ONLINE APPENDIX—Supplementary Information

MATERIALS AND METHODS

Study Participants: The DREAM trial includes 5,269 individuals ≥30 years of age with either IFG or IGT. Participants were recruited from 191 sites in 21 countries(1). IFG was defined as a fasting plasma glucose concentration between 6.1 mmol/L and 7.0 mmol/L and 2-h post load plasma glucose concentration < 11.1 mmol/L. IGT was defined as a fasting plasma glucose concentration < 7.0 mmol/L and 2-h post load plasma glucose concentration between 7.8 mmol/L and 11.1 mmol/L. Individuals were excluded if they had a history of diabetes, cardiovascular disease, or intolerance to either ACE inhibitors or TZDs. Individuals were randomized to receive 8mg/day of rosiglitazone, which was uptitrated from a starting dose of 4mg/day for the first two months. Individuals were also randomized to receive ramipril (15 mg/day) or placebo following a 2x2 factorial design.

Genotyping & Statistical Analysis: We genotyped 4,197 participants of the DREAM study, from six ethnic groups defined by self-report independent of geographic location, using an Illumina iSelect Custom Genotyping Beadchip, the IBC (ITMAT/Broad/Care) cardiovascular array(2). A complete list of the genes and SNPs captured by the IBC chip is available (http://bmic.upenn.edu/cvdsnp/). We removed SNPs with a call rate less than 90% (1280 SNPs) and individuals with less than 97% of the genotypes called (29 individuals). We performed a principal component analysis (PCA) of the proportion of alleles shared identity by state (IBS) using the software smartpca(3), and removed individuals who failed to cluster with individuals of the same self-reported ethnicity. 4,118 individuals and 43,361 SNPs passed these criteria. 32,088 SNPs passed our quality control within individuals of European ancestry and had a minor allele frequency (MAF) greater than 1%. The IBC chip was designed using a cosmopolitan tagging procedure to capture the genetic diversity present in the HapMap and Seattle SNP populations(2). As a result 11273 SNPs had a frequency <1% in our European sample.

The ethnic groups included in the DREAM trial are not equally represented and we observed differing rates of TZD-induced edema among them (Supplementary Table 1). Individuals of European and Latin American ancestry represented 75% of all genotyped samples and are the only groups (with the exception of East Asians) to have a significant increase in TZD-induced edema, although a significant overall trend was observed. In fact, the European participants account for 55% of the DREAM trial participants (n=1,921) and 65% of all cases of TZD-induced edema. To minimize the potential for population stratification and heterogeneity caused by different patterns of linkage disequilibrium (LD) among the ethnic groups, we decided to test for SNPs associated with edema in the rosiglitazone treated Europeans.

Statistical Analysis: For the scan of the individuals of European descent receiving rosiglitazone, we performed a logistic regression analysis for each SNP adjusted for age, sex, BMI, and the use of angiotensin converting enzyme (ACE) inhibitors and calcium channel blockers (CCBs). Individuals taking diuretics were removed from the analysis (n=177, n=358 in both treatment arms). To control for population substructure we included the first 10 principal components of the alleles shared identity by state among Europeans as covariates in our analyses, as described by(4), which reduced the genomic inflation factor (λ) from 1.07 to 1.03, but did not significantly affect the results (data not shown). We applied a Bonferroni correction to the 32,088 SNPs tested. The analysis was performed using PLINK(5).

To test for an interaction between the rs6123045 SNP and rosiglitazone among Europeans on edema, we performed a logistic regression analysis using the R statistical software package (www.r-project.org)(6). The model included the main effects of the SNP and rosiglitazone and their interaction term, in addition to the covariates used in the original analysis.

Survival curves for each genotypic class and the corresponding hazard ratios (HRs) were calculated from a multivariate Cox proportional hazard analysis adjusted for the same covariates, using R (www.r-project.org)(6).

We tested all common SNPs (n=57) from the NFATC2 locus in the Latin American individuals receiving rosiglitazone using the analysis described above. 59 (n=122 in both treatment arms) individuals taking diuretics were excluded from the analysis.

Deviations from Hardy-Weinberg equilibrium (HWE) were calculated for each ethnic group using an exact test(7).

The haplotype defined by the six SNPs significantly associated with TZD-induced edema in both Europeans and Latin Americans (rs6126240, rs6096453, rs4811188, rs11697582, rs2179746, rs6021276) was tested using the conditional haplotype test implemented in PLINK(5) and was adjusted for the same covariates as in the original analyses.

Supplementary Table 1: Occurrence of edema among the participants of the DREAM trial.

