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Association Between Estrogen-Related Genetic and Microbial Factors in Breast Tissue: Implications for Breast Cancer Risk

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Introduction

- Breast cancer (BC) is the most prevalent cancer, with roughly 90 - 95 percent of cases having an unknown hereditary link[1-6].
- Endogenous estrogens are a large contributor to BC development; thus, estrogen receptor genes are important to study in the context of BC[7-9] and may be used to predict genetic/hereditary BC risk.
- BC tissues are characterized by microbiome dysbiosis[10,11] and the microbiome may modulate tumorigenesis[11].
- The gut estrobolome, gut microbial genes for estrogen metabolism, may also play a role in BC development[12]. Hormone metabolism and tumorigenesis is related to gut microbiome shifts[13].
- It is currently unknown if the breast microbiome also modulates estrogen and how it relates to host gene variants in this pathway. However, the gut estrobolome, representing all microbial genes known to modulate estrogen metabolism, may be key in this connection.
- Despite the reference, there is limited research and specificity of the estrobolome.

This research aims to establish connections between genes in the pathway of estrogen metabolism and the breast microbiome in BC.

Hypothesis

Hypothesis 1: The genetic variation in estrogen receptors are associated with variations in local breast tissue estrogen modulating microbial relative abundance.
Hypothesis 2: The expression of the variants identified from the literature is associated with variations in local breast tissue estrogen modulating microbial relative abundance.

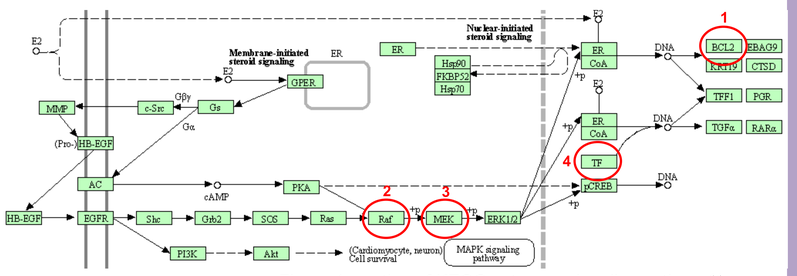

Figure 1: section of KEGG estrogen signaling pathway[14]

Investigated estrogen-pathway genes variants:

1. *Bcl-2-like protein 10 (BCL2L10)*: POS52112665
2. *Raf-1 Proto-Oncogene, Serine/Threonine Kinase (RAF1)*: POS12606048
3. Mitogen-activated protein kinase 10 (*MAPK10*): POS86011333
4. FOSL2 antisense RNA 1 (*FOSL2-AS1*): POS28387760

Scientific Approach

1. Cohort (n = 60): 15 Healthy (H), 15 Pre-diagnostic (PD), 15 Adjacent normal (AN), 15 Tumor (T)
2. Alternate allele frequency calculation from UC Davis Bioinformatics Core
3. Select variants with quality score > 55
4. Remove modifier and low impact variants (impact to protein shape and function)
5. Select variants with differing frequency (P<0.2) between PD/AN/T and H tissues
6. Associate variants with differentially abundant microbial amplicon sequence variants (ASVs) using MaAsLin2 (variant frequency & genotype with ASV)
7. Associate variants with alpha (Chao1 and Shannon index) and beta (PC1 and PC2) diversity metrics
8. Primer validation with PCR and electrophoresis
9. qPCR for primer efficiency (standard curve) and differential gene expression per gene (quantitative Ct)

