Reviewer's report

Title: Marginal Impact of Phenotypic Selection on Detecting MC4R Mutations in Italian Obese Children

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Reviewer: Anke Hinney

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Farooqi et al. (2003) described a 'MC4R obesity syndrome' pertaining to a phenotype of early onset severe obesity, increased height, advanced bone age and hyperinsulinemia. Other groups did not find this syndrome in MC4R mutation carriers (Vaisse et al., 1998; Dubern et al., 2001; Lubrano-Berthelier 2003).

However, here Santoro et al. described a mutation screen (by resequencing) of the MC4R in 240 Italian children with the described 'obesity syndrome' and in 200 normal weight controls. As the study is rather small, the selection strategy should increase the chance to find MC4R mutation carriers.

Santoro et al. detected three infrequent mutations; two of them novel. Functional studies on the novel mutations revealed that one (a nonsense mutation) was leading to a loss of receptor function, whereas the other one was indistinguishable from the wild type receptor. The frequency of functionally relevant MC4R mutations was approx. 1.6% and thus similar to previous studies in Italian obese individuals. Hence, the enrichment for the obesity syndrome did not lead to the identification of an increased number of MC4R mutation carriers.

Major Compulsory Revisions

This study adds to the large body of evidence describing mutations in the MC4R that are relevant for obesity. The 'enrichment' attempt is novel, however previous studies were contradictory, so that the a priori hypothesis might not have been too solid. This should be pointed out in the introduction and discussion.

The Q307X mutation was previously described and functionally tested by Lubrano-Berthelier et al. (Lubrano-Berthelier C, Cavazos M, Le Stunff C, Haas K, Shapiro A, Zhang S, Bougneres P, Vaisse C. The human MC4R promoter: characterization and role in obesity. Diabetes. 2003 Dec;52(12):2996-3000; hence it is not novel. The authors need to refer to this previous study and discuss their results in the light of the previous study.

Background. The part pertaining to POMC is far too long. Nowadays, nobody needs to be convinced that MC4R is a suitable candidate gene for (extreme) obesity.

Background. The authors claim that heterozygous carriers of POMC mutations develop non-syndromic obesity. This can at least not be derived from our study
as we found no association to obesity of the detected variants. An influence of the infrequent mutation on obesity can on the other hand not be excluded.

Background. The recent MC4R mutation screens on large epidemiological cohorts found mutations in the obese, but also in the normal weight individuals. So it is not a complete failure of a genotype-phenotype correlation. This sentence needs rewording.

The first sentence of Material and Methods is not understandable.

Results. The V103I polymorphism is negatively (instead of not) associated with obesity.

Results. Which ‘relatively common, but functionally irrelevant amino acid variants’ do the authors refer to? V103I and I251L are both functionally relevant (check literature) and both have an effect on BMI.


Discussion/Conclusion: It might not be too surprising that the S127L was detected in three individuals, as this mutation had previously been described by several groups (please check literature). This information needs to be added.

Minor Essential Revisions

Abstract; 2nd sentence: the ‘MC4R obesity syndrome’ is solely described by Farooqi et al. (2003); it is not a general finding. Hence, the sentence needs rewording.

Language needs to be checked by a native speaker; additionally multiple typos need correction (e.g. ‘timine’ should be ‘Thymidine’).

‘mutated subjects’ needs to be replaced with ‘individuals harbouring a mutation or variant’.

Results. ‘Given that the Y332H variant did not affect the function of the MC4R…’

Discretionary Revisions

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

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

**Declaration of competing interests:**

'I declare that I have no competing interests’