Reviewer’s report

Title: 118 SNPs of Folate-Related Genes and Risks of Selected Congenital Anomalies

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Reviewer: Jean-Louis Gueant

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The authors present a retrospective study evaluating case control association between 118 SNPs of genes related with folate pathway and the risk of spina bifida and conotruncal heart defects, using a SNPlex assay. The sample size is rather big with respectively 259 cases with spina bifida, 214 cases with conotruncal defects and 359 controls, recruited before folate fortification in US. The choice of genes is relevant. It includes genes with already described associations, such as MTHFR, MTRR, MTR, BHMT and CBS, with a rather exhaustive analysis of new SNPs. The haplotype analysis underlines the importance of gene variants involved in the re-methylation pathway of homocysteine. In conclusion, an important study, which could be improved by a minor revision

Major Compulsory Revisions

1) A calculation of population size could be included in method section, in function of study power, allele frequency and expected increased frequency in cases. In the “limitations” in last paragraph of page 9, the sample size effect should be considered according to this calculation.

2) The race/ethnicity is not matched between Spina bifida and controls (P<0.0001). This limitation concerns particularly MTHFR (dramatic differences of frequency among Hispanics, Caucasians and Afro-American. For example, see Guéant-Rodríguez, Am J Clin Nutr, 2006). This comparison between controls and SB cases should be revised using a subgroup of matched controls.

3) Correct the analysis for multiple comparisons.

4) The citation of the literature should be revised. For example, concerning the evaluation of MTRR, MTR, MTRR with Spina bifida, two case control studies have observed a significant association with MTRR in Italy (Guéant Rodriguez, Neuroscience Letters, 2003) and in France (Candito M, American Journal of Medical Genetic, 2008)...

Specific minor comments:

1) The title is not informative (“Spina bifida and heart defect” instead of “selected anomalies”)

2) Please, avoid “Wild type” (throughout the ms). It refers to a mutation rather than to a polymorphism.
3) Abbreviations of FOLR1 and FOLR2 are not given in footnote of table 1
4) Table 2 should be divided into two tables for Spina Bifida and conotruncal defects, respectively, giving the frequencies not only in controls but also in cases. Some of the SNPs have a minor allele frequency lower than 0.1.

5) Please, indicate significant results of table 3 in bold, to help the reader! Tables 4 and 5 could be limited to significant haplotypes. Please, provide P-values in the tables and indications for adjustment in footnote.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

declare that I have no competing interests