Reviewer's report

Title: Mutation analysis of the MDM4 gene in German breast cancer patients

Version: 1 Date: 12 September 2007

Reviewer: Mieke Schutte

Reviewer's report:

General

The paper by Reincke et al. reports on a whole gene mutation screen of MDM4 in 40 German familial breast cancer cases. They identified eight unique sequence variants, with two sets of coupled variants (one with 2 variants and one with 4 variants). The two variants in the MDM4 coding sequence are further analysed in 200 controls and 140 bilateral breast cancer cases. The silent V74V variant was present at equal frequency in these populations and the missense D153G variant was not identified. Thus, neither of the variants associated with their breast cancer cases and the authors conclude that MDM4 does not play a major role as a breast cancer susceptibility gene in Germany.

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

1. The assumption that MDM4 is a breast cancer susceptibility allele is rather speculative. The cohort size of 40 cases is way too small to address the hypothesis in a meaningful manner, except in the unlikely case that MDM4 mutations would turn out to be rather prevalent. The study simply does not have sufficient statistical power (on which the authors do not comment) to either include or exclude MDM4 as a susceptibility gene. No conclusions can be drawn at all, meaning that the study design is bad.

2. The follow-up mutation screens are technically not sound. First, but minor, is that the authors do not report whether the D153G variant was also identified in another independent amplification reaction. But given the follow-up screens, one could assume that this was the case. The follow-up screens are a major problem. Reactions with restriction enzymes are not always reliable. It is difficult to design good technical controls; the only reliable one in my opinion is to include an independent control DNA fragment that contains the restriction site in each reaction. A positive control as separate sample is not sufficient as the most typical cause for failures in digestions is inhibitors that are present in the DNA samples. The authors do not describe their controls at all.

3. There is a curious observation presented in Table 2. The coupled variants in introns 8, 9, 10 and the 3'UTR variant are detected as heterozygotes in half of the cases. Yet, no homozygotes are detected. This is rather unexpected at such frequencies and would suggest that there is selection against homozygosity or
that there is a technical problem. In the former case, (one of) the variants could be of interest in terms of pathogenicity. In the latter case, the study is troubled.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

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Discretionary Revisions (which the author can choose to ignore)

**What next?:** Reject because scientifically unsound

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests