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Longer Life Foundation Final Research Report: "Determination of the Genetic Contribution to Glycemic Control and CAD Outcomes in Patients with DM2 and CAD"

ABSTRACT
Diabetes mellitus (DM) currently affects more than 220 million people worldwide and its prevalence is increasing. Among persons with DM, complications of accelerated atherosclerosis, particularly acute coronary events, are the principal cause of death. To date, few studies have examined the hypothesis that genetic variation may account for variable responses to treatment and variable clinical outcomes among patients with DM and coronary artery disease (CAD).

The **B**ypass **A**ngioplasty **R**evascularization **I**nvestigation **2 D**iabetes (**BARI 2D**) is a multicenter trial examining the effects of different approaches to treatment of CAD and DM on long-term outcomes in patients with type 2 DM. Using a custom PPAR-pathway gene SNP microarray containing 3,351 single nucleotide polymorphisms (SNPs) in 223 PPAR-pathway genes, we investigated genetic associations of PPAR-pathway genes with extent of CAD among patients with DM in the BARI 2D trial. We found a specific variant in a PPAR-pathway gene that was associated with the extent of CAD in BARI 2D and validated this finding in two independent cohorts. This is the first report of a genetic variant that correlates with the extent of atherosclerosis in patients with diabetes. The physiologic pathways affected by this genetic variant may provide novel targets for further investigation and therapeutic intervention to address the accelerated rate of progression and the high risk of adverse events associated with CAD in patients with DM.

LAY SUMMARY
Sequence differences in genes that are involved in energy use by the heart have been associated with the development of both diabetes and coronary artery disease. This study attempted to determine if specific gene sequence differences were associated with better or worse outcomes in patients with diabetes and coronary artery disease. We found that one specific gene sequence difference was associated with the severity of coronary artery disease in patients with type 2 diabetes and was able to predict the extent of coronary artery disease better than any clinical factor. The physiologic pathways affected by this genetic variant may provide novel targets for further investigation and therapeutic intervention to address the accelerated rate of progression and the high risk of adverse events associated with CAD in patients with DM.

INTRODUCTION
Diabetes mellitus (DM) currently affects more than 17 million people in the U.S. and approximately 1.6 million new cases are diagnosed each year. Among persons with DM, coronary atherosclerosis is highly prevalent and accounts for the majority of deaths. In patients with coronary artery disease (CAD), patients with DM have lower 10-year survival than those without diabetes, and the extent of CAD predicts mortality. Atherosclerosis in patients with DM has an accelerated phenotype, with more

diffuse and extensive disease that shows more rapid progression, suggesting a distinctive pathogenesis. The distinct pathogenesis of the accelerated atherosclerosis observed among patients with DM is poorly understood, and the role of genetic factors is unknown.

Bypass **A**ngioplasty **R**evascularization **I**nvestigation **2 D**iabetes (BARI 2D) was a multicenter randomized clinical trial that investigated the effect of different approaches to the treatment of both CAD and DM on long term outcomes in patients with type 2 DM. All patients in BARI 2D had DM and CAD suitable for, but not requiring, revascularization. Baseline extent of CAD in BARI 2D was defined angiographically and quantified by a core laboratory. Clinical factors contributing to extent of CAD among patients in BARI 2D, defined as the number of coronary lesions ≥ 20% diameter stenosis (DS), were recently reported, however, the total variance in extent of CAD explained by baseline clinical factors was less than 10%, providing an important rationale for examining the putative role of genetic factors.

Peroxisome-proliferator activated receptor (PPAR)-pathway genes are involved in cellular processes relevant to both CAD and DM. PPARs are master regulators of lipid and glucose homeostasis, cardiac energy metabolism, vascular inflammation and cell differentiation and have been implicated in the development and progression of both type 2 DM and atherosclerosis in animal studies. We therefore hypothesized that investigating genetic variation within the PPAR gene pathway would identify novel genes involved in diabetic atherosclerosis.

Using a custom PPAR-pathway gene SNP microarray containing 3,351 single nucleotide polymorphisms (SNPs) in 223 PPAR-pathway genes, we investigated genetic associations of PPAR-pathway genes with extent of CAD among patients with DM in the BARI 2D trial. We then sought to validate the discovered associations in two additional well-phenotyped cohorts of patients with CAD and DM.

METHODS

1,043 patients (702 Caucasian; 175 African-Americans) from the **B**ypass **A**ngioplasty **R**evascularization **I**nvestigation **2 D**iabetes (BARI 2D) genetic cohort were genotyped for 3,351 variants in 223 PPAR-pathway genes using a custom targeted-genotyping array. As an example of genotyping results, Figure 1 shows the clustered genotype results (AA in red, AT in blue, and TT in green) for 122 patients for one o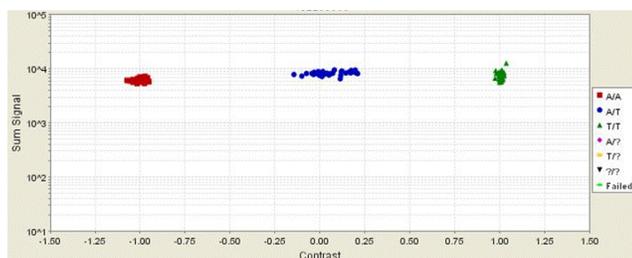f the SNPs on the PPAR-pathway custom chip. For all samples genotyped, data completeness was 99.30% and repeatability was 99.98%. Cluster analysis implemented in PLINK was utilized for screening for evidence of subtle population stratification based on pairwise identity-by-state (IBS) sharing distance among all possible pairs of the SNPs. Across the samples of our BARI 2D genetic cohort, four well-separated subgroups correlated with self-reported ethnic groups, and the two major groups of African

