Does a polymorphic glucocorticoid receptor explain inherited altered stress response and increased anxiety-type behaviors in a mouse population?

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Xu *et al.* reported in *The FASEB Journal* on a selection study in mice allegedly implicating a polymorphic glucocorticoid receptor (GR) in inherited altered stress response and increased anxiety-type behaviors (1). Xu *et al.* carried out "divergent genetic selection for altered corticosterone response to stress" and found that the resulting two lines differed for a polymorphic glucocorticoid receptor (GR^{Qn}). They then concluded that this GR polymorphism plays a role in complex mechanisms leading to lower corticosterone response to stress.

Unfortunately, the design of this study was not only needlessly complicated, but also fundamentally flawed. As a result, this experiment does not and cannot give any conclusive results.

Xu *et al.* used as their start population two already established lines that had been selected for high and low heat loss (4, 5). From these lines, they started a new selection experiment using a selection criterion that is not defined more precisely than "behavior, body weight, and, most important, serum corticosterone concentration (the highest or lowest in the same line, SH or SL selection, respectively) in response to restraint stress." In these experiments, the SL line is in fact the low heat loss line further selected for a low corticosterone response to restraint stress and the SH line is in fact the high heat loss line further selected for a high corticosterone response to stress. The SL and SH lines are not described or investigated further. Therefore, it cannot be excluded that the original heat-loss selected lines had already diverged for their allele frequencies for the GR^{Qn} gene. This divergence might have come to pass because of the selection for the heat-loss phenotype or because of genetic drift (2). In short, the results of the experiment reported by Xu *et al.* (1) cannot provide any meaningful support for the hypothesis that GR^{Qn} is involved in stress regulation. The situation would have been different if a standard selection study design using bidirectional selection from a single heterogeneous start population had been used. [Even with that design, results from non-replicated studies should be interpreted with great care: due to chance and genetic drift, selected lines may differ for genes that have nothing to do whatsoever with the character selected for (2, 3).]

The main question of whether the phenotypic differences measured between the SL and SH lines can be attributed, at least partly, to the glucocorticoid receptor polymorphism therefore remains unanswered. Using the present lines, the only acceptable indication of a contribution of the polyglutamine polymorphism would be the demonstration of a difference between genotypes *within* lines. Such an experiment is mentioned by the authors at the beginning of the discussion (p. E1808) but only as a complementary approach. By the way, a much simpler way to test the hypothesis, which does not need many years of laborious selection, would be to compare a battery of inbred strains (6) or recombinant inbred strains (7) for these characteristics.

There are some additional problems with this paper. These concern the fact that the polyglutamine track in the mouse glucocorticoid receptor had already been described before, the doubtful interpretation of the data in Figure 4*A*, and the exceptionally long series of restraint stress. However serious they may be, compared to the major flaw described above, these problems are actually rather minor. FJ

REFERENCES

1. Xu, D., Buehner, A., Xu, J., Lambert, T., Nekl, C., Nielsen, M. K., and Zhou, Y. (2006) A polymorphic glucocorticoid receptor in a mouse population may explain inherited altered stress response and increased anxiety-type behaviors. *FASEB J.* **20,** 2414–2416
2. Falconer, D. S., and Mackay, T. F. C. (1996) *Introduction to Quantitative Genetics*, Longman, Harlow, UK
3. Caramaschi, D., de Boer, S. F., and Koolhaas, J. M. (2007) Differential role of the 5-HT1A receptor in aggressive and non-aggressive mice: An across-strain comparison. *Physiol. Behav.* **90,** 590–601
4. Nielsen, M. K., Freking, B. A., Jones, L. D., Nelson, S. M., Vorderstrasse, T. L., and Hussey, B. A. (1997) Divergent selection

