Author's response to reviews

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

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Author's response to reviews:

Reply to referee 1:

This referee raised some concerns that we can address as follows:

1. MDM4 is an excellent candidate for biological reasons, and we have cited work supportive of a potential role of MDM4 in the regulation of damage response pathways and in breast cancer pathology. We therefore share the view of referee 3 that our a priori hypothesis of a role of MDM4 in breast cancer has been a reasonable choice.

We agree with the referee 1 in principle that our study size does not exclude the possibility of any rare mutations in MDM4 that may have been detected after increasing the sample size to several hundreds, and we understand the desire to see such large association studies by sequencing. At the present time, this is not yet a practicable scheme for a plethora of candidate genes. We therefore regard it as a valuable contribution to perform exploratory studies of the type presented here. The idea is to sequence the complete coding region in a limited sample and then to specifically screen identified mutations in larger case-control series. Studies of similar design and similar size are not unusual. The present work might serve to show that there are no common MDM4 founder mutations in our population, and it also presents several details that we wish to share with the scientific community. The limitations of such a study are clear and, as suggested by the referee 2, have now been discussed more extensively in a final passage of our revised manuscript.

2. We disagree with the view that the mutation screens were technically not sound, meaning that restriction enzyme analysis was a poor screening method. In case of the two gene alterations that were screened in our series, the rare
allele abolishes a recognition site for the respective enzyme. This prohibits false negative results and, because samples that remained uncut were subjected to direct sequencing, there are also no false positives. It goes without saying that positive and water controls are always included in this type of assays. The D153G mutation was verified in independent amplification reactions and, as described in our manuscript, was also confirmed in the patient´s mother.

3. We noticed that there appeared to be a slight underrepresentation of rare homozygotes for the coupled intronic variants as one would have expected two 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 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.

Reply to Referee 2:

We appreciate the positive comments and have made the suggested changes. Much of the referee´s comments has been incorporated into a final passage of our revised manuscript.

Reply to Referee 3:

We appreciate the positive comments and have made the requested corrections for p53 and MDM4 in the revised version of our manuscript.