

Objective

Combined Oral Contraceptive Pill (COCP) use has been shown to reduce the risk of ovarian cancer by 30-50% (1). While the mechanism behind this is unclear, in previous literature COCP-users have exhibited overall lower global DNA methylation levels compared to non-users (2). Our objective was to further investigate and quantify methylation changes in cervical cells with COCP use.

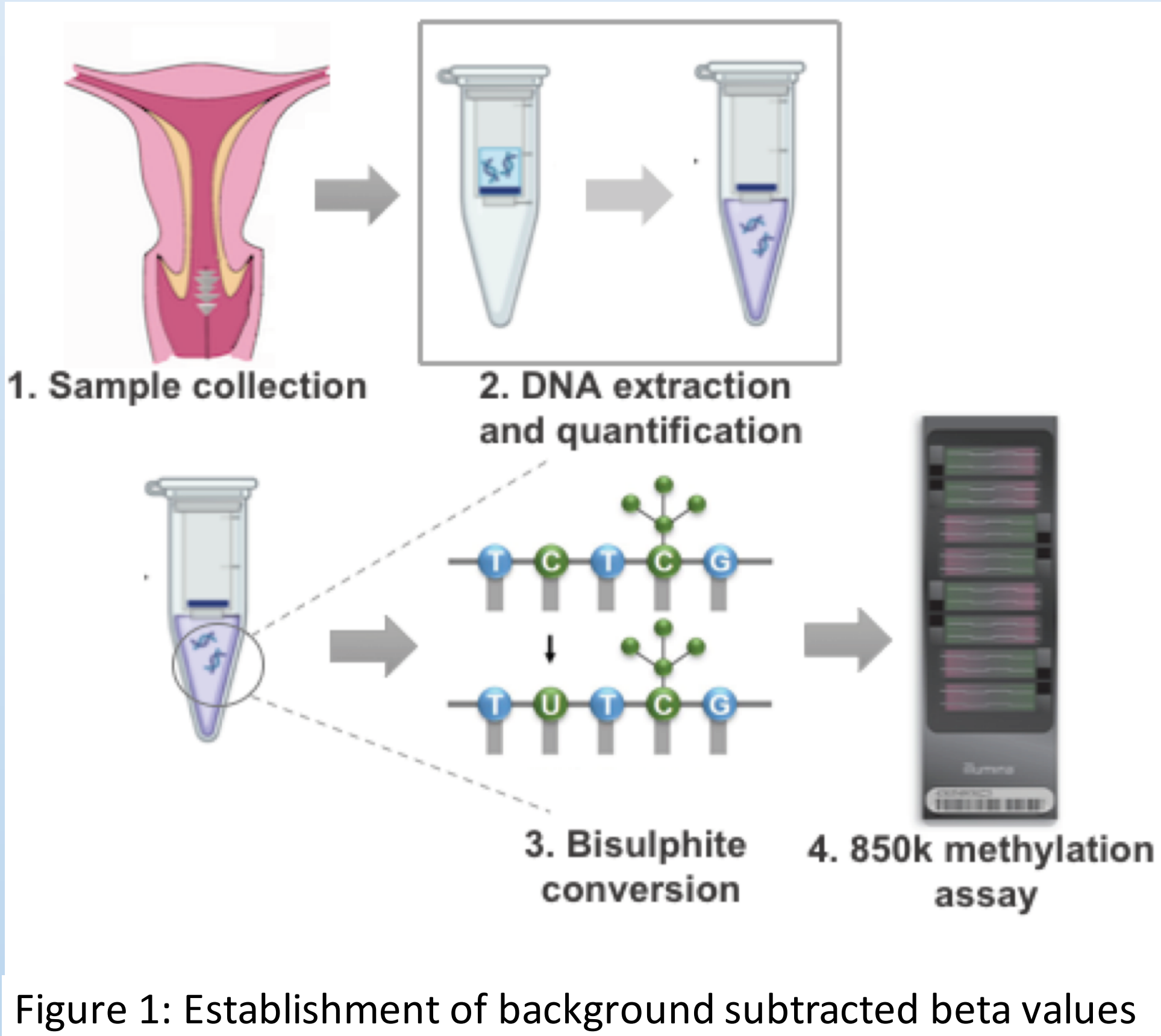


Figure 1: Establishment of background subtracted beta values

Methods

Cervical samples were collected from 153 patients attending colposcopy or gynaecology clinics at two London hospitals between January 2017 and February 2021. DNA was extracted from the samples and quantified, before bisulphite conversion and methylation assays were performed. Background-subtracted beta values (β) were calculated for each sample: a value between 1 and 0, where 1 represented 100% methylation at a CpG site and 0 represented no methylation. 27 COCP users were compared with a population of 126 COCP non-users. The average β value for COCP-users was compared to COCP non-users. β values for 16 previously identified CpG sites were calculated and COCP-users compared to non-users.