Results

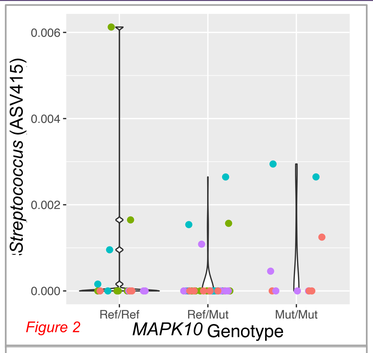

Figure 2

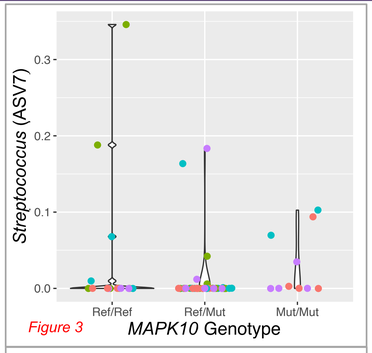

Figure 3

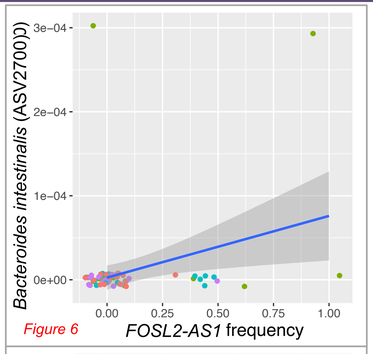

Figure 6

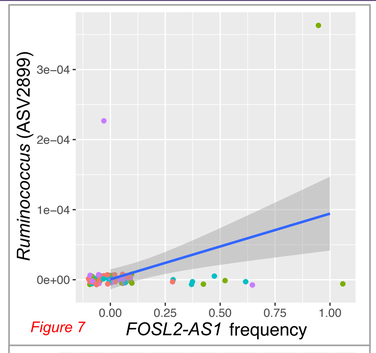

Figure 7

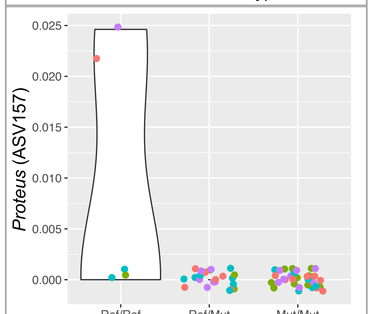

Figure 4

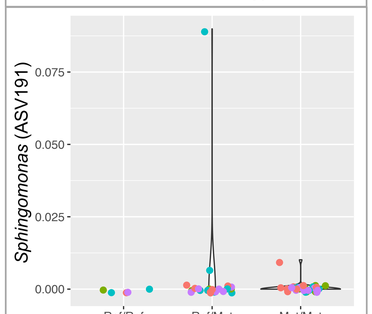

Figure 5

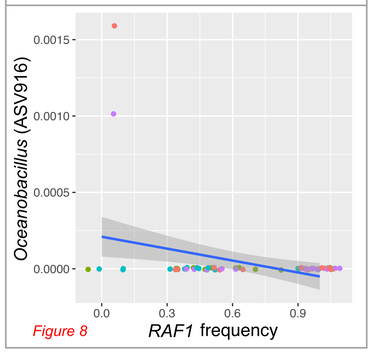

Figure 8

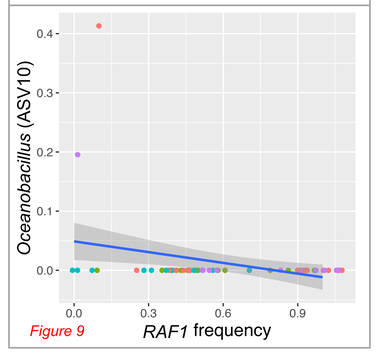

Figure 9

Figure 2 & 3: *Significant (q < 0.25) associations between MAPK10 genotype Mut/Mut with bacterial ASV415 (q < 0.001, p < 0.001) and ASV7 (q < .001, p < 0.001).* ***Figure 4 & 5:*** *Significant (q < 0.25) associations between BCL2L10 genotype Ref/Mut and Mut/Mut with bacterial ASV157 (Ref/Mut q=0.041, p < 0.001; Mut/Mut q = 0.073, p < .001) and genotype Ref/Mut with ASV191 (q = 0.077, p < 0.001).*

Figure 6 & 7: *Significant (p < 0.05) positive associations between FOSL2-AS1 alternate allele frequency with bacterial ASV2700 (q = 0.806, p = 0.016) and ASV2899 (q = 0.806, p = 0.006).*
Figure 8 & 9: *Significant (p < 0.05) negative associations between RAF1 alternate allele frequency with bacterial ASV916 (q = 0.806, p = 0.010) and ASV10 (q = 0.806, p = 0.011).*

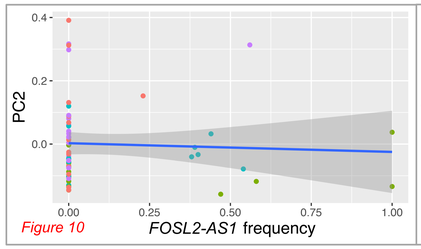

Figure 10

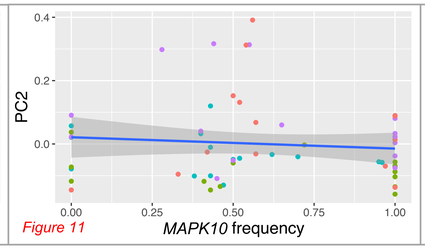

Figure 11

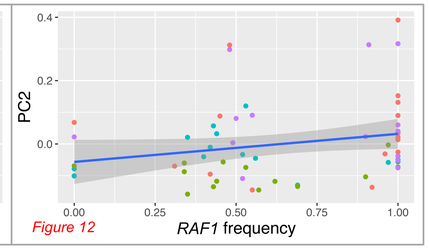

Figure 12

Figure 10 & 11: *Significant (q < 0.25) negative associations between FOSL2-AS1and MAPK10 alternate allele frequency with PC2 (FOSL2-AS1 q = 0.204, p = 0.059; MAPK10 q = 0.204, p = 0.055).*
Figure 12: *Significant (q < 0.25) positive associations between RAF1 alternate allele frequency with PC2 (q = 0.204, p = 0.031).*

Conclusions and Future Directions

- Based on q < 0.25, we found significant associations between MAPK10 genotype Mut/Mut with *Streptococcus*; BCL2L10 genotype Ref/Mut and Mut/Mut with bacterial *Proteus*; BCL2L10 genotype genotype Ref/Mut with *Sphingomonas*. Based on p < 0.05, we found positive associations between FOSL2-AS1 with *Bacteroides intestinalis* and *Ruminococcus*, and negative association between RAF1 and *Oceanobacillus*.
- Based on q < 0.25, we found significant associations between FOSL2-AS1, MAPK10, and RAF1 with PC2 beta diversity metric. FOSL2-AS1 and MAPK10 are negatively associated with PC2 and RAF1 is positively associated with PC2.
- The associations between certain estrogen pathway genes with certain bacteria suggests that the microbiome may interact with the host genome to instigate or prevent mutations in estrogen related genes. Conversely, the host genome may have modulating roles in microbiome diversity and abundances as well. Further causal investigation is needed to confirm directionality between genome and microbiome.
- In literature, the bacteria we observed as significant are also related to other cancers. *Oceanobacillus* is enriched in gastric cancer, *Sphingomonas* is enriched in thymic epithelial tumors, *Streptococcus* is enriched in colorectal cancer, *Bacteroides intestinalis* is enriched in advanced melanoma, *Ruminococcus* is associated with prostate cancer, and *Proteus* is seen to inhibit general cancer growth[15-20]. Further research is required to determine the relationship of these microbes to specific diseases or general tumorigenesis and the mechanism in which they instigate disease.
- The next step in this research involves a 18S qPCR as the housekeeping gene for normalization and finishing qPCR for all selected genes to compare gene expression between tissue types per gene.

Acknowledgements

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