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rs2981582 is associated with *FGFR2* expression in normal breast

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Breast cancer is the most common cancer in women and is associated with genetic variants in critical genes and protein pathways. Recently, several association studies have identified fibroblast growth factor receptor 2 (*FGFR2*), a gene involved in mammary gland development, as a novel gene for breast cancer risk and rs2981582, or its proxy SNP, as a candidate SNP in various populations[1–12]. Further studies suggested that two SNPs (rs2981578 and rs7895676) in strong LD with rs2981582 could influence enhancer activity by altering the Runt-related transcription factor 2 (Runx2) and/or CCAAT/enhancer binding protein beta (C/EBP β) binding affinity[13]. Accordingly, rs2981582 was observed to be associated with *FGFR2* expression in breast cancer tissues[13]. However, it is unknown whether this correlation between genotype and mRNA levels is tissue specific and whether it is present also in normal breast tissue. Indeed, it is not unusual to observe differential gene expression in the same pathway between normal and carcinoma cells[14–16].

To investigate this question, we retrieved 81 normal breast (4 Caucasian American, 8 African American, and 69 of unknown ethnicity) tissues from the University of Chicago Tissue Core Facility. RNA and DNA were extracted by RNeasy Lipid Tissue and QIAamp DNA Mini Kit (Qiagen, USA), respectively. cDNA was synthesized by High Capacity Reverse Transcription Kit (Applied biosystems, USA). *FGFR2* expression was quantified by real time PCR with *Power SYBR Green* (Applied biosystems) and primer pair 5'-CATGCGGTGGCTGAAAAAC-3' and 5'-AGGCGATCGCTCCACAACAT-3' and normalized to β -actin levels by using previously published primers[17]. The genotype of rs2981582 was determined by Taqman genotyping assay C_2917302_10 (Applied biosystems) according to the manufacturer's protocol. Three continuous integers were assigned to the three different genotypes (AA, AG, and GG) and linear regression was performed between genotype and *FGFR2* mRNA levels in SPSS 15.0 (SPSS Inc., USA).

As shown in figure 1, rs2981582 was significantly correlated with *FGFR2* expression level ($r=0.221$, $P=0.049$), consistent with the idea that this or other SNPs in strong LD with it influences *FGFR2* expression also in normal breast. Although the statistical significance of this association is marginal, it is important to note that in this case the minor allele A was correlated with lower expression levels, which is the opposite of what was reported for tumor tissues[13]. Therefore, our results suggest a differential regulation of *FGFR2* between tumor and normal breast tissue, though the reason for the difference is unclear. One possibility is that it might reflect differences in regulatory pathways in these two tissues. It might also be argued that our finding could result from the mixture of ethnic populations with different genetic

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backgrounds. However, considering the consistence of all association studies in that A of rs2981582 is the risk allele in most human populations [1–12], which suggests a common mechanism of this disease, the possibility of bias by population stratification is rather low. Additionally, there are likely to be differences in the composition of the normal and tumor breast tissues. Despite the fact that both types of samples are from human breast, normal breast is usually characterized by a lower portion of epithelial cells and more adipose cells compared to tumor tissue. Although adipose cells usually yield considerably less RNA than epithelium, they might still influence the association result. Therefore, the opposite pattern of association between SNP allele and mRNA levels may be due to differences in the regulation of gene expression in epithelial compared to adipose cells. More advanced technologies, such as laser capture microdissection (LCM) before tissue collection and RNA extraction, might provide further insight into this issue.

Our result identified a potentially distinct regulation of *FGFR2* expression in normal breast compared to tumor cells. In light of this and the complexity of splicing and tissue specific expression in this gene, we conclude that further studies are necessary to clarify the genetic contribution of variation in the *FGFR2* gene to breast cancer.

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Fig 1.

The relationship between rs2981582 genotype and *FGFR2* expression (Pearson $r = 0.221$ and $P = 0.049$). The data are normalized to β -actin and log-transformed.