, 37% included 2 children, 16% included 3 or more children, and 9% included no children. Forty-nine percent of the sample was male.

Table 1 shows the results of the regression of clamp-derived insulin sensitivity on *TCF7L2* SNP rs7903146 in the total study sample and in racial and generational subgroups. Because the patterns of genotype-specific means were similar in both races, the decision was made to combine the racial groups for analyses. In the total sample, there was no significant association between insulin sensitivity and rs7903146 ($P = .85$, F value = 0.16 on 2 *df*). There was also no significant association observed in any of the subgroups, and similar patterns of genotype-specific means (a slight increase with the heterozygous or homozygous minor allele genotypes) were seen for most subgroup analyses. Additional adjustment for BMI in the total sample and in racial and generational subgroups did not materially change the results.

4. Discussion

The results of this study differ from previous studies that found a significant association between rs7903146 and insulin sensitivity in whites [3,5,6]. Furthermore, the pattern of insulin sensitivity by genotype observed in this study (a slight increase with the heterozygous or homozygous minor allele genotypes) is opposite to other published studies [5,6] and not consistent with the association of the T allele and type 2 diabetes mellitus [5,6]. The inconsistencies between this study and others may be due to the use of the intravenous glucose tolerance test, as some surrogate measures of insulin sensitivity/resistance have been shown to have different genetic determinants than clamp-derived insulin sensitivity [8], or to smaller sample sizes in previous studies that may have led to spurious results [3,5,6]. Strengths of this study include a relatively large sample size and the use of the criterion standard measure of insulin sensitivity. Weaknesses include the small number of African Americans in the sample that limited the power of analyses in this subgroup. In conclusion, this analysis offers additional evidence that the rs7903146 SNP in *TCF7L2* is not associated with insulin resistance in whites [1,2,4]. This analysis should be repeated in larger African American cohorts to verify the lack of association in this racial group.

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