

Sibylle Loibl¹, Nicole Pfarr², Karsten Weber¹, Tanja Neunhöffer³, Sonia Villegas⁴, Albrecht Stenzinger⁵, Jenny Furlanetto¹, Bahriye Aktas⁶, Jan Budczies⁴, Frederik Marmé⁷, Laura Kahmann⁸, Carsten Denkert⁴, Wilko Weichert²

¹German Breast Group; Neu-Isenburg, Germany, ²Institute of Pathology, Technical University Munich, Germany, ³Departement of Gynaecology, Helios Clinics Wiesbaden, Germany, ⁴Institute of Pathology, Charité Berlin, Berlin, ⁵Institute of Pathology, University Hospital Heidelberg, ⁶University Women's Hospital, Essen, ⁷NCT, Section Translational Gynaecologic Oncology, Heidelberg, ⁸Clinic Landkreis Neumarkt, Parsberg, Germany

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.

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

Genes	BCPv2 Panel 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

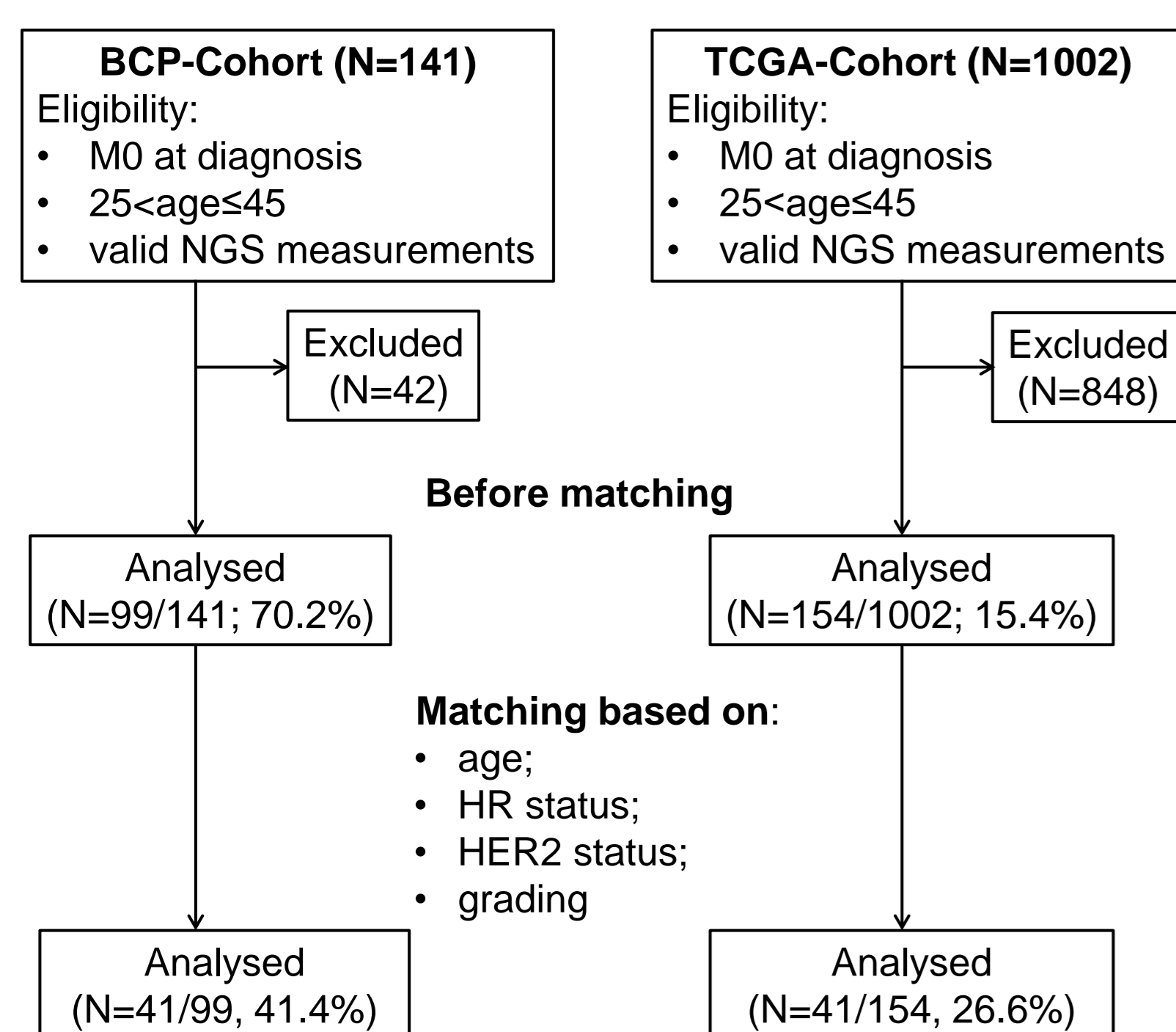


Table 2. Clinical characteristics in BCP vs. non-pregnant controls

Parameter	Category	non-matched		matched	
		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%)	12 (29.3%)
	G3	69 (69.7%)	53 (50.5%)	29 (70.7%)	27 (65.9%)
HR*	positive	43 (43.4%)	104 (72.2%)	21 (51.2%)	21 (51.2%)
	negative	56 (56.6%)	40 (27.8%)	20 (48.8%)	20 (48.8%)
HER2*	positive	13 (13.1%)	24 (16.2%)	1 (2.4%)	1 (2.4%)
	negative	86 (86.9%)	124 (83.8%)	40 (97.6%)	40 (97.6%)

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

Results

- 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 sample) 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%) in the BCP vs. TCGA cohorts, respectively (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 lymph node positive 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 compared to HR-positive patients (75.0% vs. 52.4% for BCP; 85.0% vs. 23.8% for TCGA control; Figure 3).

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

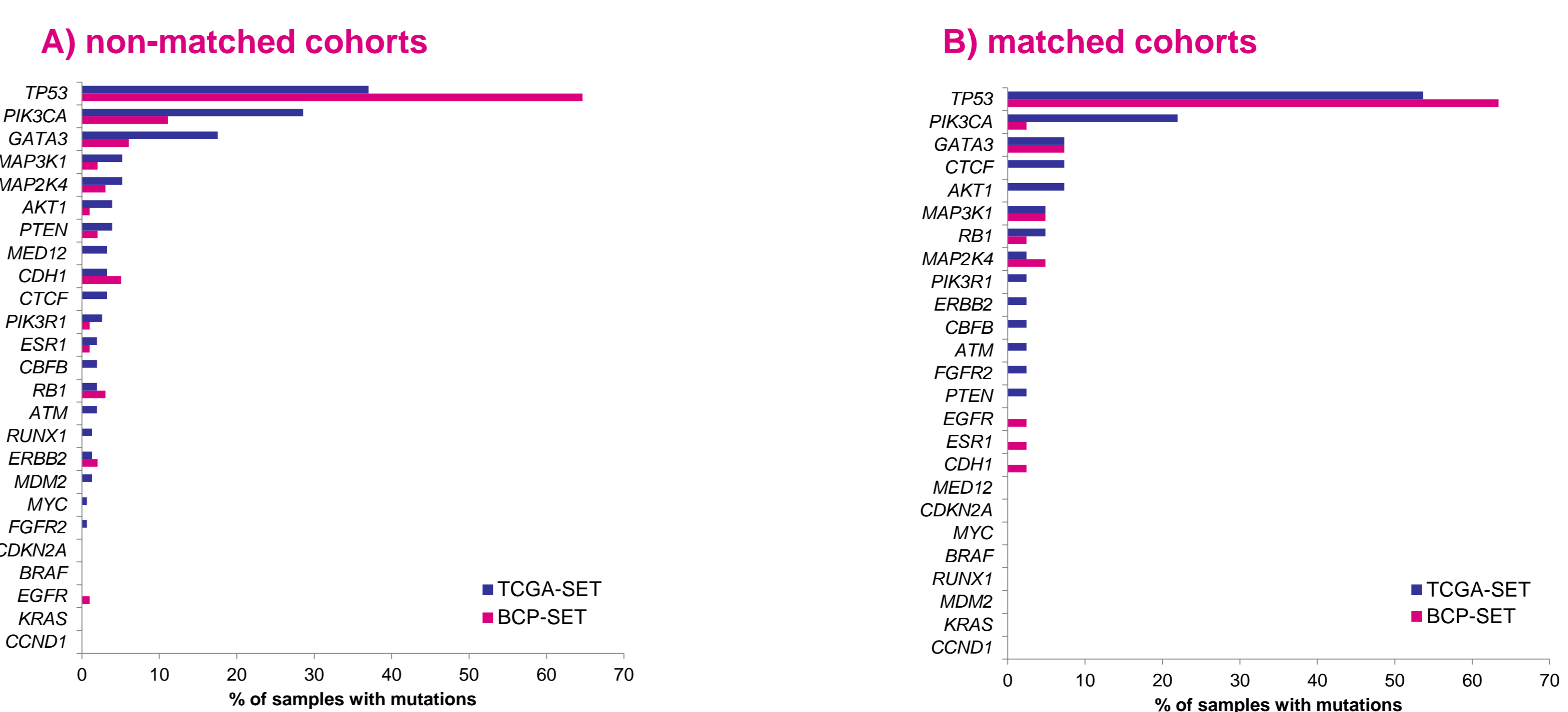
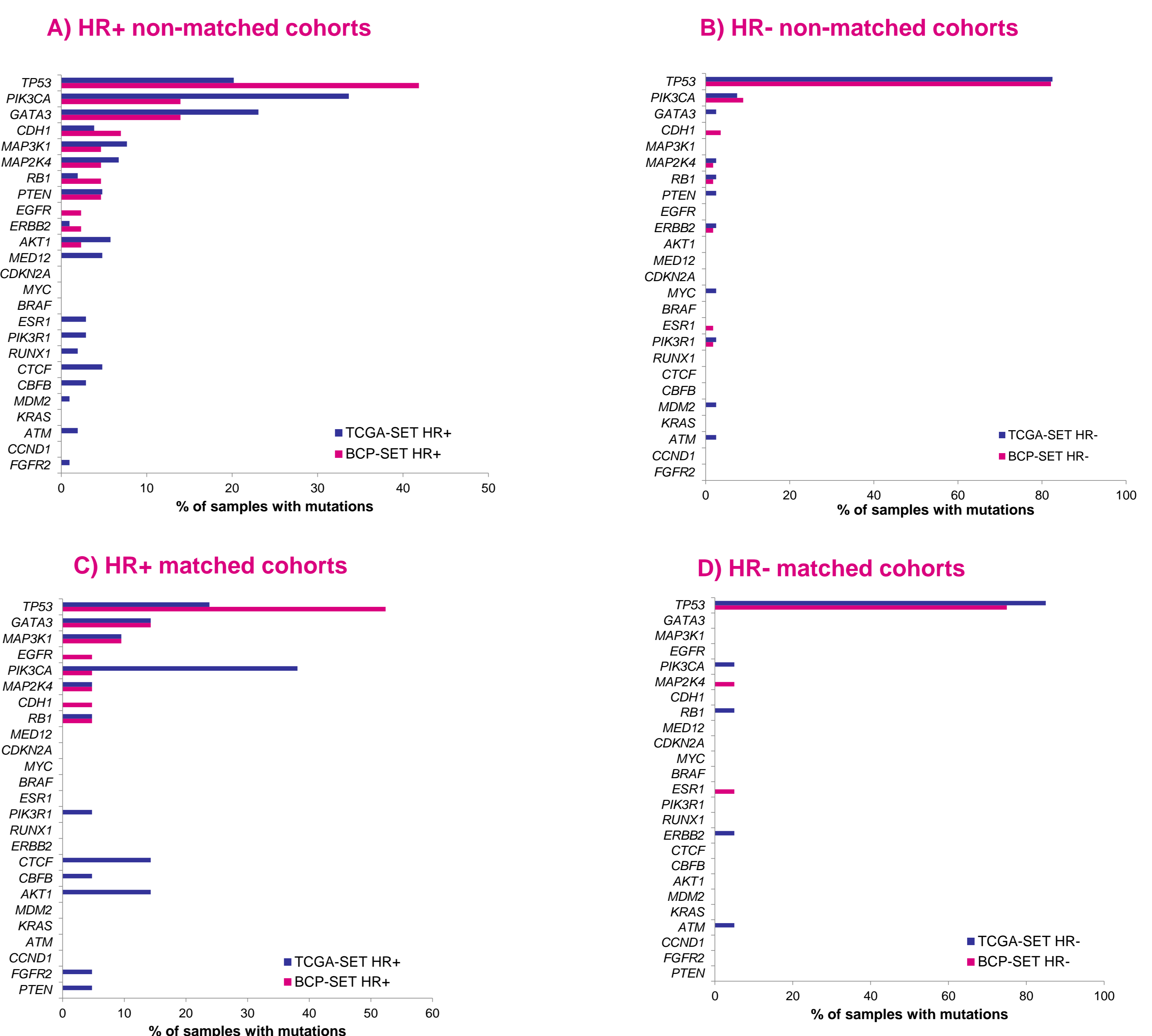


Figure 3. Mutation patterns by HR status in BCP vs. non-pregnant controls



Conclusions

Overall the mutational landscape does not seem to be different between pregnant patients and non-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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