The rationale for case-control methodology in epidemiological studies of cancer risk (response to Speirs *et al.*, 1990)

A number of studies have examined the hypothesis of an association between the genetically determined ability to metabolise debrisoquine (DBR) and lung cancer susceptibility. These include Ayesh *et al.* (1984), Caporaso *et al.* (1988), Law *et al.* (1989) and Roots *et al.* (1988). The frequency of poor metabolisers (PM) reported in these studies is in the aggregate significantly lower than that in the general population but more importantly, each of these studies included a well-defined control group thereby allowing an appropriate case-control comparison. In each study a lower percentage of PMs was found among the cases and in three of the four studies the result was statistically significant.

The results in these studies contrast with those in the recent report of Speirs *et al.* (1990) who, finding eight PMs among 82 lung cancer cases (10%), conclude that 'the value of the DBR phenotype as a marker for lung cancer susceptibility in the population at large is low'. Unfortunately, with regard to the resolution of this important question, their methodological approach is not suitable for addressing their stated goal. The major flaw in this study is the failure to define a control group and the subsequent reliance on historical controls for their statistical analysis and conclusions. The use of nonconcurrent controls is replete with theoretical pitfalls, many of which are illustrated by this study. For instance, the determination of the DBR metabolic phenotype is crucially dependent on the phenotyping procedure employed. The protocol of Speirs *et al.* (1990) differs from the standard used in earlier work in a number of respects including: dose of DBR administered (Speirs *et al.* (1990)—1 mg, other studies—12.8 mg, as hemisulphate); time of drug administration (Speirs *et al.* (1990)—not specified but presumably variable and at patient discretion, others—in the morning); diet prior to debrisoquine (Speirs *et al.* (1990)—not less than 4 h prior to last meal, others—overnight fast); medical supervision of phenotyping procedure (Speirs *et al.* (1990)—apparently at home at patient discretion and unsupervised, others—mostly in hospital under medical supervision); concurrent medications (Speirs *et al.* (1990)—drug use recorded, others—non-essential medications withheld); and laboratory method for determination of DBR and hydroxy-DBR [Speirs

et al. (1990)—combined gas chromatography-mass spectrometry with the metabolic ratio directly determined by comparison of ratios of areas for the respective peaks of DBR and hydroxy-DBR to a standard curve (the direct method), other investigators—gas chromatography with electron capture, metabolic ratio determined from values of DBR and hydroxy-DBR, (the indirect method)]. The respective merits of the different approaches are not at issue. The crucial point is that each of these differences calls into question a comparison with historical controls who were phenotyped using a different procedure.

A further point regarding the methods deserves mention. The study depends critically on an identification of PMs based on 'high' values of the metabolic ratio. Elevated ratios occur in PMs because they excrete abundant DBR and minimal hydroxy-DBR. The protocol employs less than one-tenth the usual dose of DBR and yet still, presumably, identifies detectable hydroxy-DBR for the direct determination of the ratio. A small absolute error in hydroxy-DBR will greatly influence the value of the ratio.

Speirs *et al.* (1990) note that in the study of Ayesh *et al.* (1984) their 'very fast metabolisers were under-represented in the controls (29.9%) in comparison with populations studied by Price Evans *et al.* (1980) (56.7%) or Steiner *et al.* (1985) (71.7%)'. Some years ago we reflected on the fact that the controls in this study did not apparently meet Hardy-Weinberg constraints. This led us to speculate that the problem might have resulted from the assignment of inaccurate cutpoints for distinguishing the phenotypes. We therefore employed a maximum likelihood procedure to determine new cutpoints in the phenotype distribution in the controls (Caporaso *et al.*, 1989a). In the resulting reanalysis the new phenotype distributions fit the Hardy-Weinberg equilibrium, the 126 apparent homozygous extensive metabolisers (EMs) among the 234 controls (53.8%) are in agreement with other studies, and the case-control difference remains highly significant (Caporaso *et al.*, 1989a,b). Other authors have proposed similar methods in order to generate mathematically optimised cutpoints for overlapping phenotypes (Henthorn *et al.*, 1989). The use of such a procedure is especially important when a new population is studied, a different racial group is examined, or

a new laboratory or phenotyping procedure employed. In contrast to the work of Ayesh *et al.* (1984), the lack of a control group in the Speirs *et al.* (1990) study excludes the possibility of such a corrective analysis.

Additional questions might be raised with regard to the reason for the exclusions, the mixture of incident and prevalent cases, the unusual distribution of cancer histologies, and prior therapy of patients.

In summary, well designed and executed epidemiological studies are required to advance further the debate regarding this marker of cancer susceptibility.

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