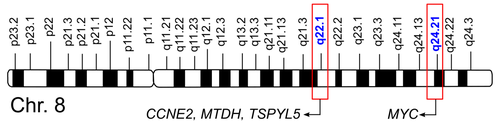
High Risk breast cancer genes at 8q22-24 and their role in over 5000 patients evaluated with the MammaPrint risk of recurrence assay

Sami Diab[1], Matei P. Socoteanu[2], Carlos A. Encarnacion[3], Cynthia R. C. Osborne[4], Carolyn B. Hendricks[5], Kristi McIntyre[6], Vibha Taneja Thomas[7], Rajith Bhaskaran[8], Josien Haan[8], Lorenza Mittempergher[8], Andrea Menicucci[9], William Audeh[9], **Joyce O'Shaughnessy**[4], FLEX Investigators' Group[10]

1. Rocky Mountain Cancer Center-Aurora, Aurora, CO; 2. Texas Oncology-Longview Cancer Center, US Oncology, Longview, TX; 3. Texas Oncology-Waco, US Oncology, Waco, TX; 4. Texas Oncology-Baylor Sammons Cancer Center, US Oncology, Dallas, TX; 5. Maryland Oncology Hematology, Bethesda, MD; 6. Texas Oncology-Presbyterian, US Oncology, Dallas, TX; 7. Texas Oncology-Flower Mound, US Oncology, Flower Mound, TX; 8. Research and Development, Agendia NV, Amsterdam, Netherlands; 9. Medical Affairs, Agendia, Inc., Irvine, CA; 10. FLEX Investigators' Group

BACKGROUND

Previous studies have shown that *CCNE2* expression is higher in patients' cancers resistant to CDK4/6 inhibitors (1). Increased expression of *CCNE2*, *MTDH,* or *TSPYL5*, genes contained within MammaPrint (MP), a 70-gene risk of distant recurrence signature, has also been implicated in breast oncogenesis, poor prognosis, and chemoresistance (2-4). These genes are located on chromosome region 8q22.1, one of the most recurrently amplified regions out of all MP genes in breast cancers (BC) (5). *MYC*, located within close proximity on 8q24.21, is overexpressed in 40% of all BC. Here we examined the expression of *CCNE2*, *MTDH*, and *TSPYL5* in relation to MP risk and the 80-gene molecular subtype signature, BluePrint (BP), and their correlation with *MYC* expression in early stage BC patients.



METHODS

CCNE2, *MTDH*, *TSPYL5,* and *MYC* mRNA expression was measured in 5022 BC samples sent to Agendia (Irvine, CA) for MP and BP testing, which included FFPE microarray full-transcriptome data. Intensities were Lowess normalized and log2 transformed. MP was used to stratify patients into Ultra Low Risk (**UL**), Low Risk (**LR**), High Risk (**HR**), and Ultra High Risk (**UH**). HR and UH are similar to MP High 1 (MP1) and MP High 2 (MP2) reported in the I-SPY2 trial, which have demonstrated the ability for these groups to predict chemo-sensitivity with significant differences in pathological complete response (6). Both MP and BP were used to classify patient samples into Luminal A, Luminal B, HER2, or Basal type. To validate the co-expression of 8q22.1 genes, normalized gene expression and matched copy number (both segmented and GISTIC) data were downloaded for 1036 BC tumor types from TCGA (7-8). Wilcoxon rank sum test was used to assess expression differences.

RESULTS

The expression of *CCNE2*, *MTDH*, and *TSPYL5* was significantly higher in MP HR patients compared to LR patients and significantly higher in UH patients compared to HR patients (**Figure 1**). Additionally, *CCNE2* and *MYC* expression was elevated in LR compared to UL patients. In contrast, there was no difference in *MYC* expression between HR versus LR or UH versus HR (**Figure 1**).

CCNE2, *MTDH*, and *TSPYL5* were the highest expressed in Basal type tumors, 83% of which were UH, followed by Luminal B type tumors while expression of these genes was lowest in Luminal A type tumors (**Figure 2**). *MYC* was significantly higher expressed in Basal type tumors but overall exhibited a smaller magnitude of differential expression across BP subtypes compared to 8q22.1 genes.

The expression of *CCNE2*, *MTDH*, and *TSPYL5* significantly correlated with each other (**Figure 3**). The highest correlations were observed between *MTDH* and *CCNE2* and MTDH and TSPYL5. There was no association between the expression of 8q22.1 genes and *MYC* in any MP risk subgroup (**Figure 3**).

In 84% (866) of the 1036 BC TCGA samples, the 8q22.1 genes were detected on the same DNA segment, of which 57% (496) were determined to have an "amplification" and 43% (370) with "no amplification". The 8q22.1 genes in samples with an amplified segment were significantly higher expressed in comparison to samples with no amplification of 8q22.1 genes (**Figure 4**).

CCNE2, _MTDH_, and _TSPYL5_, genes contained within the MammaPrint assay, have similar expression patterns and when overexpressed represent a unique subgroup of high risk breast tumors.

These data may be clinically relevant for stratifying patients in ongoing clinical trials evaluating response and resistance to targeted therapies in early stage BC.

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Contact: FLEX@agendia.com

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TABLES and FIGURES

Figure 1. 8q22-24 gene expression in MP risk groups

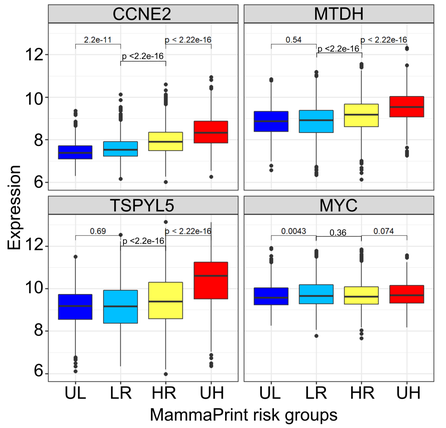


Figure 2. 8q22-24 gene expression among BP subtypes

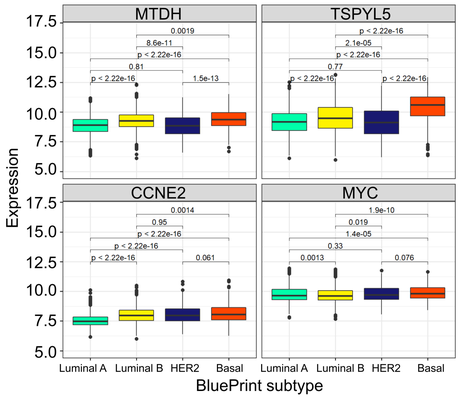
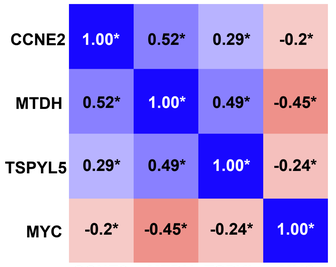


Figure 3. Gene expression correlation



Significant correlations are indicated by (*).

Figure 4. 8q22-24 copy number amplification and gene expression in TCGA samples