Title: Meta-analysis on the effect of the N363S polymorphism of the glucocorticoid receptor gene (GRL) on human obesity

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Author's response to reviews: see over
Dear Editor in Chief,

We are sending the revised version of manuscript MS: 4828794938253605 “Meta-analysis on the effect of the N363S polymorphism of the glucocorticoid receptor gene (GRL) on human obesity”.

We feel that we have properly addressed, point by point, all comments and suggestions raised by the referees. Accordingly, a number of changes have been made to improve the article. We have listed in a separate letter each of our answers to the comments of reviewer 1, 2, and 3.

Thank you for the comments which have helped to improve the manuscript.

Hoping to hear from you in due course,

Sincerely,

Dra. A. Marti on behalf of all co-authors
Comments to reviewer 1

Marti et al have conducted an study aimed to estimate the association between the N363S polymorphism of the glucocorticoid receptor gene (GRL) and obesity. A mixed approach was employed. Data from three new case-control studies were analyzed, and in addition, to increase the statistical power, a formal meta-analysis by combining data from 12 previously published studies was carried out. They conclude that although certain genotypic effects could be population-specific, there is no compelling evidence that the N363S polymorphism is associated with either average BMI or obesity risk.

In general, the study is well designed and analyzed. However, there are some aspects that require further revision to clarify the results.

Minor Essential Revisions

The major limitation of this study is the high degree of heterogeneity in the pooled studies. Such studies greatly differ in the general characteristics of the subjects as well as in the magnitude of the association. For example, if only the new data obtained from the present report were included, the OR for obesity of carriers of the 363S allele as compared with NN homozygotes was statistically significant (OR= 0.45; 95%CI: 0.24-0.85) and in the opposite direction obtained in the previous studies carried out by Lin et al (OR> 2 and statistically significant). More information about the specific characteristics of every population is needed before pooling data. Stratification of the pooled results (Fig 1-3 and Table 3) by gender or prevalence of the variant allele could be useful to understand these contradictory findings.

The discussion of the corresponding results should be included in the new version of the manuscript.

a) As the reviewer stated, we acknowledge that there is some heterogeneity in the pooled studies. As mentioned in the Methods section of the manuscript, we evaluated potential sources of heterogeneity by subset analysis.

b) The reviewer also indicated: “More information about the specific characteristics of every population is needed”. To conduct a meta-analysis we mainly rely on the reported information in the published papers, and in this case we did not have full access to some detailed information needed to further stratify studies according to relevant characteristics because most of the studies provided only the city in which the subjects were enrolled as specified in Table 3.

c) According to the reviewer’s suggestion we have performed a subset analysis by stratification of the pooled results by gender and prevalence of the N363S variant of the GRL gene which is now included in Table 4. Unfortunately, the results of this new analysis were not statistically significant and in some cases the heterogeneity persists within subgroups. New information about this issue has been included in the revised manuscript.

d) We have added a legend in Figures 1 to 3 and in Table 2 with subjects’ information for every study mentioned (Echwald et al 2001. Lin et al 1999 and 2003, two Pamplona and one German studies).

e) Furthermore taking into consideration the reviewer’s suggestion we performed a new subtype analysis (which is now added into Table 4) considering separately subjects from Europe (15 studies) or other origin (mostly Australian populations, 5 studies).

f) A recent paper has been incorporated into the reference list (van Rossum EF, Russcher H, Lamberts SW. Genetic polymorphisms and multifactorial diseases: facts and fallacies revealed by the glucocorticoid receptor gene. Trends Endocrinol Metab. 2005;16:445-50).
As stated before, we have added a legend in Figures 1 to 3 and in Table 2 with some characteristics of each study population. New information on Spanish and German subjects is also incorporated into the Methods section of the revised manuscript.
Comments to reviewer 2

General
In general this is a well written article; unfortunately the results presented are of relatively limited interest since they only rule out an effect of one particular variant and not the gene. This observation would be of much greater interest if there were strong prior support for this variant being functional with regards to obesity. Given what we now know regarding the often chaotic pattern of linkage disequilibrium (even within genes), not having full information regarding the genetic variation within a gene makes interpreting such studies in a meaningful biological manner basically impossible.

