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Letters to the Editor

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Response to Letter by Markoula et al

Response:

We have read with interest the results presented in Lazaros et al[1] and discussed by Markoula et al[2] on the relationship between risk of stroke and the genetic variations in the estrogen receptor α (ESR1), commonly known as the PvuII (−397T>C; rs2234693) and XbaI (−351A>G; rs9340799) polymorphisms. In correspondence with our findings[3] they observed no such relationship in their primary analysis of 130 ischemic stroke patients versus 240 healthy age-matched controls. However, on additional analysis in smaller subgroups the authors report an association between the −351 (XbaI) A variant and earlier age at onset of stroke in males, and the −397 (PvuII) C variant being under-represented in female stroke patients.

Markoula et al now suggest that part of the dissimilarity between the 2 studies could be explained by the use of different strategies to analyze the ESR1 genotypes. Our study used genotype analysis based on haplotypes of the 2 polymorphisms we constructed using PHASE analysis of the 6229 subjects in our cohort study, whereas Lazaros et al used so-called diplotype (combinations of the genotype at each locus) analysis in the 370 subjects from their case-control analysis. Although we acknowledge that there is some uncertainty in the estimation of haplotypes based on PHASE analysis in population based samples, this program has been widely used to reconstruct haplotypes including validation with other methods and always with very little error,[4,5] and so we think this error is minimal in our dataset. In addition, in comparing genotype data (and the associations based on them) across studies it is important to consider technical issues concerning accuracy of genotyping methods. In this respect, restriction fragment length polymorphism analysis, such as used by Lazaros et al, has a more compromised track record than current high-throughput genotyping methods such as Taqman, which we used in our study.

Yet, we think a more important issue in this discussion is the lack of power to draw meaningful conclusions in studies of a small number of subjects analyzing the subtle effects so typical for common genetic variants in complex traits such as stroke. The Lazaros et al study has only 30% of the cases compared to our original study population (and only 5% of the controls) whereas multiple analyses were performed with eventually even smaller numbers per group, because of the several subgroups based on sex, end point and genotype definition. In the absence of any replication effort or analyses in a larger cohort, such results therefore remain to be treated very cautiously.

Although we much welcome efforts to replicate initial reports on genetic associations, we urge the study population to be preferably bigger than the original sample size, and we recommend careful consideration to standardize genotype and phenotype methods and analytic designs. A better approach in this respect will therefore be to run (prospective) meta-analyses of the multiple datasets available, to determine the true effect size of a genetic variant and to study possible sources of heterogeneity across populations or by subgroups, if sufficient power is available. Guidelines to this end are currently being developed and interim versions are available.[6]

Disclosures

None.

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