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Freely associating

After publishing a paper on a genetic association between α-2-macroglobulin and Alzheimer disease (AD) last August[1], *Nature Genetics* now publishes four related *Correspondences*[2–5]. Three of these report a failure to replicate the strong association between *A2M* (the gene encoding α-2-macroglobulin) and AD; the fourth is a response from the authors of the original paper. Why do editors and reviewers consider these manuscripts worthy of publication? Is there a problem? The answer is a qualified yes.

The search for alleles that are more frequently associated with particular phenotypes than would be expected by chance is not new. Fundamental issues, however, such as the significance threshold of a true association (especially in light of multiple-hypothesis testing aggravated by publication bias for positive associations[6]), how to best analyse a given data set, and what constitutes a valid refutation, have yet to be resolved. Easily compromised by inappropriate controls and inadequate statistical methods, the standards of such association studies have improved over the years, and they are widely anticipated to contribute to the understanding of complex traits. Still, the majority of association studies are never replicated, and while that does not necessarily render the original result false, repeated non-replication is nonetheless a good reason to dismiss the association.

Given these basic questions and the lack of understanding of what makes a complex trait complex, some would argue that association studies should not be published in journals of high profile. An alternative is to place them in a special section with a 'read at your own risk' disclaimer. Neither of these are attractive options. *Nature Genetics* will continue to publish selected association studies of broad interest and high calibre. It is an editor's job to ascertain the former but s/he depends on expert advice for the latter. Plausible biological context, low *P* values, independent replication, rigorous phenotypic assessment and genotyping, and appropriate statistical analysis conspire to boost one's confidence in a proposed association. The combined features must reach a certain threshold, but even experts do not always agree on the main merits and demerits of a given study.

So what of *A2M* and AD? Which of the four studies are right and which are wrong? The answer, according to expert referees, is that all of them are right—or at least worthy of publication: the original paper, because it describes an association between *A2M* and AD in families, and the other three, because they are parts of the same story that points to uncertainties regarding the original result and association studies in general. Dow *et al.*[2] report a failure to see the association in a large sample of cases and controls. Such a population-based association study differs

fundamentally from family based studies; the magnitude of the former effect is estimated by a straightforward odds ratio, the latter by an odds ratio conditional on shared genetic and environmental influences. Both types of studies have their limitations, and some genetic associations have been suggested by one but not the other. Population-based case-control studies with appropriately matched cases and controls are a highly efficient means for detecting associations. Because related individuals are not 'independent samples', family based studies tend to be less efficient, but they are also less prone to errors stemming from admixture, given their use of unaffected family members as internal controls. Blacker *et al.* introduced a new method to analyse family data[1,7]; their sibship disequilibrium test (SDT) as well as similar tests like the sib transmission-disequilibrium test[8] (S-TDT) and the discordant alleles test[9] render both affected and unaffected sibs informative. The two other groups also used families, in both cases including some from the same National Institute of Mental Health (NIMH) pool as those analysed by Blacker *et al.* Using a different analytic strategy, Rudrasingham *et al.*[3] observed some—albeit considerably weaker—association in the NIMH sample but no significant association in their own families. Rogaeva *et al.*[4] report no significant association in either the NIMH families or their own. Blacker *et al.* suggest in their response that some of the discrepancies may be due to the use of different methodology[5].

Is it all a question of analysis and/or interpretation? On the basis of the comments from the reviewers on all manuscripts, one would have to say yes, to some extent. None of the reviewers feel that, in light of the additional data, the conclusions of the original paper are invalid. They agree that the *Correspondences* are a useful addition to the bigger picture, but several issues remain controversial. Population stratification (or admixture) errors are clearly a problem for case-control studies, but do they tend to lead to false-positive results or can they also mask an existing association? Are family-based association studies also prone to stratification errors? What is the most efficient way to analyse a given data set? The method used by Blacker *et al.* (as well as other variations on the TDT/SDT theme) have only recently been published and have yet to undergo extensive scrutiny by the community to reveal their appropriate use and limitations. Although there might be more than one 'right' way to analyse a given data set, there are ways that are clearly wrong. Given the current excitement over 'SNPs and chips', and with cost-effective high-throughput genotyping a high priority in academia and industry, more geneticists are likely to perform association studies in the future. Efforts must not be wasted on poorly designed studies and inappropriate or inefficient means of analysis. There is an urgent need for a limited and generally accepted set of methods that permit appropriate assessment and comparison of individual results.

Reporting a genetic association means taking a risk—for authors and journals. This includes undermining public confidence if results are first published and publicized, followed by non-replication or even outright refutation. On the other hand, only when results are disseminated widely will others attempt to verify or falsify them. *Nature Genetics* continues to welcome submission of association studies of high quality. Ideally, they should have large sample sizes, small *P* values, report associations that make biological sense and alleles that affect the gene product in a physiologically meaningful way. In addition, they should contain an initial study as well as an independent replication, the association should be observed both in family based and population-based studies, and the odds ratio and/or attributable risk should be high. Few studies will meet all criteria, but in order to minimize our risk, we will apply high standards. In general, we will expect manuscripts reporting genetic associations to include an estimate of the effect size and to contain either a replication in an independent sample or physiologically meaningful data supporting a functional role of the polymorphism in question. Our standards will evolve as knowledge improves on complex traits and appropriate strategies for conducting association studies.

1. Blacker, D. *et al. Nature Genet.* **19**, 357–360 (1998).
2. Dow, D.J. *et al. Nature Genet.* **22**, 16–17 (1999).
3. Rudrasingham, V. *et al. Nature Genet.* **22**, 17–19 (1999).
4. Rogaeva, E.A. *et al. Nature Genet.* **22**, 19–21 (1999).
5. Blacker, D. *et al. Nature Genet.* **22**, 21–22 (1999).
6. Altschuler, D., Kruglyak, L. & Lander, E. *New Engl. J. Med.* **338**, 1626 (1998).
7. Liao, A. *et al. Hum. Mol. Genet.* **7**, 1953–1956 (1998).
8. Spielman, R.S. & Ewens, W.J. *Am. J. Hum. Genet.* **62**, 450–458 (1998).
9. Boehnke, M. & Langfeld, C.D. *Am. J. Hum. Genet.* **62**, 950–961 (1998).