eventually reaches 100% when the top 20 genes detected by either method are compared with higher ranks of differentially expressed genes. This shows that stochastic fluctuations introduced during normalization may assign *P* values that, if ranked, may suggest discordance of results.

Williams *et al.* used a Bonferroni-like correction to adjust for the number of markers tested and to estimate numbers of expected false positives. However, Bonferroni correction is inappropriate for genome-scale microarray analyses because it is overly restrictive, assumes independence between tests and, in this context, has insufficient power to detect true effects. We[3] and others[4,5] used a permutation-based approach to calculate genome-wide corrected *P* values[6] and used the false discovery rate (FDR)[7] to estimate the number of true positives at any significance threshold[3,4,8]. FDR gives additional information because the proportion of true positives in any given data set may vary greatly depending on tissue specificity[3] and normalization procedure (**Fig. 1b**). Williams *et al.* fail to recognize the additional information that can be conferred by use of FDR. We propose that combined reporting of genome-wide corrected *P* values and FDR estimates offers a standard that could be adopted in genetical-genomics study designs.

Williams *et al.* suggest that linkage that is robust to independent normalization techniques may be more reliable than linkage that is not. In many studies, the RMA method[9] has been shown to be superior to alternatives, and in our own data set, RMA consistently gave a lower FDR than other methods (**Fig. 1b**). This encouraged us to use RMA-normalized expression data for our eQTL linkage studies in preference to MAS5-normalized data.

The analysis of Williams *et al.* does not take into account linkages of individual expression phenotypes to multiple tightly linked markers. In our study, we removed redundant linkages using a custom algorithm that defined a non-redundant eQTL data set[3]. Given Williams's definition of linkage, 'identity of transcript and eQTL in combination (*i.e.*, transcript-eQTL pairs)', small changes in the linkage statistic due to different normalization procedures may change the location of the eQTL peak of linkage. If linkage to tightly linked markers is not considered, the CAT procedure may record non-concordant linkage, as the transcript-eQTL combination may be different. This apparent lack of linkage concordance does not imply that the same eQTL is unequally detected by different normalization procedures, since linkages to tightly linked markers are likely to represent the same eQTL.

Williams *et al.* propose to "exclude genes with low-intensity signals that are likely to arise from nonexpressed transcripts, as such data lack biological plausibility". Irizarry *et al.*[2] have shown that filtering out nonexpressed genes makes little improvement on the precision of normalization algorithms such as RMA and will also remove extreme allelic effects that cause complete loss of signal on the microarray, as found for Cd36 in hypertensive rats[10]. Weak signals may also indicate underrepresentation of a specific cell type, which may point to important biological effects in heterogeneous tissues.

Finally, we believe that the analysis described by Williams *et al.* ignores the quantitative nature and continuity of the available linkage evidence, which pertains regardless of the normalization method used. We and others have reported table(s) of eQTLs ranked according to their genome-wide corrected *P* value thresholds and FDR. The evaluation of the biological relevance of eQTLs is not limited to summary tables of linkage results. Extensive discussion of the eQTL data sets is provided in our manuscript[3], and further data mining and functional validation are ongoing. By identifying thousands of mapped *cis*- and *trans*-acting eQTLs in a model system with large numbers of existing physiological QTLs, we have generated a unique and accessible resource to test the hypothesis that genetic variation in gene expression has a key role in the molecular evolution of complex traits. The analysis of Williams *et al.* permits a re-evaluation of the important elements of robust microarray data analysis.

Enrico Petretto, Jonathan Mangion, Stuart A Cook & Timothy J Aitman

UK Medical Research Council (MRC) Clinical Science Centre, Faculty of Medicine, Imperial College, London W12 0NN, UK.

Michal Pravenec

Czech Academy of Sciences and Centre for Applied Genomics, 142 20 Prague 4, Czech Republic.

Herbert Schulz, Judith Fischer & Norbert Hubner

Max-Delbruck-Center for Molecular Medicine (MDC) Berlin, Berlin-Buch 13125, Germany.
e-mail: t.aitman@csc.mrc.ac.uk

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Animal research and the search for understanding

To the Editor:

The Editorial in the May issue of *Nature Genetics* (**38**, 497–498; 2006) reported on a number of the conclusions and recommendations in our report entitled, 'The Ethics of Research Involving Animals' (http://www.nuffieldbioethics.org/). The article was an excellent summary of the ethical issues most relevant to geneticists.

The article included the following paragraph: "Its [the Report's] conclusions are designed to reinforce the UK Home Office regulations for animal experimentation, which in 1959 introduced the '3R' goals—refinement, reduction and replacement—ultimately treating animal experimentation as a problem requiring regulatory reduction."

We would like to point out that the Report's conclusions were not intended to reinforce the UK Home Office regulations for animal experimentation. It did conclude, however, that the concept of the 'Three Rs' and the moral position underlying current UK legislation could be accepted, or at least tolerated by all those holding reasonable views.

In addition, the Three Rs were first described by Russell and Burch in 1959 (ref. 1). The principle of the Three Rs became enshrined in UK legislation with the introduction of the Animals (Scientific Procedures) Act in 1986.

Sandy Thomas

Nuffield Council on Bioethics, 28 Bedford Square, London WC1B 3JS, UK.
e-mail: bioethics@nuffieldbioethics.org

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