Author's response to reviews

Title: Prevalence of Pathogenetic MC4R mutations in Italian Children with Early Onset Obesity, Tall Stature and Familial History of Obesity

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To the editor of
BMC- Medical Genetics

Dear Editor,

On behalf of the other co-authors, I'm re-submitting as original paper the manuscript titled “Prevalence of Pathogenetic MC4R mutations in Obese Italian Children with early onset obesity, tall stature and familial history of obesity.” by Nicola Santoro, Grazia Cirillo, Zhimin Xiang, Rita Tanas, Nella Greggio, Giuseppe Morino, Lorenzo Iughetti, Alessandra Vottero, Alessandro Salvatoni, Mario Di Pietro, Antonio Balsamo, Antonino Crinò, Anna Grandone, Carrie Haskell-Luevano, Laura Perrone, Emanuele Miraglia del Giudice and the Childhood Obesity Study Group of the Italian Society of Pediatric Endocrinology and Diabetes (SIEDP).

The manuscript has been modified according to the referees suggestions. In particular the title has been changed as well as a large part of the introduction and some points of the discussion session.
I confirm that all the authors of the manuscript have read and agreed to its content, that readily reproducible materials described in the manuscript will be freely available to any scientist wishing to use them for non-commercial purposes, and that we have ethical approval for any experimentation. I confirm that the manuscript is original, has not already been published in a journal and is not currently under consideration by another journal. The authors declare that they have no competing interests.

My best regards,
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ANSWERS TO THE REVIEWERS

Reviewer: Stephen O’Rahilly
Reviewer’s report:

This is a useful and generally well produced contribution to the literature regarding MC4R mutations. It describes a prevalence of 4/240 of pathogenic mutations in Italian children with severe obesity pre-selected for increased height vs ~200 lean childhood controls and shows that the one new mutant found is defective in signalling. This is a significant contribution to the literature.

Q: However, because the authors specifically selected the obese group to have increased stature, and did not compare them with a group of equivalently obese children without increased stature they can not firmly conclude, as their title does, that there are no phenotypic characteristics of MC4R deficiency other than obesity per se. Therefore the title needs to be changed to a more descriptive one e.g. “Prevalence of pathogenic MCR mutations in obese Italian children with tall stature” as the current title is insufficiently justified by the data. The authors also need to recognize this limitation in their discussion. I view this issue as representing a Major Compulsory Revision.

A: We really thank dr O’Rahilly for his constructive comments and suggestions. The title has been changed according to his suggestion in “Prevalence of Pathogenetic MC4R mutations in Italian Children with Early Onset Obesity, Tall Stature and Familial History of Obesity”. Moreover, in the discussion session has been specified that the lack of a group of equivalently obese children without increased stature constitutes a limit of our study. See the Discussion on page 11 from line 10 to line 12.

Q: The authors also discuss the issue of whether MC4R mutations are more likely to lead to early-onset rather than late-onset obesity and cite a variety of papers that seem to suggest that previous indications that MC4R mutations
predominantly lead to early onset obesity may be incorrect. The authors need to be cautious here because in many of the papers describing obese adults there is no objective data on whether the obese adults had been obese as children or not. Some comments to that effect would improve the balance of the discussion. I view this as a Minor Essential Revision.

A: This comment has been included in the Discussion. See page 10 from line 12 to line 14.

Reviewer: Anke Hinney
Reviewer's report:
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
Q: 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.

A: We are in debt with dr Hinney for her observations which make more complete our paper. According to her suggestions Background and Discussion have been changed. See Background on page 3, from line 20 to line 23 and Discussion on page 10 from line 8 to line 11.

Q: 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.

A: As correctly observed by dr Hinney, the Q307X mutation has been already described by Lubrano-Berthelier et al., but it had not been characterized. This has been pointed out in the discussion (page 9, line 3 and line 4) and the new reference has been included (ref. 21).

Q: 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.

A: The introduction has been changed accordingly, with a reduction of the part concerning the POMC.

Q: Background. The authors claim that heterozygous carriers of POMC mutations develop non-syndromic obesity. This can at least not be derived from our study (ref. 7); 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.

A: A large part in the introduction dealing with POMC has been deleted and the sentence including the above cited reference has been rewritten in order to not generate any confusing message.

Q: 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.

A: The sentence has been rewritten see the Background on page 3 from line 20 to line 23

Q: The first sentence of Material and Methods is not understandable.

A: This sentence has been deleted.

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

A: This has been changed.

Q: 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.

A: The sentence was too confusing, that is why it has been deleted.

Q: Discussion. Young et al. (Young EH, Wareham NJ, Farooqi S, Hinney A,
A: This reference has been included (Ref n 30).

Q: 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.
A: This has been pointed out. See the Discussion session on page 11 from line 4 to line 6.

Minor Essential Revisions

Q: 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.
A: This has been changed. See the abstract line 2.

Q: Language needs to be checked by a native speaker; additionally multiple typos need correction (e.g. ‘timine’ should be ‘Thymidine’).
A: ‘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…’
A: These changes have been made

Reviewer: Giles Yeo
Reviewer's report:
In essence, the manuscript by Santoro et al provides us with the functional analysis on 2 new MC4R non-synonymous mutations, one of which, Q307X is a nonsense mutation and is consequently completely inactive. The functional analyses are well performed. However, my main problem is with the conclusion that MC4R mutations are not identified by a ‘particular phenotype’. I would have to disagree with this entirely, as patients with MC4R mutations have a very clear phenotype that has been well reported by Farooqi et al 2003, NEJM.

Q: Perhaps the authors meant to say that the prevalence of MC4R mutations in a highly selected Italian cohort of severely obese children is lower than in other areas of Europe? I would like to see the text of the abstract and conclusions changed to reflect this.
A: We deeply understand the point of dr.Yeo. According to the suggestion of dr Yeo and dr O'Rahilly the paper has been substantially changed, softening our primary hypothesis and giving more evidence to the data concerning the prevalence of MC4R mutations in a particularly selected population of obese children. The Title of the paper has been changed in a more descriptive one, the
conclusions of the paper and of the abstract have been rewritten. Please see the abstract from line 18 to line 21 and conclusion session on page 12 from line 3 to line 6.