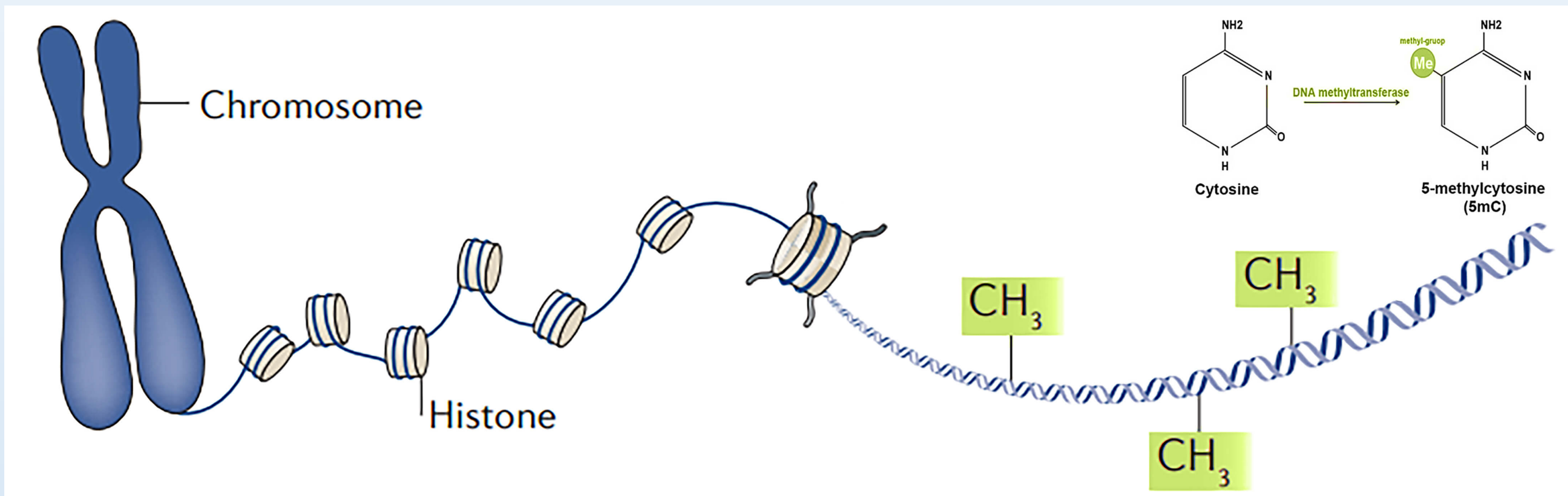


Figure 2: DNA methylation

Results

There was a net gain of methylation in COCP-users when compared to COCP non-users. COCP users had a median β value of 0.544 whilst COCP non-users had a median β value of 0.538. This result was statistically significant ($p < 0.05$).

However, COCP users’ samples were hypomethylated at certain CpG sites across different chromosomes compared to COCP non-users, including genes CASC4, FLT1, KIAA1549L, OR10A3, INPP5A, CUX1, SH3BP4, and MAB21L3 ($p < 0.01$). The difference in methylation levels (β values) in these genes, whilst statistically significant, was minimal (smallest difference in values 0.003, largest difference in values 0.103).

Table 1: Beta values for CpG sites

Target ID	CHR	MAPINFO (bp)	Reference Gene	Median β		Difference in β between COCP-users and non-users (95% CI)	P-values
				COCP-users	COCP non-users		
cg20970380	1	116676103	MAB21L3	0.831	0.876	-0.046 (-3.218 - 3.127)	0.00040
cg00837485	1	218774366	MIR548F3	0.776	0.738	0.039 (-3.574 - 3.651)	0.00047
cg00882757	1	248127300	OR2AK2; OR2L13	0.678	0.638	0.040 (-1.939 - 2.019)	0.00086
cg23683528	2	235860449	SH3BP4	0.006	0.012	-0.006 (-1.143 - 1.131)	0.00074
cg25060765	6	7680990	-	0.570	0.547	0.023 (-2.796 - 2.842)	0.00077
cg02548323	7	101556938	CUX1	0.562	0.665	-0.103 (-3.600 - 3.394)	0.00092
cg23357150	10	134470225	INPP5A	0.845	0.846	-0.001 (-3.303 - 3.301)	0.00093
cg06834698	11	7961985	OR10A3	0.868	0.886	-0.018 (-3.108 - 3.073)	0.00066
cg22937632	11	33563946	KIAA1549L	0.685	0.724	-0.038 (-1.734 - 1.657)	0.00092
cg26183967	11	72871645	-	0.686	0.607	0.080 (-1.781 - 1.940)	0.00089
cg08407503	12	30842614	IPO8	0.887	0.861	0.026 (-1.556 - 1.609)	0.00055
cg22877969	13	28950883	FLT1	0.422	0.464	-0.042 (-1.783 - 1.698)	0.00089
cg06043360	13	74785598	-	0.898	0.872	0.025 (-0.891 - 0.941)	0.00060
cg12491688	15	44580831	CASC4	0.026	0.029	-0.003 (-0.879 - 0.873)	0.00089
cg15784881	16	3820865	CREBBP	0.813	0.809	0.004 (-3.009 - 3.017)	0.00051
cg14643763	20	2153724	-	0.465	0.394	0.072 (-2.225 - 2.368)	0.00089

CHR, Chromosome; COCP, Combined oral contraceptive pill; CI, Confidence intervals; bp, base pairs.

