Reviewer's report

Title: Association of MTHFR Gene Polymorphisms with Breast Cancer Survival

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Reviewer: Ji-Yeob Choi

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General

This study presented an interesting analysis of the functional MTHFR genetic polymorphisms with outcome of breast cancer patients after treatments in mixed population. My major reservations are that the statistical analysis is not sufficiently rigorous to rule out confounding, that the authors claim interactions between MTHFR genetic polymorphisms and races in a model rather than stratification analysis by races with different allele frequencies in each race, that the haplotype estimate from two SNPs were not suitable from different ethnicity with different allele frequency since haplotype estimate depends on the allele frequency, and that small subjects had been collected for 10 years, causing low participation rate and selection bias.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Methods

Study Population: comparably small eligible subjects (n=248) has been collected in several hospitals (n=5) for 10 years. Although authors excluded patients having some criteria, the participation rate still appeared low. How many subjects were listed on to participate to obtain the 248 women and what percentage was excluded? This should be provided in the Study Population section.

Authors mentioned about the treatment in patients in Discussion (2nd paragraph, page 13), but should describe them in Methods.

Genotyping: genomic DNA for genotyping was from several different sources including 28 tumors and non-tumor tissues. Tumor DNA might be different from non-tumor tissues or buffy coat DNA, thus need to discuss that.

Statistical analysis:
What kinds of method did you used to estimate haplotypes reconstruction and frequency estimate?

Results

Each table should present how many events (death) there were in each stratum because small sample size might result in non-significant association. For example, the associations of MTHFR on breast cancer survival were statistically significant in only ER negative, which may be related to poor prognosis (more events) in ER negative group rather than ER positive group.

In table 2, the frequencies of each race looked different significantly, and should add p-value for difference (such as by chi-square test). Authors showed estimated haplotype from genotype data, but the population frequencies of haplotypes, as well as the diplotype configuration of each subject, are estimated from a set of genotypes of the subjects in a sample from the population, therefore, the haplotype estimated from genotype data should be estimated after stratified by races. For estimation of haplotype, linkage disequilibrium between SNPs should be addressed.

If there are significant differences of allele frequencies between races, the analysis should stratify the data by races rather than add interaction term in the model, especially in case that the relationship between variables was in opposite direction (Szklo and Nieto, Epidemiology Beyond the Basics, Chapter 6-7). How could authors interpret the results of difference of allele frequencies with opposite direction of outcome?

Discussion
As authors described the role of MTHFR in the last paragraph on page 10, a lot of factor could influence the outcome of breast cancer related to MTHFR genetic polymorphisms. It should be very hard to interpret the results of opposite direction on breast cancer survival by C677T and A1298C, that both were reported to decrease enzyme activity, in mixed races with different allele frequencies as well as different prognosis profiles.

What next?: Reject because scientifically unsound

Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.