Reviewer’s report

Title: The risk allele of SNP rs3803662 and the mRNA level of its closest genes TOX3 and LOC643714 predict adverse outcome for breast cancer patients

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Reviewer: Christina Justenhoven

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Manuscript Title
“The risk allele of SNP rs3803662 and the mRNA level of its closest genes TOX3 and LOC643714 predict adverse outcome for breast cancer patients” by Gudmundsdottir et al.

Reviewer Comments

The SNP rs3803662 is located in LOC643714 and close to the TOX3 gene. It is one of the well-established breast cancer susceptibility loci. The authors investigated the association of this SNP as well as mRNA expression level of LOC643714 and TOX3 with histo-pathological tumor characteristics and survival of breast cancer patients. They showed an association between the SNP and lobular tumors as well as short overall survival. The mRNA expression of LOC643714 and TOX3 was positively correlated. Low expression was associated with high Ki67 levels and basal subtype, whereas high mRNA expression was associated with positive lymph nodes, ER and PR positive tumors as well as shorter overall and distant metastasis free survival. The authors concluded that rs3803662 as well as LOC643714 and TOX3 expression affect breast cancer progression and tumor subtypes.

Major Concerns:

• The manuscript would benefit from additional careful reading and revision of the text (see minor issues).
• Discussion: A potential physiological explanation for the observed association between TOX3 and LOC643714 and tumor characteristics as well as survival is missing.
• Discussion: The study is quite small but well designed. The exploratory character of the study should be discussed and it should be mentioned that these findings provide the basis for further validation studies including larger sample sets.
• Supplementary Table S1: please include %

Minor Issues:

• Abstract, Methods: please change “normal DNA” to “constitutional DNA” or
“DNA isolated from blood and normal tissue”

- Background, paragraph 2, line 1: change “SNP is within” to “SNP lies in”
- Background, paragraph 2, line 4: change “is a 133 kb” to “are located in a 133 kb”.
- Background, paragraph 2, line 6: change “strongest association” to “strongest effect” (comment: the word “association” has been used two times in this sentence).
- Background, paragraph 2, lines 8 and 9: change “It has been found to be a regulator …and to interact…” to “It is involved in the regulation of…and interacts…”
- Background, paragraph 2, lines 11 and 12: change “…regulator that does not bind to DNA on its own but enhances activity, mediated by other transcription factors…” to “co-regulator that enhances activity of transcription factors…”
- Background, paragraph 3, line 1: “…lower TOX3 mRNA has…” to “…lower TOX3 mRNA level has…”
- Background, paragraph 3, lines 2 and 3: “…a dose-dependent manner with the number of risk alleles.” to “…allele-dependent manner.”
- Background, paragraph 3, lines 3 and 4: “However, the only study of a mechanism for TOX3 in breast cancer showed that increased expression…” to “So far, one study showed an effect of TOX3 expression on breast cancer in that increased level…”
- Background, paragraph 3, line 5: “…current…” to “…present....”
- Methods, paragraph 3, subheading: change “RNA isolation and measurement of mRNA” to “RNA isolation and quantification”
- Methods, paragraph 3, lines1 and 2: change “…TOX3 are part of a larger study [36] from which the array data are available through GEO as GSE22133.” to “…TOX3 were retrieved from a study including 359 breast tumors [36] via GEO (GSE22133, include URL here)”
- Methods, paragraph 3, lines 2 and 3: change “…to measure RNA levels …” to “… to confirm the array data RNA levels....”
- Methods, paragraph 3, lines 3 and 4: change “…in total RNA in samples from the same fresh frozen breast tumors that were used to produce the gene expression arrays describes above.” to “…total RNA was isolated from the identical fresh frozen breast tumors which were used in the first study”
- Methods, paragraph 3, line 21: change “To be able to include…” to “To include…”
- Methods, paragraph 5, line 1: change “…to test for deviations…” to “…to test genotype frequencies for deviations…”
- Methods, paragraph 5, line 10: change “…performed in the statistical…” to “…performed using the statistical …”
- Results, paragraph 1, subheading: change “Carriers of the risk allele of the
rs3803662 have shorter OS than non-carriers” to “Carriership of the rs3803662 risk allele is associated with shorter OS”

- Results, paragraph 1, lines 3 and 5: change “The only significant association that was identified was that patients who carried the risk allele more often had tumors of the lobular histological type than the ductal type (p=......)” to “We observed a significant association between patients who were carriers of the risk allele and occurrence of lobular tumors (p=......)”

- Results, paragraph 2, subheading: change “The risk allele of the rs3803662 was associated with a decreased in TOX3 mRNA in ER positive tumours” to “rs3803662 is associated with decreased mRNA expression in ER positive tumors”

- Results, paragraph 5, subheading: change to “Elevated TOX3 mRNA expression in breast tumors is associated with poor survival”

- Discussion, paragraph 2, line 3: change “…than in the…” to “…than the…”

- Discussion, paragraph 2, lines 4 and 5: change “…the risk allele appeared more often in lobular…” to “…the risk allele was more frequent in patients with lobular…”

- Discussion, paragraph 2, line 10: change “information about” to “information on”

- Discussion, paragraph 2, lines 10 and 11: change “…the risk allele significantly shortened overall…” to “…the risk allele was significantly associated with poor overall…”

- Discussion, paragraph 4, line 3: change “A reduction of TOX3 or LOC643714 mRNA…” to “A decreased level of TOX3 or LOC643714 mRNA…”

- Discussion, paragraph 5, line 7: change “…came from…” to “…were related to…”

- Discussion, paragraph 6, line 1: change “regulate” to “regulates”

- Discussion, paragraph 6, line 9: change “…they did not see any association…” to “…they observed no association…”

- Discussion, paragraph 7, line 8: change “…whole…” to “…entire…”

- Discussion, paragraph 8, line 3: change “…databases and thus it is possible that compilation of the data eliminated the effect.” to “…databases we suggest that the effect potentially vanished due to variability in study design and data acquisition.”

- Discussion, paragraph 8, lines 4 to 6: change “Also, and as our data suggest, the effect of expression may differently affect tumor subtypes and thus should be analyzed accordingly.” to “We assume that mRNA expression of our candidate genes may differently affect breast tumor subtypes, these findings provide a suitable basis for further validation studies.”

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

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

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

**Declaration of competing interests:**

no competing interests