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LETTER TO THE EDITOR

Do polymorphisms of 5,10-methylenetetrahydrofolate reductase (MTHFR) gene affect the risk of childhood acute lymphoblastic leukemia?

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Abstract. Meta-analysis has become an important statistical tool in genetic association studies, since it may provide more powerful and precise estimates. However, meta-analytic studies are prone to several potential biases not only because the preferential publication of "positive" studies but also due to difficulties in obtaining all relevant information during the study selection process. In this letter, we point out major problems in meta-analysis that may lead to biased conclusions, illustrating an empirical example of two recent meta-analyses on the relation between MTHFR polymorphisms and risk of acute lymphoblastic leukemia that, despite the similarity in statistical methods and period of study selection, provided partially conflicting results.

Key words: Childhood, Leukemia, Meta-analysis, MTHFR, Polymorphism

Recently, in "A meta-analysis of genotypes and haplotypes of methylenetetrahydrofolate reductase gene polymorphisms in acute lymphoblastic leukemia" (ALL), Zintzaras et al. [1] reported that both C677T and A1298C variants, in a recessive model, might be associated with a reduced risk of childhood ALL, but also noted that small studies tended to show stronger effect sizes than larger studies, suggesting publication bias. Theoretically, genuine association, differences in ethnical background, phenotype misclassification, disease heterogeneity, and gene-nutrient interactions are some of several potential factors that might explain such findings. However, in this case, failure in obtaining/including all published reports should also be considered. In fact, a number of important "negative" studies were either missed or excluded due to different exclusion criteria by Zintzaras et al., since, by February 2006, at least four additional reports on *MTHFR* polymorphisms and ALL risk were available: three published between 2003 and 2005 [2–4], and one published in April 2006 [5], but that was available online (earlier access) in September 2005. Of these four missed/excluded papers, two examined adult ALL [2, 3], two evaluated childhood ALL [4, 5], and three used validated methods of genotyping [3–5], whereas all four were case-control in design and reported no statistically significant results, indicating no relation between the studied variants and a reduced risk of ALL. Carefully analysis of both genetic aspects and epidemiological issues of each missed/excluded paper based on quality scores validated in previous meta-analyses [6] suggests that such studies are of moderate to high quality. Importantly, one of them is the largest case-control study (~2,000 subjects) performed thus far on the relation between *MTHFR* polymorphisms and risk of childhood ALL [4].

When considering childhood ALL only, results derived taking into account the two missed/excluded childhood ALL papers [4, 5] and those described by Zintzaras et al. [1] still agree with a putative presence of publication bias (Egger's test, $p = 0.03$). However, pooled estimates are no longer statistically significant: $OR_{C677T} = 0.87$; 95% CI = 0.72–1.06, $p = 0.17$ and $OR_{A1298C} = 0.80$; 95% CI = 0.56–1.16, $p = 0.24$ under fixed- and random-effects models, respectively (recessive model). These results are in line with a recent study testing the interaction between child's *MTHFR* genotype and maternal folate supplementation during pregnancy [7] and an additional meta-analysis that shows no evidence for a protective effect of *MTHFR* polymorphisms on the susceptibility of childhood ALL [8].

How two meta-analyses [1, 8] investigating the same hypothesis, quite similar in methods and performed almost at the same time might yield different conclusions?

One possible explanation might be differences in inclusion/exclusion criteria. In this respect, Zintzaras et al. stated in their paper that they identified 31 articles using their search criteria but selected only nine studies for meta-analysis on the basis of a few

inclusion-criteria. Therefore, it is indeed possible that Zintzaras et al. retrieved the four additional studies here presented, but chose not to include them because of different selection criteria. Differences in the selection criteria may lead to a narrow selection of papers and, as a result, bias not only the pooled estimates, but also, more importantly, the qualitative results of the overall analysis.

While we cannot rule out this possibility, since Zintzaras et al. did not report which studies were excluded in their screening, one may argue that such differences in qualitative results between Zintzaras et al. [1] and Pereira et al. [8] could be attributed to a failure in obtaining all relevant studies by the former meta-analysis.

In fact, Zintzaras et al.'s electronic searches were carried out using solely Medline. Despite to the fact that the four missed/excluded papers are retrieved by Medline, a large body of evidence demonstrates that a number of studies can be missed even by skilled Medline searches [9, 10], suggesting that the use of this database uniquely is not sufficient to retrieve all studies for a meta-analysis.

Hence, meta-analysts may easily overcome the possibility of failure in obtaining all available literature by searching multiple databases (e.g., Medline, EMBASE, Web of Science and LILACS), contacting leading researchers and experts in the field and performing meta-analyses on issues that at least one of the authors has a previous background in the specific hypothesis to be tested.

In any case, our empirical observations of how two similar meta-analyses can yield different conclusions have three broader implications. First, a cautious interpretation of results from meta-analyses whose search strategies were based on Medline only is required. Second, we need urgently a more objective tool for comprehensive literature appraisals with reproducible inclusion criteria in order to reduce bias and provide the fullest amount of evidence. Third, that editors and reviewers should demand mandatory searches in at least two electronic databases to improve the quality and reliability of future meta-analyses in both clinical research and genetic epidemiology.

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