Variation at the NFATC2 Locus Increases the Risk of Thiazolidinedione-Induced Edema in the Diabetes REduction Assessment with ramipril and rosiglitazone Medication (DREAM) Study.

Short Title: NFATC2 is associated with Edema in DREAM

Sweneke D. Bailey, BSc1 Changchun Xie, PhD2,3 Ron Do, MSc1 Alexandre Montpetit, PhD4 Rafael Diaz, MD5 Viswanathan Mohan, MD, PhD, DSc, FRCP6 Bernard Keavney, MD, FRCP7 Salim Yusuf, MD, DPhil, FRCPc2,3,8 Hertz C. Gerstein, MD, MSc, FRCPc2,3,8 James C. Engert, PhD1,9, and Sonia Anand, MD, PhD, FRCPc2,3,8 on behalf of the DREAM investigators.

1. Department of Human Genetics, McGill University, Montreal, Quebec, H3A 1B1, Canada
2. Population Health Research Institute, McMaster University, Hamilton, Ontario, L8L 2X2, Canada
3. Department of Clinical Epidemiology and Biostatistics, McMaster University, Ontario, L8N 3Z5, Canada
4. McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, H3A 1A4, Canada
5. Estudios Clínicos Latino americá, Jujuy 1415, Rosario 2000, Argentina
6. Madras Diabetes Research Foundation, Gopalapuram, Chennai, 600 086, India
7. Institute of Human Genetics, University of Newcastle, Newcastle Upon Tyne, NE1 3BZ, UK
8. Department of Medicine, McMaster University, Hamilton, Ontario, L8S 4L8, Canada
9. Department of Medicine, McGill University, Montreal, Quebec, H3A 1A1, Canada

Address reprint requests to:
Dr. James C. Engert
Email: jamie.engert@mcgill.ca.

Additional information for this article can be found in an online appendix at http://care.diabetesjournals.org

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**Objectives:** Thiazolidinediones are used to treat type-2 diabetes. Their use has been associated with peripheral edema and congestive heart failure (CHF), outcomes that may have a genetic etiology.

**Research Design and Methods:** We genotyped 4,197 participants of the multi-ethnic DREAM trial with a 50k SNP array, which captures approximately 2000 cardiovascular, inflammatory, and metabolic genes. We tested 32,088 SNPs for an association with edema among Europeans who received rosiglitazone (n=965).

**Results:** One SNP, rs6123045, in *NFATC2* was significantly associated with edema (OR=1.89, 95% CI:1.47-2.42, p=5.32x10^{-7}, corrected p=0.017). Homozygous individuals had the highest edema rate (HR=2.89, p=4.22x10^{-4}) when compared to individuals homozygous for the protective allele, with heterozygous individuals having an intermediate risk. The interaction between the SNP and rosiglitazone on edema was significant (p=7.68x10^{-3}). Six SNPs were significant in both Europeans and Latin Americans (p<0.05).

**Conclusion:** Genetic variation at *NFATC2* locus contributes to edema among individuals who receive rosiglitazone.

Although changes in lifestyle can prevent or delay diabetes(1), the majority of patients require multiple therapeutic strategies to prevent or treat the disease. Thiazolidinediones (TZDs) are a class of drugs used in the treatment of diabetes that derive their insulin sensitizing effects from the activation of the peroxisome proliferator-activated receptor γ (PPARγ)(2). TZDs can effectively control glycemia among diabetics(3). However, their use has been shown to cause an increase in peripheral edema and CHF(4; 5). Edema is the most commonly reported adverse drug reaction associated with TZDs and this has been partly attributed to the 6% to 8% increase in plasma volume that occurs with their use(6). In addition, the observed increase in CHF associated with rosiglitazone may derive from a shared etiology.