Figure 1. Clustered genotype results for 122 patients for a single SNP on the PPAR-pathway custom targeted-genotyping chip.

Americans and Caucasians were distinguished. Within Caucasian and African American, no further population stratification or genetic drift was detected according to multidimensional scaling plots.

Angiographic endpoints were determined by a core laboratory. The primary endpoint for our analysis was number of lesions ≥ 20% DS documented on the coronary angiogram. Myocardial jeopardy index and number of lesions ≥ 70% DS were also determined for variant(s) achieving significance in replication cohorts. Number of lesions and myocardial jeopardy index were evaluated as continuous variables with kernel density estimators used to plot their overall distributions. Continuous variables were compared by student t tests or Wilcox tests (depending on distributional properties); categorical variables were compared by chi-square tests. Stepwise linear regression was used to identify baseline factors associated with number of lesions (log transformed) and myocardial jeopardy index.
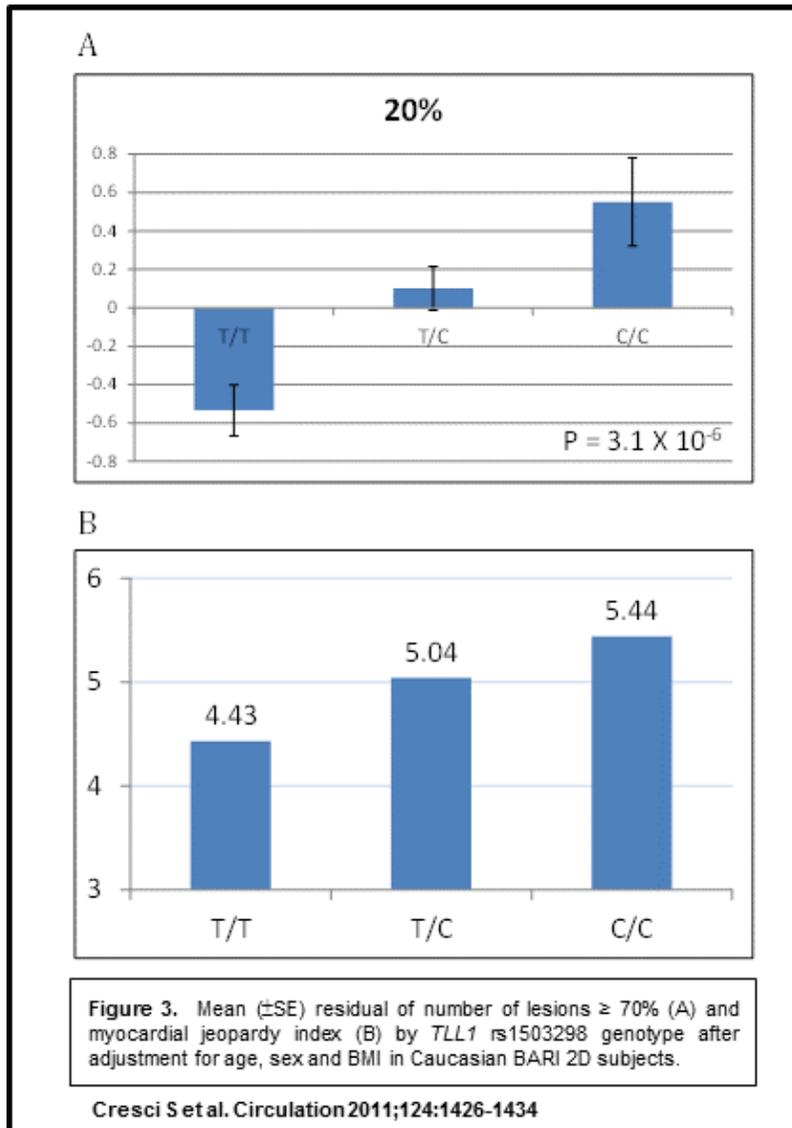
RESULTS