Design

Case-control study investigating DNA methylation changes associated with COCP use.

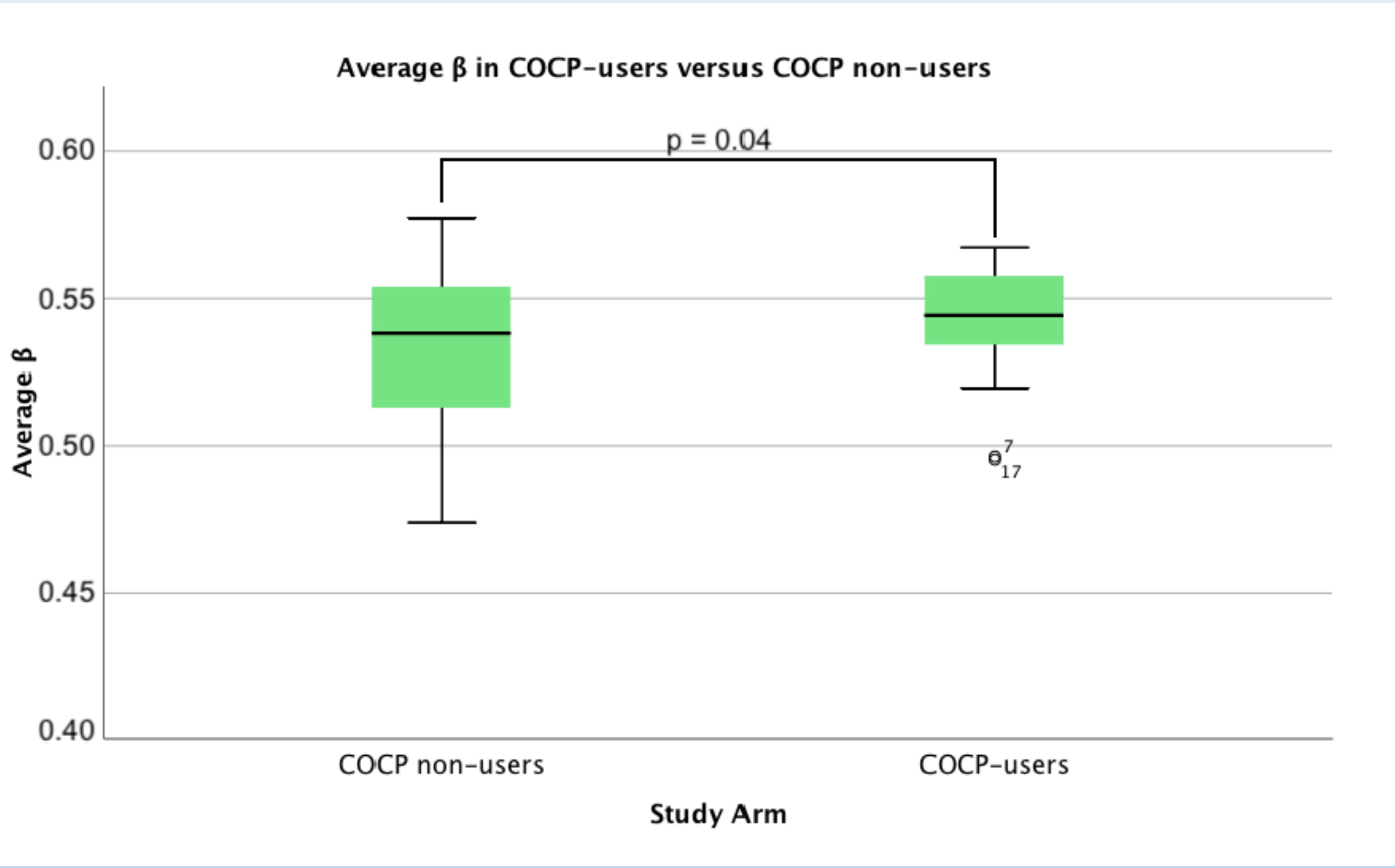


Figure 3: Boxplot of average beta values in COCP-users and non-users

Conclusion

The mechanism by which COCP use provides protection against carcinogenesis remains unclear. Our study adds to the current body of evidence suggesting there is a relationship between COCP use and methylation levels, and suggests target CpG sites for further investigation. This warrants future research in a larger cohort with more established COCP use; we anticipate our small changes in methylation levels may be more significant after long-term COCP use.

References

1. Iversen. L et al. Association between contemporary hormonal contraception and ovarian cancer in women of reproductive age in Denmark: prospective, nationwide cohort study. BMJ. 2018;362(8169):3609. Available from: doi: 10.1136/bmj.k3609

2. Campesi. I et al. Oral contraceptives modify DNA methylation and monocyte derived macrophage function. Biology of Sex Differences. 2012;3(1):4-15.Available from: doi: 10.1186/2042-6410-3-4.