

# Unveiling Survival Signatures: Assessing Contributions of Transcriptional Outliers on Breast Cancer Survival



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5, 6

## Introduction | Abstract

Despite their potential as prognostic biomarkers and therapeutic targets, outlier genes with unique expression patterns in cancer have not been comprehensively characterized in the context of patient survival. The integration of outlier gene signatures with clinical measurements enables more nuanced profiling of tumor-specific heterogeneity. Leveraging TCGA RNA-Seq and METABRIC microarray data, we constructed univariate and multivariate Cox proportional hazards models to investigate the impact of outlier genes on patient survival. Our findings shed light on the role of outlier genes as proxies for patient survival, demonstrating their relevance in characterizing breast cancer survival patterns. Exploration of clinical variables in association with outlier genes and their molecular mechanisms can unveil avenues for therapeutic interventions targeting tumor-specific transcriptional variations. Future endeavors include an extension of this methodology across cancer types and advancing pattern identification in pan-cancer outlier contributions for clinical and molecular data.

## Methods | Schematic Workflow

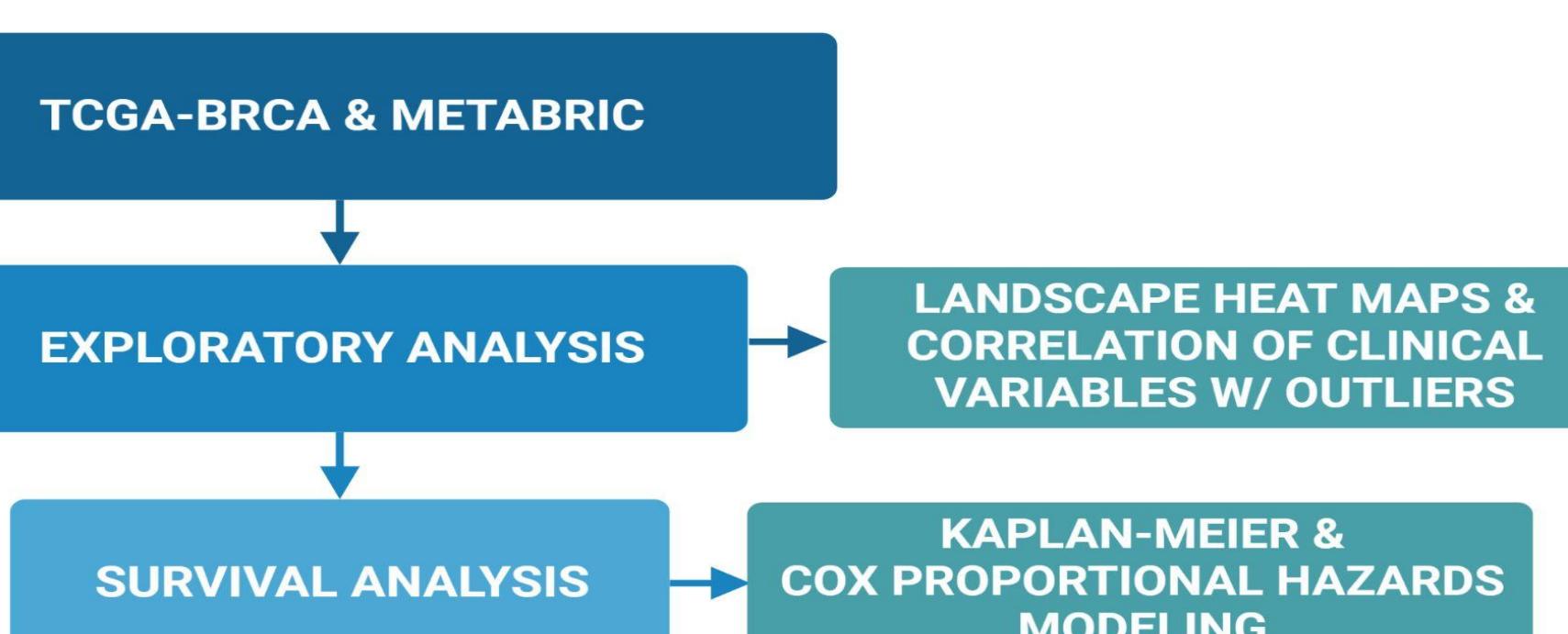
### Methods / Schematic Workflow

#### TCGA-BRCA Dataset:

- RNA-seq data from  $n = 1085$  breast cancer patients, with 1187 identified outlier genes
- RNA abundance levels represented as Fragments Per Kilobase Million (FPKM)

#### METABRIC Dataset:

- Microarray data from  $n = 1991$  breast cancer patients, with 4782 identified outlier genes
- RNA abundance levels represented as probe intensity values



## References

- Cancer Genome Atlas Research Network; Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, Ellrott K, Shmulevich I, Sander C, Stuart JM. The Cancer Genome Atlas Pan-Cancer analysis project. *Nat Genet*. 2013 Oct;45(10):1113-20. doi: 10.1038/ng.2764. PMID: 24071849; PMCID: PMC3919969.
- Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, Speed D, Lynch AG, Samarajiwa S, Yuan Y, Gräf S, Ha G, Haffari G, Bashashati A, Russell R, McKinney S; METABRIC Group; Langerod A, Green A, Provenzano E, Wishart G, Pinder S, Watson P, Markowitz F, Murphy L, Ellis I, Purushotham A, Børresen-Dale AL, Brenton JD, Tavaré S, Caldas C, Aparicio S. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature*. 2012 Apr 18;486(7403):346-52. doi: 10.1038/nature10983. PMID: 22522925; PMCID: PMC3440846.

## TCGA-BRCA & METABRIC Outlier Landscape

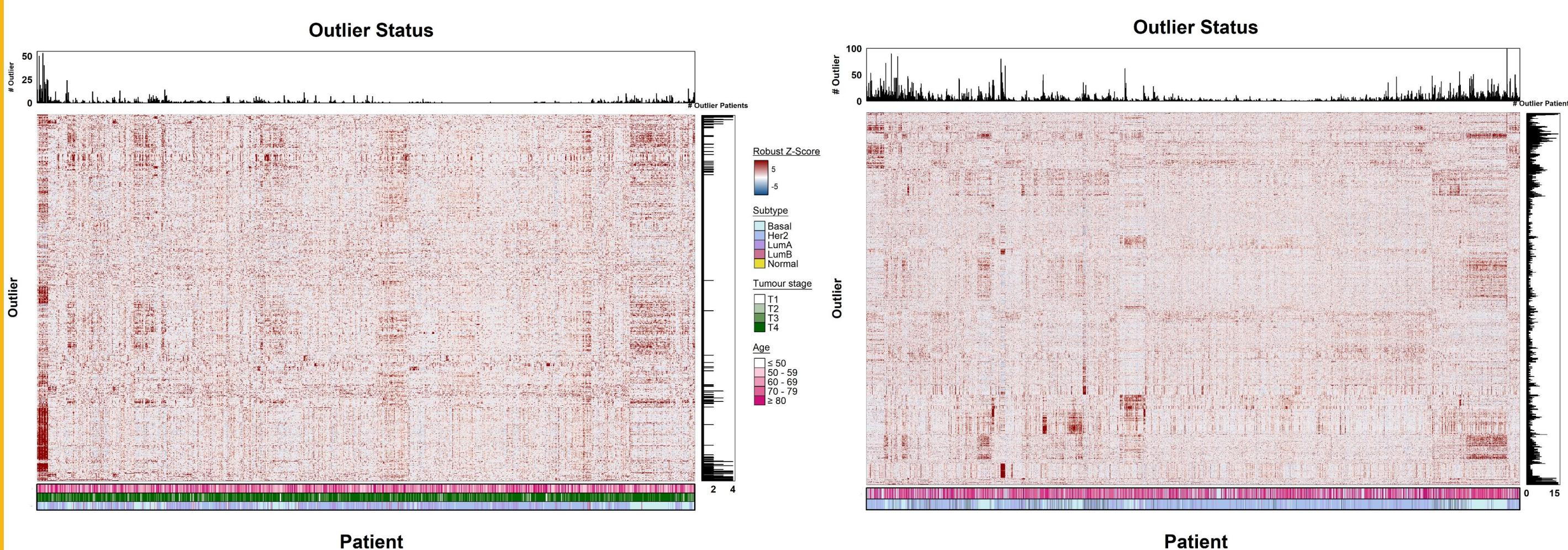


FIGURE 1. TCGA-BRCA Outlier Heat Map. Clustering of outliers by gene and patient

## Survival Analysis: Outlier Gene Count and Molecular Subtype

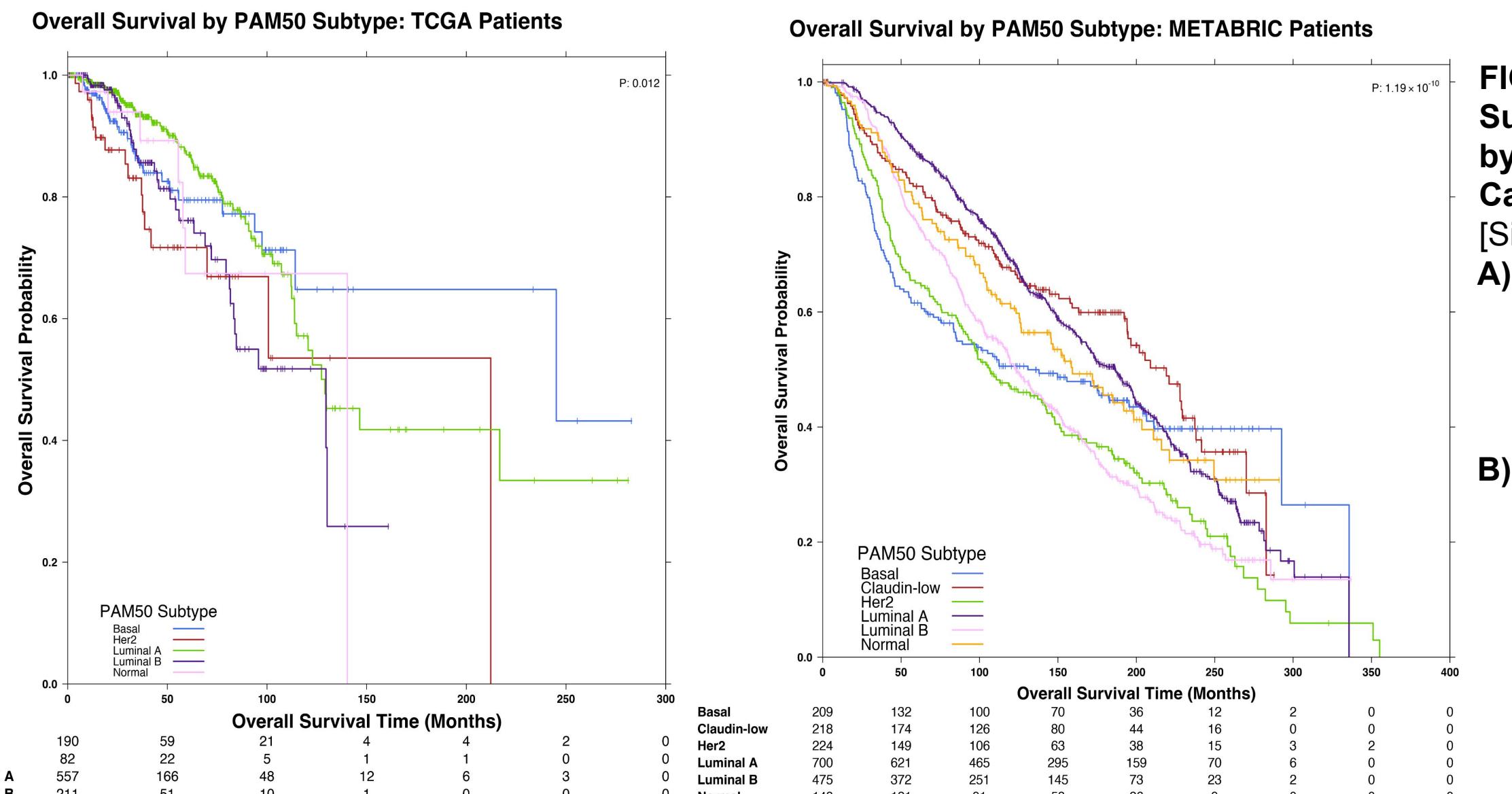


FIGURE 5. Kaplan-Meier Survival Curves Stratified by PAM50 Molecular Cancer Subtype [Significance Level:  $\alpha = 0.05$ ]

- A) TCGA-BRCA: Kaplan-Meier survival curves and log-rank test comparison for subtype strata  
B) METABRIC: Kaplan-Meier survival curves and log-rank test comparison for subtype strata

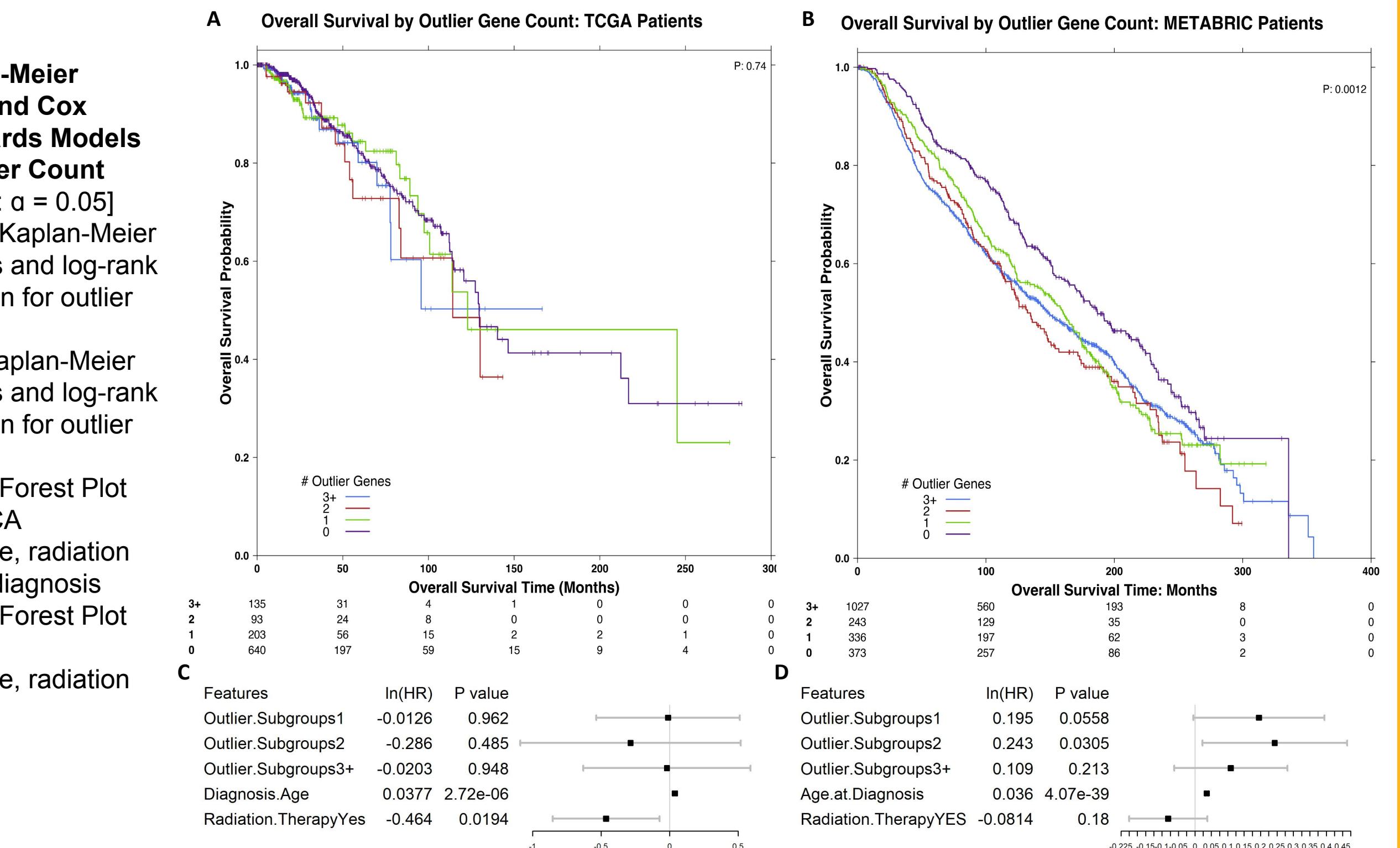


FIGURE 6. Kaplan-Meier Survival Curves and Cox Proportional Hazards Models Stratified by Outlier Count [Significance Level:  $\alpha = 0.05$ ]

- A) TCGA-BRCA: Kaplan-Meier survival curves and log-rank test comparison for outlier strata  
B) METABRIC: Kaplan-Meier survival curves and log-rank test comparison for outlier strata  
C) CoxPH Model Forest Plot for TCGA-BRCA  
Covariates: age, radiation therapy, prior diagnosis  
D) CoxPH Model Forest Plot for METABRIC  
Covariates: age, radiation therapy

## Conclusion

- ER and PR status became increasingly negative with increasing outlier gene count.
- HER2 status became increasingly positive with increasing outlier gene count.
- METABRIC displayed reduced survival probability with increasing outlier gene count early post-diagnosis. TCGA had limited follow-up, requiring further investigation.
- Both diagnosis age and radiation therapy showed significant associations with survival in METABRIC covariate-adjusted Cox models, while diagnosis age was significantly associated in TCGA.
- METABRIC revealed significant outlier genes post-FDR correction. Limited significant outliers in TCGA; longer follow-up may clarify.
- Future endeavors include exploration of outlier genes' survival patterns in other cancer types for pan-cancer insight generation, as well as replication of survival analyses in cohorts with extended patient follow-up.

## Results | Discussion

## Clinical Variable Correlation

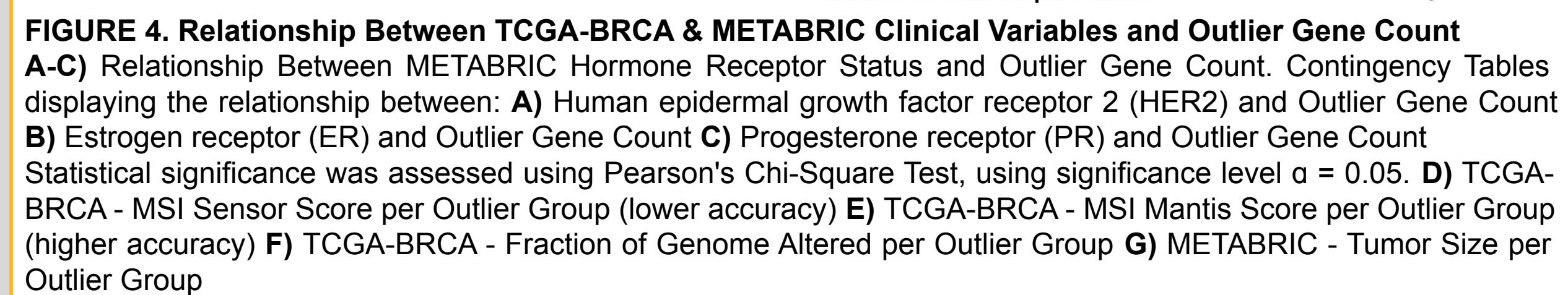
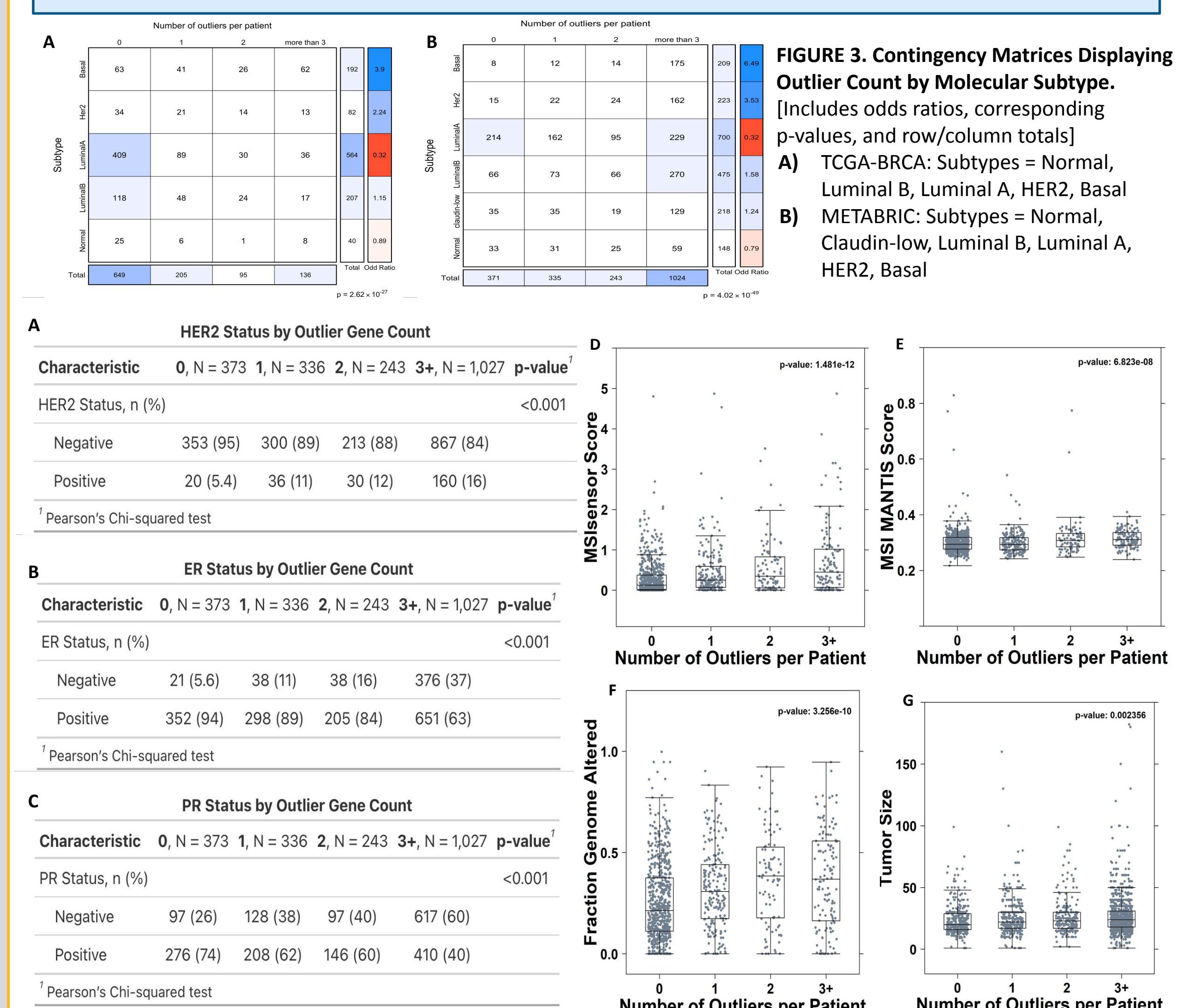


FIGURE 4. Relationship Between TCGA-BRCA & METABRIC Clinical Variables and Outlier Gene Count  
A-C) Relationship between METABRIC Hormone Receptor Status and Outlier Gene Count. Contingency Tables displaying the relationship between: A) Human epidermal growth factor receptor 2 (HER2) and Outlier Gene Count  
B) Estrogen receptor (ER) and Outlier Gene Count C) Progesterone receptor (PR) and Outlier Gene Count  
Statistical significance was assessed using Pearson's Chi-Squared Test, using significance level  $\alpha = 0.05$ . D) TCGA-BRCA - MSI Sensor Score per Outlier Group (lower accuracy) E) TCGA-BRCA - MSI Mantis Score per Outlier Group (higher accuracy) F) TCGA-BRCA - Fraction of Genome Altered per Outlier Group G) METABRIC - Tumor Size per Outlier Group