In Caucasian BARI 2D subjects, single SNP analysis identified one SNP (rs1503298) that was highly significantly associated (P = 5.5 x 10^{-6}) with number of coronary lesions ≥ 20% DS (Figure 2), even after stringent Bonferroni correction for all 3,351 SNPs. This association was validated in the diabetic subgroups of two independent cohorts, the TRIUMPH post-myocardial infarction registry and the prospective Family Heart Study of individuals at risk for CAD. This SNP is located in intron 12 of the gene that encodes Tolloid-like 1 (*TLL1*), a metalloproteinase that regulates bone morphogenetic protein-2 (BMP-2) and transforming growth factor β. This association remained significant after including all baseline clinical covariates and principal components in the model. The mean (±SE) residual of number of lesions ≥ 20% by *TLL1* rs1503298 genotype after adjustment for age, sex and BMI in Caucasian BARI 2D subjects is shown in Figure 3A. To put this data into a clinically relevant context, an average BARI 2D subject (a 63 year old male with a BMI of 30) with the TT genotype would have 4.43 coronary lesions ≥ 20% DS, with the CT genotype would have 5.02 coronary lesions ≥ 20% DS, and with the CC genotype

Figure 2. Results of tests for a trend in the association between number of lesions ≥20% and each single nucleotide polymorphism on a custom chip in whites in Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D). Red line depicts the threshold for statistical significance after strict, conservative Bonferroni correction for multiple comparisons.

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would have 5.46 coronary lesions ≥ 20% DS (Figure 3B). Of note, in general linear regression modeling, this SNP explained more variance of the phenotype (number of coronary lesions ≥ 20% DS) than the previously determined clinical factors. *TLL1* rs1503298 explained 2.75% of the variance as compared to sex (1.61%), age (0.12%), and BMI (0.01%). Subjects with *TLL1* rs1503298 CT genotype had 22% more coronary lesions ≥ 20% DS as compared with those with TT genotype and those with the CC genotype had 37% more coronary lesions ≥ 20% DS.
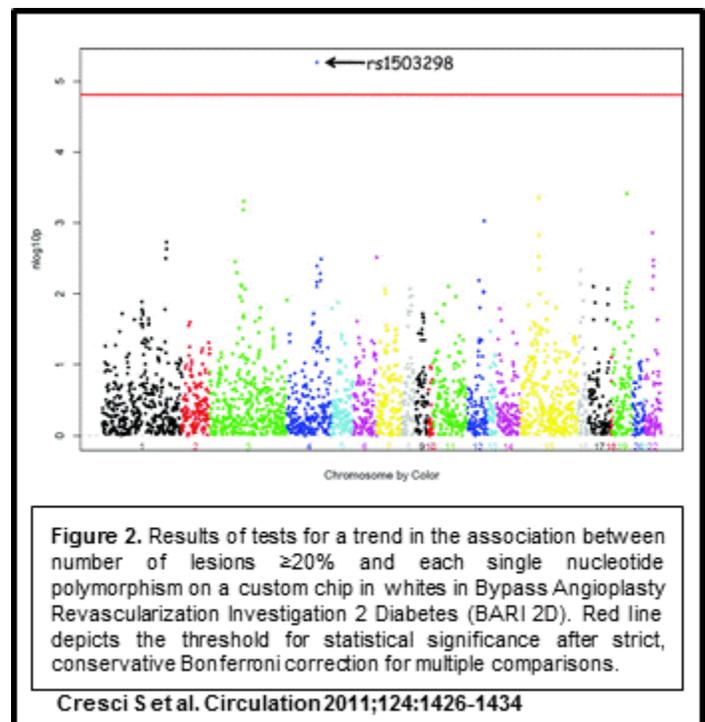
The *TLL1* rs1503298 polymorphism showed strong associations not only with the primary endpoint of number of lesions ≥ 20% DS, but also with number of angiographically severe (≥70% DS) lesions, and with myocardial jeopardy index, a semi-quantitative method used to estimate the amount of potential myocardial ischemia attributable to the location and severity of coronary lesions in an individual; these measures of extent of severe CAD have previously been shown to correlate with prognosis in patients with CAD. To our knowledge, this is the first demonstration of a significant association of genetic variation specifically with extent of the atherosclerotic disease that develops among patients with DM, where atherosclerosis has been known to display a distinctively aggressive phenotype.

Figure 3. Mean (±SE) residual of number of lesions ≥ 70% (A) and myocardial jeopardy index (B) by *TLL1* rs1503298 genotype after adjustment for age, sex and BMI in Caucasian BARI 2D subjects.

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DISCUSSION

The observation of an association between extent of CAD in patients with DM and this particular gene in the PPAR-pathway regulated processes is noteworthy. *TLL1* encodes a protein, Tolloid-like1, that has been identified within the cascade of cellular processes related to vascular inflammation and calcification. Since vascular calcification is a prominent feature of the phenotype of diabetic atherosclerosis, it is intriguing to speculate that genetic variability in *TLL1* is may have strong biologic plausibility as a contributor to its pathogenesis. Furthermore, the physiologic pathways affected by this genetic variant may provide novel targets for further investigation and therapeutic intervention to address the accelerated rate of progression and the high risk of adverse events associated with CAD in patients with DM.

FUTURE PLANS

These data will be extended to investigate whether the *TLL1* variant that is associated with extent of CAD is also associated with clinical outcomes among patients with diabetes and CAD, including myocardial infarction and mortality, and potentially form the foundation for an R01 submission.