We read with interest the comments offered by Drs. Hughes and Bradley (1) on our systematic review (2). Four single nucleotide polymorphisms (SNPs), rs9332739 and rs547154 in the complement component 2 gene (C2) and rs4151667 and rs641153 in the complement factor B gene (CFB), were pooled. Hughes and Bradley point out that we omitted the most common variant, rs12614. In fact, rs12614 is in high linkage disequilibrium (LD) with rs641153, which was included, and the major allele of both of these SNPs is in the range of 90% (population code, CEU, in the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/)). Moreover, our review was initiated in September 2010, at which point only 4 studies had published associations with rs12614, whereas 14 studies (n = 11,378) were available for rs641153. While it is true that both SNPs are better analyzed as a haplotype, these data were simply not available for pooling.

Hughes and Bradley also point out that we obtained and pooled new data that were not previously published. While it is recommended that contact with authors be completed as part of a comprehensive meta-analysis, we acknowledge that these additional data were not previously published and peer reviewed and, hence, do not have the same level of transparency. However, given that sample collections often increase over time and that the instrumentation for genotyping is continually improving, we thought that it would be advantageous to use the most recent information; this is a subjective decision.

We also agree that the allele frequencies given by Kaur et al. (3) were exactly opposite to those expected and were suggestive of strand flipping. However, we specifically queried this with the lead author on 2 separate occasions and were assured it was not.

Hughes and Bradley do make an interesting suggestion that SNPs in high LD should be used as a gauge of genotyping quality in HuGE reviews. This is an interesting idea but difficult to put into practice as the $r^2$ parameter they propose as a measure of LD has some unusual properties. Although $r^2$ is a measure of LD, it is also linked to the allele frequency; even small differences in allele frequencies between 2 linked SNPs can reduce the $r^2$ dramatically. Way (4) explored these effects and found that, at a baseline allele frequency of 10%, even a difference in allele frequency between 2 SNPs as small as 2% can drop the $r^2$ value below 0.8. This degree of allele frequency difference is consistent with what could be expected for sampling error. Furthermore, when we look at 2 linked diallelic SNPs, giving 4 possible haplotypes, the absence of 1 haplotype dramatically reduces $r^2$, despite the 2 loci being in high LD as measured by $D'$. In fact, this is the situation for rs12614 and rs641153, where the low frequency of 1 haplotype means that the $r^2$ is 0.01 but the $D'$ is 1.

Hughes and Bradley also suggest consideration of genotype call rate restrictions as an inclusion criterion for meta-analysis. This would be more appropriate when focusing on genetic variants per se, as considered within the context of a genome-wide association study or other specific genetic analysis where large numbers of SNPs are evaluated (5).

The concerns raised by Hughes and Bradley reflect the limited ability of a meta-analysis based on summary data to tease out inconsistencies best identified at the individual level. We agree that SNPs in LD should be evaluated, but this will not necessarily be straightforward. A move to make genetic data sets publicly available, as in the Database of Genotypes and Phenotypes (http://www.ncbi.nlm.nih.gov/gap), is a step in the right direction for greater transparency.

ACKNOWLEDGMENTS
Conflict of interest: none declared.

REFERENCES


Ammarin Thakkinstian1, Gareth J. McKay2, Julie Silvestri3, Usha Chakravarthy3, and John Attia4 (e-mail: ammarin.tha@mahidol.ac.th)
1 Section for Clinical Epidemiology and Biostatistics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand
2 School of Medicine, Dentistry, and Biomedical Sciences, Centre for Public Health, Queen’s University of Belfast, Belfast BT12 6BA, Northern Ireland, United Kingdom
3 Centre for Vision and Vascular Sciences, Royal Victoria Hospital, Queen’s University of Belfast, Belfast BT12 6BA, Northern Ireland, United Kingdom
4 Centre for Clinical Epidemiology and Biostatistics, University of Newcastle, Hunter Medical Research Institute, and Department of General Medicine, John Hunter Hospital, Newcastle 2305, Australia

DOI: 10.1093/aje/kwt068; Advance Access publication: April 9, 2013