LETTER TO THE EDITOR

Matching in case-control studies

Comments on Iqbal MP et al. 2005. J Mol Genet Med, 1, 26-32.

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Sir,

As in all case-control studies, the reported lack of association between methylenetetrahydrofolate reductase (MTHFR) 677C>T mutation and coronary artery disease in Pakistan (Iqbal et al, 2005) is highly dependent on the matching of cases and controls ('comparison' group). The various constituent populations of Pakistan are characterized both by a long-standing tradition of intra-community (*biraderi*) marriage (Shami et al, 1994; Hussain, 2005) and average consanguineous marriage rates of 50% in the present generation (National Institute for Population Studies, 1992; Bittles et al, 1993; Yaqoob et al, 1993). Not surprisingly, these two factors have resulted in significant inter-community genetic differentiation (Wang et al, 2000; Mohyuddin et al, 2001). Further, a recent study by Ismail et al (2004) showed that parental consanguinity was a major independent risk factor for acute myocardial infarction in young Pakistani male adults (OR 3.80, 95% CI 1.13-12.75).

It is therefore puzzling that, in selecting their cases and 'comparison' group, Iqbal et al appear to have ignored genetic substructure as an important potential variable, instead relying on sex, BMI, age and socio-economic class as the primary factors to be controlled. Failure to consider or discuss possible inter-community differences in the prevalence of specific MTHFR genotypes, and more especially to allow for the influence of the known high levels of first cousin marriage in Pakistan on myocardial infarction, make interpretation of the negative findings obtained very problematic.

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AUTHORS' RESPONSE

Sir,

We wish to thank Professor Bittles for his most helpful and relevant comments on our recently published paper reporting lack of association between *MTHFR* 677C>T dimorphism and coronary artery disease (CAD) in Pakistan (Iqbal et al, 2005).

For this project, we designed an association study of a case-control type. In such designs, it is indeed absolutely crucial for investigators to exercise utmost care in matching cases and controls (incidentally, we refer to the 'control' group as a 'comparison' group, as one cannot be sure that they are indeed true controls – any one of them may develop the disease later in life). We would like to clarify that the "genetic substructure" of the cases and controls was taken into account. Our population subjects belonged to five of the major ethnic groups of Pakistan, e.g. Punjabi, Balochi, Sindhi, Pathan, Urdu-speaking (Mohajirs). Incidentally, readers may wish to know that Pakistan hosts in fact eighteen major, distinct ethnicities (Qamar et al, 2002). The frequency of the 677T mutant allele was quite small (2-3% of homozygotes for mutant) and distributed evenly in all these five ethnic groups. Although we observed no difference in allele frequencies amongst the different ethnicities, statistical analyses of the data with such small numbers was not likely to yield meaningful conclusions. As no

particular ethnic group was found to harbour deviating mutant allele frequency, we decided to combine them all in a "Pakistani population". The study could be repeated independently on the various ethnicities of the country, although we doubt that the findings would be any different.

Professor Bittles is right in stating that consanguinity is very common in Pakistan, as it reaches an overall level of nearly 50% in the general population (Yaqoob et al, 1993). Although information about consanguineous marriages of parents was not part of our questionnaire, we wish to reiterate that all patients and controls in our study were unrelated. Therefore, any effect of consanguinity must similarly affect both cases and controls, as we ensured that they were part of the same general population. On this score, association study designs lead to dubious results mostly if genotypic distributions of markers deviate from Hardy-Weinberg equilibria – which is not the case in our present study, where *MTHFR* 677C>T genotypes do occur in Hardy-Weinberg proportions.

The first report about association of parental consanguinity with myocardial infarction by Ismail et al (2004) was on a population of young male adults (15-45 years), while our study population comprises both males and females whose ages range between 30 to 74 years. It remains to be ascertained whether the findings by Ismail et al (2004) are applicable to older Pakistani populations as well. Chronic, degenerative disorders (such as, among many others, CAD, hypertension, type II diabetes mellitus and stroke) result from intricate interactions between the effects of both genetic and environmental factors. It seems that the major gene effects underscoring these disorders are transmitted in recessive manners (Frossard et al, 1998). As consanguinity raises homozygosity levels, this may explain why diseases such as CAD are more prevalent in consanguineous societies such as Pakistan's, thus corroborating the findings of Ismail et al (2004) (this fact has been amply demonstrated in the case of monogenic diseases, where countries such as Pakistan have provided scores of families with rare hereditary diseases which have led to the identification of the causative genes).

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