Author's response to reviews

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

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Reply to referee 1:

We have incorporated the referee´s suggestion into our manuscript as follows:

1. Study size calculations depend on both allele frequencies and the expected risk which is difficult to assess for MDMX in a precise manner. We already included a final passage in our revised manuscript that addresses the power limitations. We now extended this passage in writing more specifically that “…it is also possible that more families with the D153G mutation may be detected in an extended case-control series. However, several thousands of samples would have to be screened to confirm, for instance, a 2-3 fold increase in risk for a variant with a carrier frequency of less than 1 %.”

2. We now included into the manuscript that samples that remained uncut were subjected to direct sequencing to avoid false positives (Material and Methods), that positive and water controls were always included (Material and Methods), and that the D153G mutation was verified in independent amplification reactions and was also confirmed in the patient´s mother (Results).

3. Our results indicated a MAF of 0.20-0.25, thus 2-3 rare homozygotes might have been expected. We now included into the Discussion that “…we noticed that there appeared to be a slight underrepresentation of rare homozygotes for the coupled intronic variants as one would have expected 2-3 homozygotes among the sequenced cases. This is not a technical problem since we identified a homozygote among a few additional samples after limited sequencing of these portions of the gene. We think that it might be a spurious observation due to a relatively small number of fully sequenced samples but we cannot formally exclude the possibility that there is selection. The data provided here will enable subsequent studies that specifically address the distribution of the three main haplotypes and the corresponding genotypes in large case-control settings.”