	Rosiglitazone			Placebo			
Ethnic Group	**Edema**		**Total**	**Edema**		**Total**	**P-value**
Europeans	253	(26.2%)	965	154	(16.1%)	956	5.92×10^{-8}
Latin Americans	71	(19.8%)	358	46	(15.1%)	368	7.22×10^{-3}
South Asians	16	(8.3%)	192	9	(4.7%)	193	0.144
African American	18	(17.6%)	102	14	(14.1%)	99	0.497
Native North American	9	(20.0%)	45	16	(27.6%)	58	0.373
East Asian	7	(28.0%)	25	1	(4.0%)	25	0.049*
Other	16	(25.4%)	63	16	(25.8%)	62	0.958
Total	390	(22.3%)	1750	256	(14.5%)	1761	8.63×10^{-7}

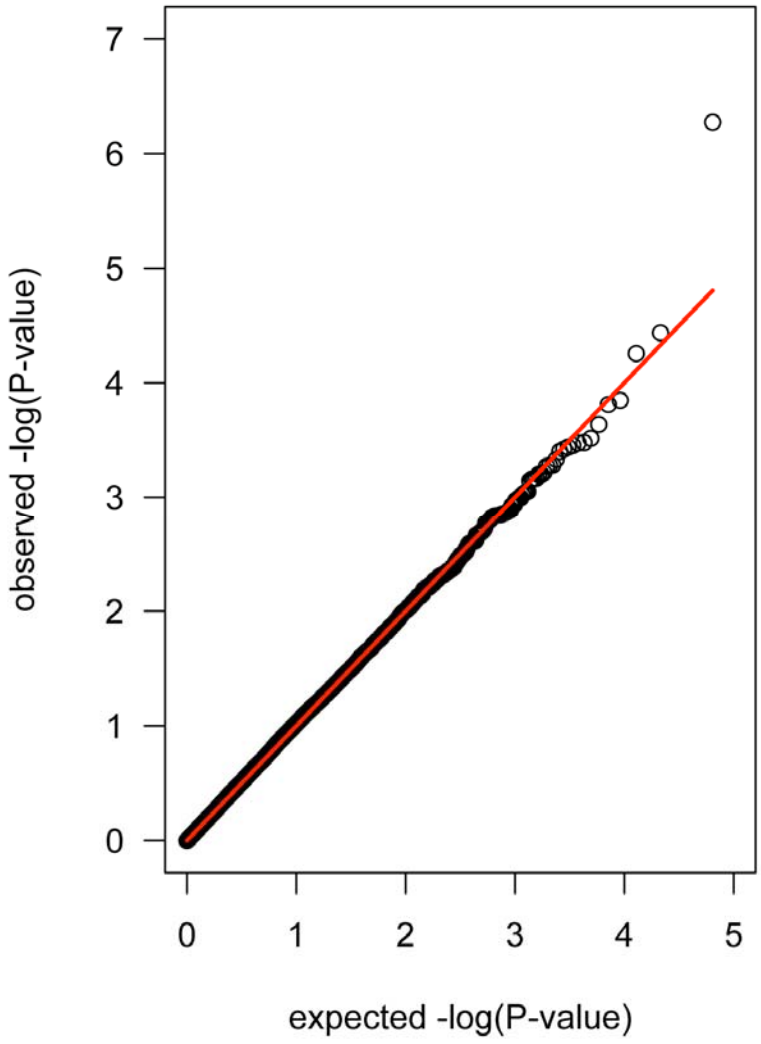
P-values were calculated from a χ^2 test except for (*), which is from a Fisher's exact test. Individuals taking diuretics were excluded.

Supplementary Table 2: Clinical characteristics of the European participants included in the study.

	Rosiglitazone (n=965)	**Placebo (n=956)**
Age	55.9 (10.4)	55.5 (10.5)
BMI	31.0 (5.4)	30.9 (5.5)
Women	538 (55.8%)	557 (58.2%)
ACE inhibitors	491 (50.8%)	495 (51.8%)
CCBs	91 (9.4%)	111 (11.6%)

Data are mean (SD) or number (%). ACE = Angiotensin Converting Enzyme. CCBs = Calcium Channel Blockers. Excludes individuals who were taking diuretics.

Supplementary Figure 1: QQ-plot of the SNPs associated with peripheral edema in the European subset of the rosiglitazone treatment arm. The -log of the observed P-values are plotted against the -log of the expected P-values. P-values were calculated from a logistic regression analysis adjusted for age, sex, BMI, ramipril, thiazides, aldosterone inhibitors, diuretics, and the first 10 principal components of the proportion of alleles shared IBS among the European individuals.



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