Reviewer's report

Title: BMI-independent association of obesity risk SNPs in PCSK1 with insulin sensitivity and proinsulin conversion

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Reviewer: Jose Florez

Reviewer's report:

In this study, Heni, Haupt et al. examine two missense single nucleotide polymorphisms (SNPs) in the gene that encodes the prohormone convertase 1 (PCSK1) for association with obesity traits, proinsulin to insulin processing, and insulin sensitivity measures in a cohort of 1,498 non-diabetic German subjects. The two SNPs (rs6232 and rs6235, encoding N221D and S690T, respectively), which are not highly correlated by linkage disequilibrium, are relevant because mutations in this gene give rise to childhood obesity and multiple endocrine abnormalities. Previous work by others has shown an association of rs6232 with obesity, and demonstrated a 10% decrease in enzymatic activity caused by this variant.

All subjects underwent a 75-gram oral glucose tolerance test, from which areas under the curve (AUC) and insulin sensitivity were derived. Genotyping was of good quality and statistical methods were generally appropriate. SNP rs6232 was examined under a dominant genetic model because of the low frequency of its minor allele. To account for multiple hypothesis testing, the authors applied a Bonferroni correction factor that considered 2 independent SNPs and 3 sets of phenotypes.

The authors found no association of either SNP with obesity related traits. There were no associations with fasting proinsulin, but the minor alleles were associated with higher stimulated proinsulin (by AUC) and with the conversion index obtained by dividing proinsulin AUC over insulin AUC. There were no associations with insulin secretion indices. Interestingly, the minor allele at rs6232 was also associated with lower fasting insulin, lower insulin resistance by homeostasis model assessment (HOMA-IR), and higher insulin sensitivity as in Matsuda and de Fronzo. The authors conclude that these SNPs do not contribute greatly to obesity, but are associated with an impaired proinsulin to insulin conversion; they further postulate that the risk allele at rs6232 also induce an unexpected increase in insulin sensitivity, and speculate as to how that may be the case.

This paper therefore makes three major claims:

1. Two missense SNPs at PCSK1 (one of which has been shown to impair enzymatic activity) influence proinsulin to insulin conversion.
2. These SNPs are not associated with obesity measures in this cohort.
3. The proinsulin-raising allele may also increase insulin sensitivity through unknown mechanisms.

With respect to #1, the finding is relevant but expected. With regard to #2, as the authors acknowledge their cohort is likely underpowered to replicate the previously reported result; as recently presented in international meetings (e.g. ASHG October 2009, oral abstract by Speliotes et al.) the GIANT Consortium has demonstrated an association of PCSK1 SNPs with body mass index at P ~ 10e-5 – though not genome-wide significant, more convincing than the negative result shown here. Finally, a major problem concerns the unexpected result described in #3. If a genetic variant truly impairs prohormone convertase activity, as shown in a prior publication, then carriers of that variant will display relatively higher levels of proinsulin and lower levels of endogenous insulin (as shown here). But the primary reason is a conversion defect, not heightened insulin sensitivity. Because insulin is used in the numerator for HOMA-IR and in the denominator for the Matsuda insulin sensitivity index, it is natural that the proinsulin-raising and insulin-lowering allele will also seem to be associated with lower HOMA-IR and higher insulin sensitivity; but in this scenario, these indices do not accurately reflect the insulin resistant state of the organism, because there is a primary insulin synthetic defect. In order to eliminate such confounding, statistical adjustment is not enough: one ought to study measures of insulin sensitivity that are derived from the exogenous administration of insulin, as in the hyperinsulinemic euglycemic clamp.

Therefore, this paper seems to be reduced to one interesting but expected result, an ultimately false negative statement on obesity, and a novel claim on insulin sensitivity that is based on a physiological measure that seems inappropriate in this context, and should therefore be removed as currently framed.

Major compulsory revisions:

1. Adjust the obesity analyses and their interpretation to the likely fact that PCSK1 confers a modest effect on obesity when analyzed in large enough samples, as recently reported by the GIANT Consortium
2. Remove the analyses for insulin sensitivity as being inadequate in this context
3. Report linkage disequilibrium between the two SNPs
4. The decision to analyze rs6232 under a dominant model seems arbitrary, as a low minor allele frequency does not influence the mode of risk transmission. An additive model should be provided, and the use of a dominant model justified on biological/genetic rather than statistical grounds
5. Discussion, 2nd paragraph: please change the first sentence to reflect that alleles (and not SNPs) are associated with higher stimulated proinsulin levels, as you correctly do in the rest of the manuscript

Discretionary revisions:

The authors might consider including a figure plotting insulin secretion vs
proinsulin levels by genotype group, to indicate whether the presence of the risk allele impairs the ability of beta cells to secrete mature insulin (see Ingelsson et al. Diabetes 2010 online, Fig. 1 as an illustration).

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I am currently participating in a genome-wide association study of proinsulin levels.