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doi: 10.1096/fj.08-0102LTR

for heat loss in mice: II. Correlated responses in feed intake, body mass, body composition, and number born through fifteen generations. *J. Anim. Sci.* **75,** 1469–1476
5. Nielsen, M. K., Jones, L. D., Freking, B. A., and DeShazer, J. A. (1997) Divergent selection for heat loss in mice: I. Selection applied and direct response through fifteen generations. *J. Anim. Sci.* **75,** 1461–1468
6. Grupe, A., Germer, S., Usuka, J., Aud, D., Belknap, J. K., Klein, R. F., Ahluwalia, M. K., Higuchi, R., and Peltz, G. (2001) In silico mapping of complex disease-related traits in mice. *Science* **292,** 1915–1918
7. Peirce, J. L., Lu, L., Gu, J., Silver, L. M., and Williams, R. W. (2004) A new set of BXD recombinant inbred lines from advanced intercross populations in mice. *BMC Genet.* **5,** 7

Additional evidence showing an additive effect of glucocorticoid receptor polymorphisms on anxiety-type behavior, stress response, and body weight in a population of mice with low heat loss background

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We have recently reported differences between two mouse lines, which were generated by selection for high (SH) or low (SL) stress response, in distribution frequency of polymorphic forms of glucocorticoid receptors (GR), one with 8 (GR^{wt} or GR^{8q}) and the other with 16 (GR^{Qn} or GR^{16q}) glutamines (1). The SH and SL lines of mice were developed, respectively, from two lines (Replicate #1), previously selected for high (MH) or low (ML) heat loss (2, 3), because the difference in corticosterone response to restraint stress between the MH and ML lines was observed only in Replicate #1, but not in mice of Replicates #2 and #3 (unpublished data). The SH and SL lines of mice should still have the genetic bases of the MH and ML mice because we intentionally carried out our selection within the existing lines. After we identified the polymorphic form of GR in the 4th generation of SH and SL mice, we screened all three replicates of MH and ML lines to determine whether there is any linkage to or pleiotropy with heat loss. Although the GR polymorphism was also observed in MH and ML lines, the distribution frequencies of the two forms of GR were not different between the MH and ML mice in Replicates #2 and #3, which had no difference in corticosterone responses to stress as observed in our prescreening prior to our SH and SL line establishment and subsequent selection. However, the mice in Replicate #1, which showed the differences in corticosterone response to stress and were used as base population for SH and SL selection, had similar GR allelic distribution as that found after stress-response selection in SH and SL mice (**Table 1**). This indicates that GR polymorphism is not linked to heat loss but may play a role in the mechanism of altered stress response.

We agree with the comments made by Mormede *et al.* that the experiment yet to be done is to determine the stress responsiveness between mice with different GR genotypes within the same SH or SL lines. However, there were not enough mice in certain GR genotypes, as shown in our manuscript, and we did not have much success in generating sufficient numbers of mice with three GR allelic combinations within the same SH or SL line in next three generations of selections subsequent to our report. This was because most of the litters with "$GR^{8q/8q}$" in SL line and those with "$GR^{16q/16q}$" in the SH line died at a very young age or because some females produced no litter at all, which was probably due to their advanced age at mating. In two recent generations, we have used younger breeders based on GR genotypes without going through the previously described selection process and have been more successful. We are now able to evaluate all three GR genotypes within the SH and SL lines. Here we report data obtained from the 10th generation of SH and SL mice with all three GR allelic combinations within the same line. Based on genotyping using the GR primers as we previously described (1), a total number of 144 mice (72 per line) were used in this study and were divided into three groups (8 mice per GR genotype per group from each line): control, acute restraint (30 min restraint before the maze test); and repeated restraint was performed (two sessions of restraint before the maze test: 30 min restraint per day for consecutive 3 d and repeated once after 4 non-stress d). Each of the mice was tested in an elevated plus maze for 5 min and results were recorded using a Noldus EthoVision® automated video-tracking system as described previ-

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doi: 10.1096/fj.08-0103LTR