Reviewer's report

**Title:** A comprehensive investigation of variants in genes encoding adiponectin (ADIPOQ) and its receptors (ADIPOR1/R2), and their association with serum adiponectin, type 2 diabetes, insulin resistance and the metabolic syndrome.

**Version:** 1 **Date:** 12 November 2012

**Reviewer:** David Meyre

**Reviewer's report:**

In this report, KE Peters and colleagues assessed the association of tagged SNPs in the ADIPOQ, ADIPOR1 and ADIPOR2 genes with serum adiponectin level and metabolic traits in two population-based studies and one T2D case sample of European-Australian individuals. They found not surprisingly an association between SNPs in ADIPOQ (but not in ADIPOR1 and ADIPOR2) and adiponectin level and no association of the SNPs in the three genes with metabolic traits.

**MAJOR COMPULSORY REVISIONS**

1-Gene selection: I do not see the interest to study the association of SNPs in ADIPOR1 and ADIPOR2 genes with adiponectin level. Indeed multiple studies already tested this hypothesis and did not find any significant association. Choosing SNPs in the 11 loci (in addition to ADIPOQ) identified by the five GWAS for serum adiponectin level would have been a far more informative strategy.

2-The authors may test which of the nine SNPs is an independent contributor to adiponectin variation using conditional regression models. Several of the nine SNPs are likely to be in modest linkage disequilibrium with each other and may represent redundant association signals.

3-Were the T2D case cohort and the two population-based cohorts recruited in different geographical area of Australia? If yes, did the authors adjust the tests for population stratification? Population stratification, if not properly taken into account, can lead to false negative and false positive results.

4-Methods: ‘A random (10%) sample was analyzed in duplicate, with reproducibility found to be 98%’. As the authors double-genotyped 64 SNPs, we expect them to describe a range of concordance rate (e.g. SNP individual concordance rates were comprised between 97.1 and 100%) rather than a single value. Is the 98% concordance rate a mean value? A general rule in the field is to discard SNPs that did not display a concordance rate > 99%. Please be more specific.

5-Methods: I do not think that adjusting adiponectin genetic association tests for BMI make any sense. Previous studies have shown an association between
SNPs in ADIPOQ and obesity-related traits (Bouatia-Naji et al Diabetes 2006, Siitonene et al BMC Med Genet 2011, Yu et al Obesity 2012), so BMI cannot be considered as a confounding factor in this specific context.

6-I do not think relevant to include the T2D case study in the meta-analysis of quantitative traits. As the authors acknowledge themselves, important discrepancies are found between the T2D sample and the two population-based studies (higher BMI, lower adiponectin…). The disease status and anti-diabetic treatments have a major impact on QT values. I recommend using this cohort only in the T2D case control study.

7-17% of the SNPs genotyped using the Illumina Golden Gate technology do not pass the quality control criteria. How the authors explain this abnormally high rate of SNP genotyping failure?

8-Discussion: ‘Despite putative effects of the adiponectin receptors on tissue adiponectin activity, we found no evidence that tSNPs in ADIPOQ, or ADIPOR1/R2 were associated with insulin resistance, MetS or T2D. This supports the findings of three large studies [4, 32, 41]’. I do not agree with this comment. Dastani et al (PLOS Genet 2012) (ref 32) evidenced that a multi-SNP genotypic risk score for adiponectin-decreasing alleles (including a SNP in ADIPOQ) was associated with BMI, WHR, fasting insulin, HOMA-IR, 2-hour post OGTT glucose, T2D, triglycerides and HDL-cholesterol. In order to gain statistical power in their analysis, the authors may assess the association of these metabolic traits using a multiple SNP genotype risk score that includes SNPs independently associated with adiponectin level. If an association is found between the gene score and a specific metabolic trait, a post-hoc analysis further adjusting for serum adiponectin may help to understand if the association between SNPs in ADIPOQ and the metabolic trait is mediated by circulating adiponectin (mendelian randomization).

MINOR ESSENTIAL REVISIONS

9-Abstract: please detail the sample size of the three cohorts.

10-Is HOMA-IR normally distributed? Logarithm transformations of HOMA-IR have been reported in literature (Cauchi et al Diabetes 2006).

11-Do haplotypes explain more variation of serum adiponectin than single SNPs tagging the same LD blocks?

12- The lack of replication of the association between adiponectin and several SNPs previously identified in GWAS is intriguing. As several rare population-specific coding variants have been reported to be strongly associated with adiponectin level (Vasseur et al HMG 2002, Croteau-Chonka et al HMG 2012, Warren et al Diabetes 2012) one explanation for the observed discrepancies may be synthetic associations (the fact that population-specific rare variants are in partial linkage disequilibrium with common variants) (Dickson et al PLOS Biol 2010). This may be mentioned in the discussion.
13-The in silico functional tests for SNPs have little value without confirmatory in vitro studies. Please discard the discussion about in silico experiments, as the discussion is too long.

14-Please do not cite and discuss the results of the reference 36. This study is clearly underpowered and has not scientific value.

15-As the power of the present study is limited, the lack of association of SNPs in ADIPOQ with metabolic traits must be interpreted with caution in the discussion (negative result or lack of power to evidence a subtle genetic effect).

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests