# Comparison of the mutational landscape of breast cancer during pregnancy and non-pregnant controls

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## Background

Breast cancer during pregnancy (BCP) is a rare coexistence and is associated with contradicting results about its biology and prognosis[1,2]. Little is known about the impact of pregnancy on breast cancer biology at the genomic level. Based mainly on classical immunohistochemistry and mutational analysis in one small dataset[3,4] it is believed that BCP during pregnancy is biologically not different from breast cancer diagnosed outside pregnancy.

**The aim of the study is to compare the pattern of somatic mutations between pregnant and non-pregnant patients with breast cancer using a dataset of pregnant patients enrolled in BCP study and non-pregnant controls obtained from TCGA database.**

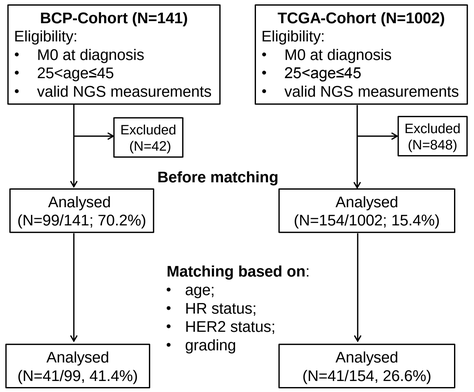
## Materials and Methods

The BCP study (GBG 29; BIG 03-02) is a multicenter observational study for breast cancer during pregnancy. Formalin-fixed paraffin embedded (FFPE) core biopsies taken before therapy were retrospectively analysed for somatic mutations using an Ion Torrent: Proton/PGM sequencing platform (Figure 1). The samples were assayed on a custom designed Breast Cancer Panel (BCPv2)[5] that comprises 236 amplicons split into two primer pools and covers hotspot regions of 138 exons of 25 genes (Table 1). Raw data analyses were performed using the Ion Torrent Suite Software (version 4.4). Only non-synonymous mutations that have not been reported as being of germline origin were processed further. All statistical tests were by default 2-sided, significance level was set to $\alpha$=0.05.

### Table 1. Gene panel

| | BCPv2 Panel | |
|---|---|---|
| **Genes** | **Exons** | **Amplicons** |
| **GATA3** | 4-6 | 6 |
| **PTEN** | 1-9 | 19 |
| **FGFR2** | 3,7,12 | 3 |
| **CCND1** | 1,3,4 | 3 |
| **ATM** | 37 | 2 |
| **KRAS** | 2-4 | 5 |
| **MDM2** | 4,7,11 | 3 |
| **RB1** | 2,3,8,13,14,16-18,20-22 | 12 |
| **AKT1** | 3 | 1 |
| **CBFB** | 3-5 | 3 |
| **CTCF** | 3,4 | 5 |
| **CDH1** | 2-16 | 29 |
| **TP53** | 3-10 | 15 |
| **MAP2K4** | 3-9 | 9 |
| **ERBB2** | 7,8,14,17,19-21 | 9 |
| **RUNX1** | 5-9 | 10 |
| **PIK3CA** | 2,5,8,10,14,21 | 11 |
| **MAP3K1** | 2-20 | 43 |
| **PIK3R1** | 2-16 | 25 |
| **ESR1** | 1,4,8 | 3 |
| **EGFR** | 18-21 | 8 |
| **BRAF** | 11,15 | 3 |
| **MYC** | 2,3 | 3 |
| **CDKN2A** | 1,2 | 4 |
| **MED12** | 2 | 2 |
| **25 Genes** | **138 Exons** | **236 Amplicons** |

### Figure 1. Consort statement



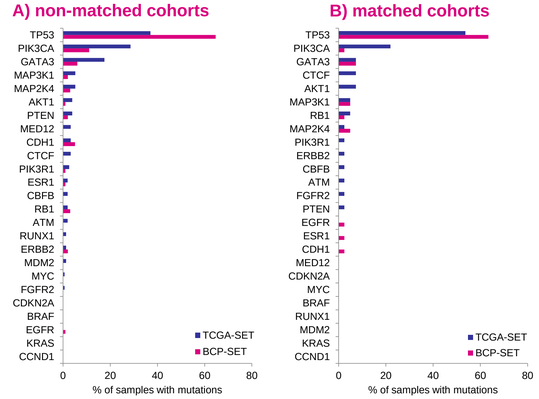

## Results

### Table 2. Clinical characteristics in BCP vs. non-pregnant controls

| | | non-matched | | matched | |
|---|---|---|---|---|---|
| **Parameter** | **Category** | **BCP-cohort** | **TCGA-cohort** | **BCP-cohort** | **TCGA-cohort** |
| **Age, years** | median | 34 | 40 | 37 | 38 |
| | min-max | 26-43 | 26-45 | 28-43 | 26-45 |
| **Tumor size** | T1-2 | 82 (82.8%) | 130 (85.0%) | 37 (90.2%) | 34 (82.9%) |
| | T3-4 | 17 (17.2%) | 23 (15.0%) | 4 (9.8%) | 7 (17.1%) |
| **Nodal status** | negative | 48 (49.5%) | 61 (39.6%) | 23 (57.5%) | 14 (34.1%) |
| | positive | 49 (50.5%) | 93 (60.4%) | 17 (42.5%) | 27 (65.9%) |
| **Grading*** | G1-2 | 30 (30.3%) | 52 (49.5%) | 12 (29.3%) | |
| | G3 | 69 (69.7%) | 53 (50.5%) | 29 (70.7%) | |
| **HR*** | positive | 43 (43.4%) | 104 (72.2%) | 21 (51.2%) | |
| | negative | 56 (56.6%) | 40 (27.8%) | 20 (48.8%) | |
| **HER2*** | positive | 13 (13.1%) | 24 (16.2%) | 1 (2.4%) | |
| | negative | 86 (86.9%) | 124 (83.8%) | 40 (97.6%) | |

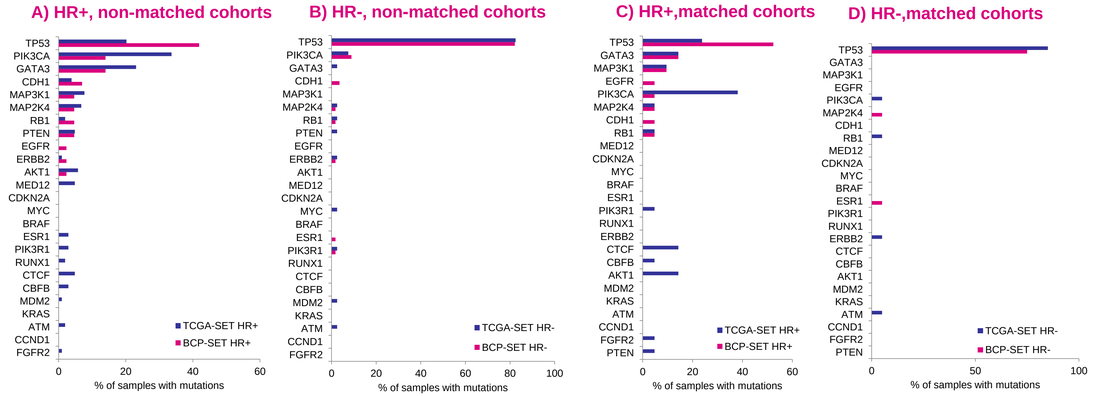
*Numbers in matched BCP-set vs.TCGA-set are identical by definition of the matching

### Figure 2. Mutation patterns overall in BCP vs. non-pregnant controls




- Comparison of the mutational patterns between BCP and non-pregnant controls (TCGA cohort) before any matching showed overall 102 mutations (average 1.03 mutations per samples) in BCP dataset vs. 195 (average 1.27 mutations per sample) in the TCGA. The most frequent somatic mutations for both cohorts were detected in *TP53* (65% vs. 37%), *PIK3CA* (11% vs. 29%) and *GATA3* (6% vs. 18%; Figure 2).
- Exact matching (1:1) in BCP and TCGA cohorts was performed based on age (26-30 vs. 31-35 vs. 36-40 vs. 41-45), HR (positive vs. negative), HER2 (positive vs. negative) and grading (G1/2 vs. G3) and yielded 41 patients from both datasets (Table 2).
- In the matched cohorts BCP patients had significantly less frequently N+ tumors as compared to non-pregnant controls (p=0.046) with no significant difference for *TP53* (p=0.502) and *GATA3* (p=1.000) mutational status whereas *PIK3CA* mutations were detected in only 2.4% of the pregnant patients vs. 22.0% of the non-pregnant controls (p=0.015; Figure 2). Within HR subgroups, overall *TP53* was the most frequently mutated gene with higher mutational rate in HR-negative subgroup (52.4% vs. 75.0% for BCP; 23.8% vs. 85.0% for TCGA control; Figure 3).

### Figure 3. Mutation patterns by HR status in BCP vs. non-pregnant controls




## Conclusions

Overall the mutational landscape does not seem to be different between pregnant patients and no-pregnant controls The imbalances in *PIK3CA* mutational rate after matching might be explained by a remaining bias caused by differences in sensitivity or specificity of methods used to detect mutations or differences in variables not used for matching. Further comparisons using other datasets, looking into gene expression patterns are currently conducted.

## References

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