An aim of the Diabetes REduction and Assessment with ramipril and rosiglitazone Medication (DREAM) trial was to determine if rosiglitazone could prevent progression to diabetes among individuals with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT)(7). Consistent with previous findings, a significant increase in edema and CHF among individuals receiving rosiglitazone was observed(7). The identification of genetic variants that predispose individuals to edema or CHF could lead to pre-therapeutic screening procedures. To determine whether genetic variation contributes to the etiology of TZD-induced edema, we tested common SNPs capturing approximately 2,000 cardiovascular/metabolic genes in DREAM trial participants receiving rosiglitazone.

**MATERIALS AND METHODS**

The DREAM trial has been described in detail elsewhere(7). We tested 32,088 SNPs for an association with TZD-induced peripheral edema in 965 European individuals receiving rosiglitazone. Edema was defined as the presence of pitting edema at both ankles reported at any clinic visit and includes individuals that withdrew from treatment due to edema. We used logistic regression for...
each SNP adjusted for age, sex, BMI, the use of angiotensin converting enzyme (ACE) inhibitors and calcium channel blockers (CCBs). Individuals taking diuretics were excluded from all analyses. The first 10 principal components of the alleles shared identity by state (IBS) were included as covariates.

To test for interaction between the SNP and rosiglitazone, we performed a logistic regression analysis that included the main effects of the SNP, rosiglitazone, and their interaction term. Survival curves for each genotypic class and the corresponding hazard ratios (HRs) were calculated from a Cox proportional hazard analysis. A detailed description of all materials and methods is available in the supplementary information.

RESULTS

In our genetic sub-study of DREAM, we observed an increase in edema among individuals who received rosiglitazone (n=390 (22.3%)) versus placebo (n=256 (14.5%)). Among the Europeans, 253 (26.2%) individuals receiving rosiglitazone experienced edema compared to 154 (16.1%) receiving placebo (p=8.63x10^-7) (Supplementary Table 1 in the online appendix available at http://care.diabetesjournals.org). The clinical characteristics of the Europeans were not significantly different between the rosiglitazone and placebo arms (Supplementary Table 2).

We tested 32,088 SNPs against edema in the Europeans receiving rosiglitazone. One SNP, rs6123045, in the nuclear factor of activated T-cells cytoplasmic calcineurin dependent 2 (NFATC2) gene was significantly associated with edema (OR=1.89, 95% CI:1.47-2.42, p=5.32x10^-7, corrected p=0.017)(Figure 1A). The distribution of the observed versus expected p-values is shown in Supplementary Figure 1. We observed a significant interaction between rs6123045 and rosiglitazone treatment on edema in Europeans (p=7.68x10^-3). The effect of rs6123045, while in the same direction, was not significantly associated with edema in the placebo group (OR=1.16, p=0.29)(Figure 1B).

A Cox proportional hazards analysis revealed individuals homozygous for the risk allele had a decrease in the time to the first report of edema in comparison to individuals heterozygous or homozygous for the protective allele (HR=1.76, 3.43x10^-5 and HR=2.89, p=4.22x10^-4 respectively)(Figure 1B). The effect appears to be additive as heterozygous individuals had an increased rate of edema, compared to homozygous protective individuals (HR=1.64, p=0.11).

Among Europeans receiving rosiglitazone, rs6123045 was not significantly associated with diabetes or death or with cardiovascular endpoints, including CHF, myocardial infarction, stroke, angina or a composite of these outcomes (data not shown). rs6123045 was not significantly associated with TZD-induced edema in Latin Americans. However, six SNPs in NFATC2 were significant in both Europeans and Latin Americans (Figure 1A). A haplotype defined by these SNPs is significant in both populations (OR=0.45, 95% CI:0.30-0.66, p=2.26x10^-5 and OR=0.34, 95% CI:0.13-0.90, p=1.47x10^-2 in Europeans and Latin Americans respectively)(data not shown). All significantly associated SNPs were in HWE (p>0.05).