There are three known polymorphisms in the GRL gene -BclI, ER22/23EK, N363S- with the last two involved an aminoacid sequence change. The N363S variant increases the trans-activating capacity both in vivo e in vitro, and it has been shown to be associated with increased sensitivity to glucocorticoids in vivo. Several studies have reported an association of this variant N363S with measures of increased glucocorticoid effects such as more body fat, bigger insulin response to dexamethasone and less lean-body mass. Moreover, as reviewer 3 indicated “variants with amino acid sequence alterations might be reasonably expected to show an effect if one exist”. Therefore, we chose to study this variant based on a good rationale for expecting some association. We planned and designed the study shortly after the paper by Lin et al 1999 was published in BMJ stating that “the Ser363 variant of the glucocorticoid receptor confers a virtually absolute likelihood of being overweight—unlike most markers of overweight, which confer only a slight increase in likelihood. The allele is relatively common”. We initially performed the study in 124 German obese trios, later we completed the study with 178 obese and 256 lean German subjects. Also in Spain we recruited and screened for the N263N polymorphism two populations in Pamplona (Navarra, consisting of an adult- (313 subjects) and a children- (370 subjects) case-control studies.

Major Compulsory Revisions
At minimum the authors should consider typing all known variants in this gene, or even better to re-sequence a sufficient number of individuals to determine this for themselves. Including such information into an analysis as presented here would then allow the authors to either detect an effect of this gene or to state with greater confidence that this gene does not affect variation in obesity related traits in the populations examined.

The reviewer suggests “to type all known variants or even better to re-sequence a sufficient number of individuals”. The purpose of our work was to compile a high number of studies (a large population sample) and examine them using a formal statistical methodology (meta-analysis) in order to detect or rule out the possibility that a sound association is present between a chosen gene variant and the risk of obesity. We
pooled data from different studies including our own results to rigorously assess any association between the allelic variants and the phenotype of interest and to ascertain the likelihood and magnitude of that potential association. Meta-analyses greatly increase the power and thus reduce the probability of false negative findings.

Interestingly, the three polymorphisms described are mutually exclusive (i.e. they never occur on the same allele). This naturally occurring phenomenon can simplify the analysis of association studies with these variants (Van Rossum et al, 2005).
Comments to reviewer 3

General

This article examines the association of the N363S polymorphism in the GRL gene to obesity in a meta-analysis, including previously unpublished data from the authors. Nevertheless, the N363S polymorphism is rare, and although it changes the amino acid sequence, there is meager evidence for the functional significance of this amino acid change. Thus, while the study does not rule out a possible effect of other variants for GRL, it does make a strong case that this polymorphism is not a significant player. Because no other common amino acid sequence alteration is known for GRL, this variant might be reasonably expected to show an effect if one exists.

Minor Essential Revisions

The GRL gene is also referred to as NR3C1 (nuclear receptor subfamily 3, group C, member 1) by the HUGO Gene Nomenclature Committee, and reference to this name should be made in the text, together with the chromosomal position of the gene on 5q31.3.

There has been some work to understand the functional significance of the N363S polymorphism, and it is important to summarize what is known of the functional significance of this variant.

The authors mention other polymorphisms that have been studied for GRL, but do not adequately explain why they did not study these for this investigation. By what rationale did they choose to limit their study?

a) According to the reviewer’s suggestion, information on the name of this gene recommended by the HUGO Gene Nomenclature Committee and its chromosomal position has been incorporated into the manuscript.

b) New information on the functional significance of this variant has been incorporated into the revised version of the manuscript. The N363S variant increases the trans-activating capacity both in vivo e in vitro, and it has been shown to be associated with increased sensitivity to glucocorticoids in vivo (Van Rossum 2005). Some authors indicated that carriers of this polymorphism N363S seem to have an easier fat storage caused by hypersensitive insulin secretion in response to GCs (di blasio, Hiuzenga). Moreover, the change from asparagine to serine creates a potential phosphorylation site that might be relevant for DNA binding by the GR but this effect has not been elucidated (Van Rossum 2005).

c) Information on this topic was included in our answer to reviewer 2, but here is also reproduced:

There are three known polymorphisms in the GRL gene -BclI, ER22/23EK, N363S- with the last two involved an aminoacid sequence change. The N363S variant increases the trans-activating capacity both in vivo e in vitro, and it has
been shown to be associated with increased sensitivity to glucocorticoids \textit{in vivo}. Several studies have reported an association of this variant N363S with measures of increased glucocorticoid effects such as more body fat, bigger insulin response to dexamethasone and less lean-body mass. Moreover, as reviewer 3 indicated “variants with amino acid sequence alterations might be reasonably expected to show an effect if one exist”. Therefore, we chose to study this variant based on a good rationale for expecting some association. We planned and designed the study shortly after the paper by Lin et al. 1999 was published in BMJ stating that “the Ser363 variant of the glucocorticoid receptor confers a virtually absolute likelihood of being overweight—unlike most markers of overweight, which confer only a slight increase in likelihood. The allele is relatively common”. We initially performed the study in 124 German obese trios, later we completed the study with 178 obese and 256 lean German subjects. Also in Spain we recruited and screened for the N263N polymorphism two populations in Pamplona (Navarra, consisting of an adult- (313 subjects) and a children- (370 subjects) case-control studies.