Student: Lucy de Jong

Title: Human amylase gene copy number variation and breast cancer development.

Supervisor(s): Dr Logan Walker

Sponsor: Elaine Jensen and the late Janet Collerton, Cancer Society Oxford and Kaiapoi Groups

Introduction:

The enzyme amylase is produced by the salivary glands and the pancreas, and its function is to break down starch into simpler sugar molecules, like glucose and maltose. The gene that codes for salivary amylase is called *AMY1*, and the number of copies of this gene ranges from 2 to ~17 copies between different people. A study in 2014 by Falchi *et al.* published in the journal *Nature Genetics* revealed an inverse relationship between copy number of amylase and Body Mass Index (BMI). This means that individuals with low copy number of *AMY1*, are more likely to have high BMI and develop obesity. The mechanism of action for this relationship remains unknown. The BMI of the New Zealand population has been increasing over the last few years, and this puts our population at risk of diseases such as breast cancer. Furthermore, high BMI is associated with the risk of estrogen receptor (ER) negative and/or progesterone receptor (PR) negative breast tumours which have poor prognosis. ER and PR status may be influenced by changes in expression of various genes, however the molecular link between the expression of these receptors and BMI is unclear.

Aims:

1. To establish a reliable method of measuring *AMY1* copy number.
2. To identify gene(s) whose expression changes are associated with breast tumour subtype.
3. To measure AMY1 copy number of breast cancer patients and compare this with tumour subtype.

Methods:

To measure copy number of *AMY1* I used two quantitative Polymerase Chain Reaction (qPCR) techniques with SYBR Green and TaqMan chemistry. DNA from two cell lines with known *AMY1* copy number were used as references to assess DNA from three further cell lines in which the copy number was unknown.

To explore gene expression changes in breast tumours from obese patients, I bioinformatically assessed data from a previously published study (Creighton et al. Breast Cancer Res Treat 2011) using the online data repository NextBio (http://www.nextbio.com/b/nextbio.nb). The top five genes that are differentially expressed in tumours from obese breast cancer patients were identified through this analysis. I then assessed the expression of these genes in a total of 401 breast tumours in relation to ER and PR status using data from The Cancer Genome Atlas and the online tool cBioPortal (http://www.cbioportal.org/).

Results:

I found that the SYBR Green technique was more accurate for quantifying *AMY1* copy number so used this approach to DNA from 25 breast cancer patients. Of these patients, one carried 4 copies of *AMY1*, three carried 5 copies, five carried 6 copies, eight carried 7 copies, two carried 8 copies, five carried 9 copies, and one carried 11 copies. There was no clear association between *AMY1* copy number and tumour grade, ER or PR status. A clearer picture may emerge with a larger sample size. Of the five genes tested for association, *GRIA2* and *AGTR1* were shown to be downregulated in obese patients compared with healthy weight patients, and is associated with ER- ($P=1x10^{-11}$ for *GRIA2*, $P=2x10^{-33}$ for *AGTR1* and PR- ($P=1x10^{-8}$

for *GRIA2*, P=1x10^{-21} for *AGTR1*) tumours. These results mean that a breast tumour from an obese patient is more likely to have lower *GRIA2* and *AGTR1* gene expression compared to a patient with normal weight, and is more likely to be ER- and/or PR-, which makes the cancer more difficult to treat.

Conclusion:

This research project has successfully developed a reliable method of measuring gene copy number using qPCR, and subsequently used it to measure *AMY1* copy number in 25 breast tumour samples. Using data from previous studies, we were able to predict that those patients with lower copy number are more likely to be overweight, and therefore more likely to have ER- and/or PR- breast tumours with reduced expression of *GRIA2* and *AGTR1*. This project opens up new questions to explore and may be the beginning of a much more comprehensive investigation into the role of genes, such as *GRIA2* and *AGTR1,* in breast tumours from women with low *AMY1* copy number and/or high BMI.