## Survival Analysis: Outlier Gene Contributions

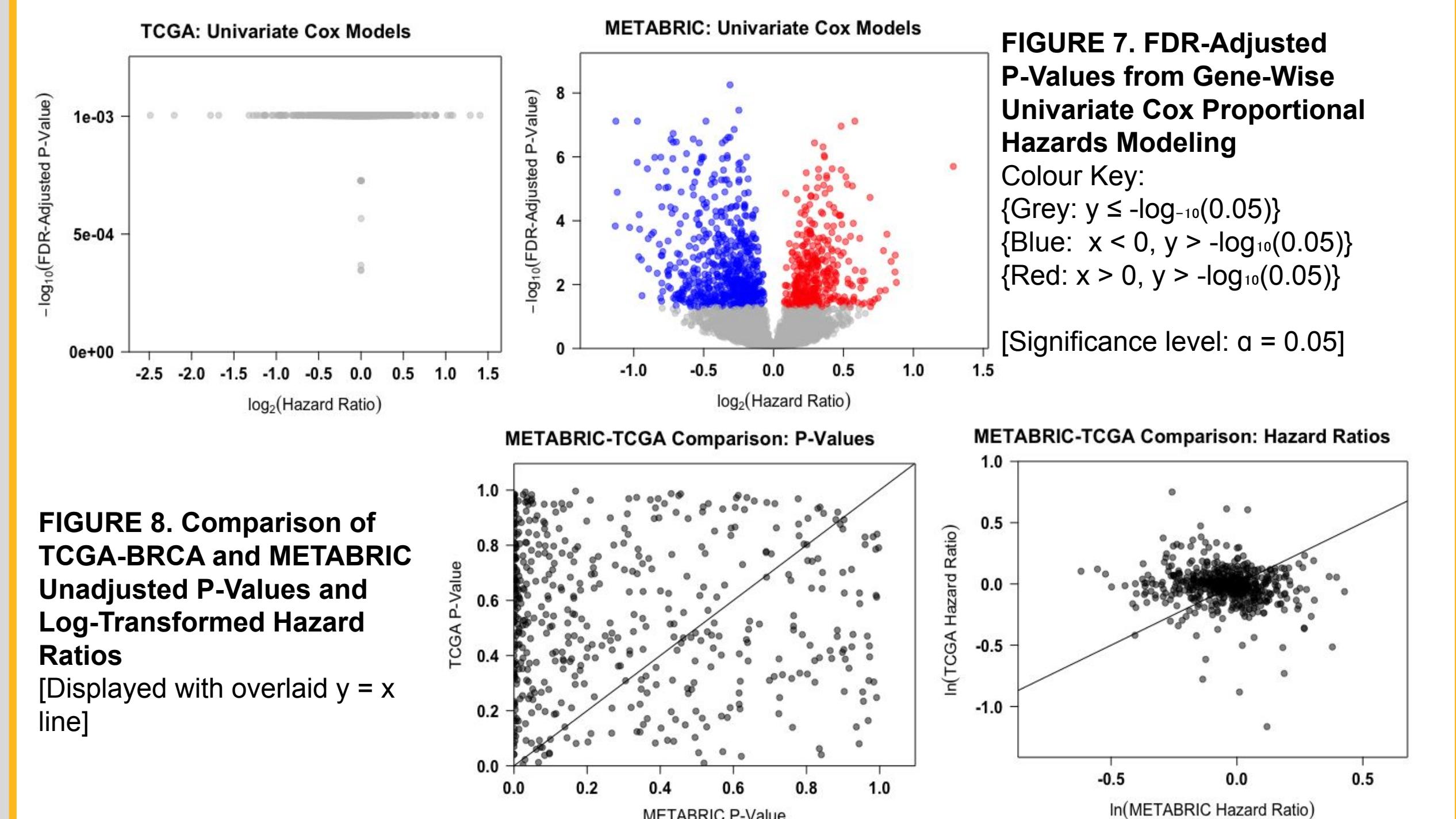


FIGURE 8. Comparison of TCGA-BRCA and METABRIC Unadjusted P-Values and Log-Transformed Hazard Ratios  
[Displayed with overlaid  $y = x$  line]

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