Author’s response to reviews

Title: The genetic susceptibility to type 2 diabetes may be modulated by obesity status: implications for association studies

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We thank both reviewers for their relevant comments. We have made the requested changes (when possible) to improve the manuscript.

Reviewer 1: Johannes A Maassen

Q1- use of word "insulin resistance" genes (table 3). These genes are not true insulin resistance genes, such as those encoding for components of the insulin receptor signalling pathway. They are more related to adipocyte function. The authors may consider choosing another name.

R1. We agree with Reviewer 1. In Table 3, Figure 1 and in the main text, “Insulin action” was therefore preferred to “insulin resistance” to qualify this category of genes.

Q2- Though BMI associates with the risk for developing diabetes, waist-hip ratio shows a stronger association. I was wondering whether the authors have waist-hip ratio data of their cohorts and whether a genetic association analysis has been made based on these data.

R2. Waist / hip ratio indeed shows a stronger association with type 2 diabetes and is a better proxy for insulin resistance than BMI. Unfortunately, the data on this anthropometric parameter were not available in 1,958 diabetics (69% of the T2D cases).
Because we think that it is a very interesting point, we added the following phrase in the Discussion section, pages 9 and 10: “In our study, data on waist / hip ratio were not available in most T2D cases. However, further analyses of the issue should consider this anthropometric parameter when analyzing interactions between obesity and T2D.”

**Reviewer 2: Vincenzo Trischitta**

**Q1.** In order to say that a gene variant acts differently in non-obese vs obese individuals, a formal significant interaction has to be observed. As the authors clearly state, this is the case only for the ENPP1 K121Q and the TCF7L2 rs7903146 SNPs. Thus the Abstract and the entire first part (i.e. first page) of the Discussion, has to be re-written using more caution. In general, when discussing their own data the authors should stress those on ENPP1 and TCF7L2 rather than keep saying that also the effect of HNF1A, GCK, SLC30A8, PPARG and ADIPOQ is modified by the absence/presence of obesity.

**R1.** Because our results have not been completely validated, we definitely need to be cautious with our affirmations and make suggestions instead. We changed most affirmations into suggestions (e.g. “is” into “may be”) throughout the “Discussion” section. However, saying that the effect of HNF1A, GCK and SLC30A8 may be modified by the absence / presence of obesity is not totally incorrect. Even if the genotypic distribution is not statistically different between obese and non-obese subjects, we always noticed a significant association with T2D in only one group but not in the other group, except for TCF7L2. However, we clearly stated that a significant heterogeneity was only found for TCF7L2 and ENPP1 variants and suggested for ADIPOQ and HNF1A SNPs. We assume that more power (more individuals) would have probably changed some trends into significant values. Laying stress on ENPP1 and TCF7L2 would deeply reduce our message that tends to suggest a more general phenomenon.
Q2. Previous studies are not always properly quoted. In details, a) When referring to ENPP1 (Background, lines 6, 7) the very recent study of Mc Ateer et al (Diabetes. 2008, 57:1125-30) has to be quoted. This is the largest meta-analysis so far carried out and, of note in this specific context, suggests that the gene effect is mediated by BMI. b) When referring to PPARG the study by Florez et al (J Clin Endocrinol Metab. 2007, 92:1502-9) has to be quoted. In this paper which analyzed the prospective DPP study, the authors clearly show that the protective effect of the Ala12 variant is larger in leaner people. A finding which is in line with the meta-analysis by Ludovico et al (reference 4 of this paper). Since both papers suggest just the opposite of what suggested by the authors, this issue has to be deeper discussed.

R2. In the revised manuscript, these references were modified as requested. Currently, some dissension exists regarding the BMI modulation of the Pro12Ala SNP in T2D risk. Contrary to what was reported by Ghoussaini and colleagues [1], other studies of the PPARG Pro12Ala SNP suggest that the protective effect of the Ala allele may be greater when control BMI is lower [1, 2]. The present analysis was specifically designed to detect interactions by analyzing a large number of European subjects with no genotypic heterogeneity and characterized by a wide range of BMI. In the “Diabetes Prevention Program”, 55 percent of participants were Caucasian, 45 percent were minorities, most were obese, and most had a family history of T2D [2]. Similarly, Ludivico and colleagues performed a combined analysis of Asian, North American, and European populations. Interestingly, the genetic effect on T2D was found to be ~ 30% stronger in Asians than in the two other more corpulent populations [3]. We therefore suggest that population heterogeneity may be the primary contributor to the overall observed BMI effect as supported by the ability of BMI to statistically explain the heterogeneity between these populations but not within Europeans [3]. Large ethnically-matched studies would be
necessary to know if such interaction is found in non-European subjects. This point was discussed in the “Discussion” section, pages 8 and 9.

References:


Q3. Given the important role mediated by obesity on the risk of T2D determined by both ENPP1 and TCF7L2, it would be important to understand whether these two genes have any effect on BMI itself either in the 4 different groups singly examined (NG non obese, NG obese, T2D non obese, T2D obese) or in the NG (non-obese + obese) and the T2D (non-obese + obese) groups. What one could expect to find is that, because of ascertainment bias (virtually always present in case-control design); variants which increase the risk of T2D by interacting with obesity would be associated with lower BMI in controls and vice versa.

R3. In our study, the association with T2D was calculated using a logistic regression model adjusted for age, gender, and BMI in order to avoid ascertainment bias. However, we added a table (Supplementary Table 4) and a comment in the “Results” section, page 7, presenting the effects of *TCF7L2* and *ENPP1* SNPs on BMI, by obesity and glycemic status. The only statistically significant associations show an effect of the *TCF7L2* variant on BMI in the T2D group only. We previously found that *TCF7L2* is probably not a
risk factor for obesity in European populations [1]. However its effect on T2D risk is modulated by obesity.

Reference:


Q4. In the abstract, it would be better to show also the OR in the non-obese group for ENPP1 (as done for TCF7L2). Once again, these are the two genes showing interaction with obesity and, therefore, data should be clearly shown in the abstract.

R4. The TCF7L2 genetic variant was the only SNP to be associated with type 2 diabetes in both non-obese and obese groups, although the effect sizes were clearly different between both groups. However the ENPP1 polymorphism was found to be only associated in obese subjects but not in non-obese individuals. In the abstract of the revised manuscript, we presented the significant as well as the non-significant associations with type 2 diabetes for all studied genetic variants that were differentially associated in obese and non-obese subjects.

Q5. Background line 2: “pathology” should be changed with “disease”.

R5. We made the requested changes

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