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In reply—We are pleased by Rudrasingham and colleagues¹ confirmation in the NIA family sample of our finding of a genetic association between the gene encoding α -2-macroglobulin (*A2M*) and Alzheimer disease (AD) in the NIMH sample². We also do not find it surprising that the case-control studies conducted by Rudrasingham *et al.*, Rogava *et al.* and Dow *et al.* did not find an association^{1,3,4}.

The findings above highlight some differences between family based and case-control association studies. First, many but not all family based studies are free of biases resulting from population admixture^{5–7}. Second, as Rudrasingham *et al.* point out, the family based design estimates the magnitude of the effect in the context of other shared familial factors (genetic or environmental). Because family based association studies estimate an odds ratio conditional on being from the same family, which is expected to be larger⁸, they cannot be used to estimate power for case-control design (as all three papers here have done^{1,3,4}).

The statistical methods needed to properly analyse family data when unaffected

siblings serve as controls^{5,6,9,10} are new and unfamiliar to many geneticists. This is probably responsible for some of the apparent discrepancies among the analyses of the three overlapping samples from the NIMH set^{1–3}. Differences are also due to the distribution of somewhat different NIMH samples to each group. In response, we present the results of both proper and improper statistical analyses using 120 NIMH families, an expansion of our original 104 (ref. 1).

Rudrasingham *et al.*¹ estimate the effect of the *A2M**2 allele on AD using the crude odds ratio (OR) in discordant NIMH and NIA sibpairs without taking family relationship into account, yielding estimates that are biased downwards⁸. To obtain an unbiased estimate, the Mantel-Haenszel OR (ref. 11) or equivalently conditional logistic regression^{10,12} should be used. Contrary to the assertion of Rudrasingham *et al.*, these methods yield unbiased estimates irrespective of sibship size¹⁰ (but estimates of statistical significance may be somewhat inflated^{11,12}, especially if large families are genetically linked to one allele). Using our 120 families from the

NIMH sample, the crude OR in discordant sibpairs is 1.68 (95% CI, 0.94, 2.99; $P=0.13$); however, the more accurate OR based on conditional logistic regression using all siblings is 2.31 (1.27, 4.19; $P=0.006$), and using only sibpairs yields a similar OR of 2.50 (1.10, 5.68; $P=0.029$).

The sibship disequilibrium test⁶ (SDT) in the enlarged NIMH sample is shown (Table 1). Unlike Rogava *et al.*'s analysis in 143 NIMH families³, but consistent with Rudrasingham's analysis of their overlapping NIMH subset¹, our 120 families continue to show highly significant evidence of association of *A2M**2 with AD. When we stratified the sample by site³, we observed a consistent trend at all three sites, but only two remained statistically significant, as expected with reduced power. If the sample is limited to discordant sibpairs, however, power is substantially reduced, especially in the stratified analysis, leading to reduced significance. Such restriction is unnecessary because the SDT remains valid in sibships of arbitrary size⁶.

Notwithstanding the disparate findings contained in these reports, the weakly positive signal from the NIA family based study¹, along with our extended analyses in the NIMH sample, lend further support to a genetic association between *A2M**2 and AD. Moreover, the biological plausibility of this association remains high: three linkage studies have observed peaks in this region^{13–15} and there is evidence implicating *A2M* in AD pathogenesis (for example, $A\beta$ clearance²). Additional studies will be

Table 1 • Sibship disequilibrium test on an enlarged NIMH sample

All eligible families	No. families	No. subjects	B, C	Pvalue
	120	437	12, 34	0.0016
Site 1	45	145	6, 9	0.61
Site 2	34	119	4, 14	0.031
Site 3	41	173	2, 11	0.022

B, number of families favouring transmission of *2 to unaffecteds; C, number of families favouring transmission of *2 to affecteds.

required to determine whether the *A2M**2 deletion is pathogenic or in disequilibrium with another mutation. We urge additional exploration of the relationship between *A2M* and AD in other family samples and more definitive studies of the biological role of *A2M* in AD.

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Distribution and early development of microarray technology in Europe

With interest, we read a review in *The Chipping Forecast*¹ on options available for obtaining expression data using microarrays. It provided a detailed listing of all distributors of biological or technical material related to this expanding field of gene technology in the United States. Although American providers of minimal EST clone sets and high-density filters were discussed, several public organizations within Europe that create and distribute standardized reference material for genome research—while indicated on a listed internet site—were not discussed in detail.

The first systematic use of reference libraries spotted in microarrays onto membranes was introduced by a European laboratory, the Genome Analysis group at the Imperial Cancer Research Fund in London. Already in the late 1980's, robotic tools were used to produce such high-density microarrays in large quantities and experimental procedures for their use in genome analysis were developed. These included experiments for fingerprinting and partial sequencing by oligo hybridization^{2–6}, integrated genome analysis^{7–10}, hybridization high-density screening^{11–15}, high-resolution mapping¹⁶ and expressed sequence catalogues^{17,18}. At that time a distribution service for such high-density filters and clones from reference libraries, the Reference Library System (RLDB), was also established and used by many laboratories worldwide¹⁹.

One of the biggest institutions in Europe to distribute biological materials like microarrays is the successor of the RLDB, the Resource Centre (RZPD) of the German Human Genome Project (DHGP), which offers several of the products that are described in detail in David Bowtell's review. The Resource Centre is a non-profit organization founded by the Federal

Ministry of Education, Science, Research and Technology (BMBF) for the support of genome research and is maintained by the Max-Planck-Institute for Molecular Genetics, Berlin, and the Deutsches Krebsforschungszentrum, Heidelberg.

The Resource Centre constructs new clone libraries, collects available libraries and copies and distributes them as high-density spotted hybridization filters or clone pools for PCR screening. The experimental data obtained by the users are included in the Primary Database operated by the centre. This concept enhances the exchange of data and information in the scientific community and is widely accepted, as more than 4,500 registered customers from academia and industry from all over the world request biological material and information.

The Resource Centre is one of two official European distributors of IMAGE clones. It distributes them not only as single clones and on high-density filters but also offers the RZPD Human Unigene Set, with 33,000 unique IMAGE clones selected on the basis of the Unigene Cluster 102 (National Centre of Biological Information, NCBI). All clones integrated into this gene set are free of T1 phage contamination. This is guaranteed by an assay system that was developed at the Resource Centre and then transferred to other centres and companies. All clones are also free of contamination by other microorganisms as determined by a unique in-house test system.

As a non-profit organization, the Resource Centre charges prices only to recover the costs for producing its materials. Clones from the IMAGE collection and the RZPD Human Unigene Set can be ordered for DM 40 (~\$24) per clone. A set of two high-density clone filters for expression studies with lysed colonies is available

for DM 1,340 (~\$800), whereby a certain number of clones identified by screening RZPD filters are free of charge. A screening service is also available. Filters with PCR products are in preparation. A similar RZPD Unigene Set for mouse and rat is currently in development. It is planned to distribute glass microarray slides for more detailed expression analysis.

The Resource Centre can be contacted by e-mail (info@rzpd.de) or fax (+49 30 32639111). For details of materials and services offered, registration and request forms, as well as up-to-date prices, see the RZPD web site (<http://www.rzpd.de>).

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