DISCUSSION

We demonstrate that variation within NFATC2 contributes to TZD-induced edema. rs6123045 was significantly associated with TZD-induced edema among Europeans when corrected for multiple testing. The significant interaction between rs6123045 and rosiglitazone treatment on edema (p=7.68x10^-3) among the European DREAM participants highlights the contribution to the etiology of TZD-induced edema. rs6123045 was not
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significantly associated with TZD-induced edema in Latin Americans. However, six SNPs in the same region are associated with TZD-induced edema in both populations and define a shared haplotype.

Previous studies of either TZD-induced or dual PPAR agonist-induced edema(8-11) were smaller in size (n≤730) and tested a smaller number of genes (≤222). None of these studies analyzed the NFATC2 gene. Twenty of the 38 SNPs previously associated with edema are captured by the IBC array and we were thus able to directly test them. However, we were unable to replicate any of these associations in Europeans (p>0.05).

The NFATC2 gene encodes a cytoplasmic component of the NFAT transcription complex. Four NFAT cytoplasmic component proteins (NFATc1-c4) are known and these are translocated to the nucleus after being dephosphorylated by the phosphatase calcineurin(12). Treatment of cardiomyocytes with rosiglitazone inhibited endothelin-1 induced calcineurin activity, suppressed the nuclear translocation of NFATc4, and enhanced the association of PPARγ with calcineurin/NFATc4(13). The constitutive activation of either calcineurin or NFATc4 in mice leads to cardiac hypertrophy and heart failure(14). In addition, Nfatc2 null mice are protected from calcineurin induced cardiac hypertrophy(15). In the context of these findings, our results are provocative and constitute a step towards elucidating the etiology of the CHF associated with TZD use(5).

Identifying the specific genetic variants interacting with TZDs and resulting in edema, or cardiovascular events, may have important clinical consequences and enable genetic variant directed use of the TZD drugs among people with dysglycemia.


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REFERENCES


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**Figure Legends**

**Figure 1. A)** Results of the association analysis between SNPs and TZD-induced peripheral edema at the *NFATC2* locus. The -log of the p-values are plotted against SNP location. P-values were calculated from a logistic regression analysis adjusted for age, sex, BMI, use of ramipril and CCBs, as well as the first 10 principal components of the alleles shared IBS among the European and Latin American individuals. Individuals taking diuretics were excluded from the analysis. The dashed lines indicate Bonferroni corrected and nominal significance. **Inset:** Results of the initial association scan of 32,088 SNPs and TZD-induced peripheral edema in n=965 Europeans receiving rosiglitazone. The -log of the p-values are plotted against SNP location for each chromosome. **B)** Survival curves estimated from the cox proportional hazards model of time to the first occurrence of edema according to the rs6123045 genotype. European individuals homozygous for the risk allele (CC) have an increase in the rate to the first report of edema in comparison to the individuals heterozygous (CT) or homozygous (TT) for the protective allele (adjusted HR=1.76, 3.43x10^-5 and adjusted HR=2.89, p=4.22x10^-4 respectively). **Inset:** The effect of the rs6123045 SNP on peripheral edema among European individuals receiving rosiglitazone or placebo. 33.6% (158/470) of individuals homozygous for the risk allele, 20.8% (84/403) of heterozygous individuals and 12.9% (12/93) of individuals homozygous for the protective allele developed edema while receiving rosiglitazone compared to 18.0% (85/473), 13.8% (56/404), and 16.5% (13/79), respectively, receiving placebo. The per-allele odds ratio and 95% confidence intervals of the logistic regression analysis are shown.
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A. 

B. 

Fraction Free of Edema

Time (years)

Number of Events by Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total</th>
<th>Rosapultare</th>
<th>Pachyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>255</td>
<td>159(62.2%)</td>
<td>96(37.8%)</td>
</tr>
<tr>
<td>CT</td>
<td>154</td>
<td>73(47.3%)</td>
<td>81(52.7%)</td>
</tr>
<tr>
<td>TT</td>
<td>79</td>
<td>41(52.6%)</td>
<td>38(47.4%)</td>
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