

Glucocorticoid Receptor Gene and Coronary Artery Disease: Right Idea, Wrong Gene Variant?

To the Editor:

Recent data reported by Lin et al¹ suggest that coronary artery disease (CAD) is associated with an Asn363Ser variant in exon 2 of the glucocorticoid receptor gene (*GRL*). The study is based on the notion that a dysfunctional glucocorticoid receptor may add to the adverse health effects of excessive cortisol concentrations.

In a previous study, comprising Anglo-Celtic descent with a strong heritability for hypertension and formerly designed to test the relationship between *GRL* and essential hypertension, the authors found an association between the Ser363 variant and elevated body mass index (BMI).² However, no association or linkage with hypertension was detected.³ This lack of effect on blood pressure has been reported previously in a Dutch cohort.⁴

The authors fail to provide valuable pieces of information regarding previously published observations. The fact is that the only studies indicating an association between the Ser363 variant and obesity, measured as BMI, is that of Lin et al² and Huizenga et al.⁴ In the referenced study of the Newcastle Heart Project,⁵ a significant association of the Ser allele was detected with increased waist-to-hip ratio (WHR) and not BMI in white men. To see an association with WHR in this report the authors needed to correct for a number of other factors that associate with central obesity, hence the multivariate approach (Christopher P.F. Redfern, personal communication, 2001). More importantly, however, is that a majority of studies indicates that the ATT to GTT point variation in exon 2 of the *GRL* is neither associated with obesity nor with obesity-related metabolic and circulatory perturbations.⁶

The Asn363Ser variant is located in the transactivation (τ) domain, and phosphorylation of serine residues is important in DNA binding by glucocorticoid receptors (GRs). However, a series of studies clearly indicates that this variant lacks any effect on the function of the receptor *in vitro*.^{4,7-9} Still, Lin et al¹ suggest that the underlying mechanism for the observed association with CAD might be that "adipocytes having the S363 variant of GR could be more sensitive to glucocorticoids. . ."

During the past decade, mutations affecting liability to obesity have been discovered at a phenomenal rate.¹⁰ The current literature linking obesity to genetic variants is teeming with reports of associations that cannot be replicated. Explanations for this lack of reproducibility are well rehearsed and typically include poor study design, incorrect assumptions about the underlying genetic architecture, and simple overinterpretation of data.

In this regard, the authors of this paper apply a case-control strategy of association study for characterizing the genetic contribution to CAD and obesity. This approach, however, is inherently more susceptible to identifying gene variants that prove to be spuriously associated with diseases.¹¹ Furthermore, findings beyond the study design and study objective are most likely to be misleading and spurious in nature.² Although publication is a crucial portion of the scientific process, investigators and editors should be encouraged to avoid publishing non-hypothesis-driven results, especially in excellent journals,² to prevent their unfortunate persistence into the present time.

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Response

We are perplexed by Dr Rosmond's letter, which simply restates what we already said in our paper,¹ and then dismisses most genetic association studies.

Our Introduction cites *all* positive and negative studies of the glucocorticoid receptor (GR) N363S variant with obesity, body mass index (BMI), and/or waist-to-hip ratio (WHR). Moreover, a morbidly obese group in Sydney further supports N363S in obesity.² Neither WHR nor BMI accurately reflects the metabolic disturbance associated with obesity, but they do classify obesity status. Regardless, a person with BMI >40 kg/m² (as in our recent study²) is obese, no matter what the marker. Although WHR indicates central obesity, it is affected by gender and age. Indeed, both BMI and WHR will grossly associate with, but neither will finely mirror, the metabolic changes.

There are also data on a *BclI* restriction fragment length polymorphism (RFLP) in obesity. Interstudy differences are commonplace, so what Rosmond says is hardly news. In the case of GR and hypertension, most data are negative. Despite finding occasional weak, gender-specific associations for some polymorphisms, our overall conclusion favored no association.³ For N363S, this applied to our original study,³ as well as to hypertension in coronary artery disease (CAD),¹ obesity, and diabetes.² Although others have studied indices of CAD, ours is the first to examine CAD itself. This involved 556 patients, with a highly significant finding for N363S (case versus control=0.15 versus 0.04; $P=0.00005$), rising to 0.45 for carriers with unstable angina.¹

We nowhere claim that N363S is functional. Nor do we say that position 363 is a proven phosphorylation site. We cite some of the *in vitro* studies and Rosmond adds others. However, they are limited by choice of cell and cannot mimic *in vivo* physiology. We stated which amino acids have shown phosphorylation and that N363S is merely a *potential* phosphorylation site.¹ Our statements apply to *any* variant in linkage disequilibrium (LD) with N363S. Thus "adipocytes having the S363 variant . . ." is correct, because it does not say that S363 itself is the reason that these cells might be "more sensitive to glucocorticoids".

Rosmond's inference that our study is misleading and spurious is grossly biased and could be leveled at almost any association study.

There is much debate about optimum design and no consensus has yet been reached.^{4,5} In the meantime, journals have the right to publish papers judged as scientifically sound in the context of prevailing views. Association studies have the potential to be spurious for a variety of reasons, such as population stratification.⁶ That is why we also examined N363S in morbid obesity² and obesity in CAD,¹ in diabetes,² and in hypertensive^{2,7} and healthy^{2,7} populations. Moreover, in the largest, most fully characterized group (CAD), S363 was associated with lipid elevation, thus providing a basis for us to speculate on mechanism.¹

Rosmond's reasons for lack of study reproducibility are incomplete. He fails to acknowledge other possibilities. For example, a positive association could be real, but specific to a certain population,⁴ because of different environmental influences on genotype. The negative genomic finding in a Melbourne study involved linkage, well-known to be less powerful.

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