PWE-094

DNA METHYLATION AS A BIOMARKER OF PROGRESSION IN BARRETT'S CARCINOGENESIS

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Introduction Barrett's oesophagus (BO) increases the risk of developing oesophageal adenocarcinoma (OAC) by around 40-fold. OAC incidence rates have increased sixfold since the 1970s but the bleak prognosis for this disease is improved by early detection. The intermediate dysplastic stage between BO and OAC is the most reliable marker of progression. However the histological presence of dysplasia is subjective due to known sampling bias along with a high inter and intraobserver variability.

Aberrant DNA methylation is shown to be characteristic of certain tumours and is known to occur early during transformation. We therefore hypothesise that DNA methylation changes take place early during carcinogenesis in BO patients which could be utilised as progression biomarkers. The study aims were therefore to identify genes showing a variation in methylation through progression and to then validate them on an independent dataset.

Methods DNA methylation levels were assessed across 27 578 CpG loci covering 14 000 genes in 24 of each BO and OAC test samples using arrays. Both supervised clustering (GSEA software) and unsupervised clustering (R software) were used to identify genes with a statistically significant difference in methylation in BO versus OAC. Genes present in both the lists were selected for validation. Internal validation and external validation on an independent set of 48 BO, 31 BO with dysplasia and 68 OAC samples was carried out using pyrosequencing assays.

Results Supervised clustering came up with 2822 hypermethylated and 645 hypomethylated genes ($p \leq 0.001$) and unsupervised with 1563 hypermethylated and 610 hypomethylated genes ($p \leq 0.01$). Genes successfully validated include *RGN* (senescence marker protein-30), *SLC22A18* (tumour suppressing subtransferable candidate 5), *PIGR* (polymeric immunoglobulin receptor), *GJA12* (gap junction protein, γ2) and *TCEAL7* (transcription elongation factor A – like 7). After internal validation (spearman's correlation $p < 0.001$, coefficient=0.9), external validation was carried out and a highly statistically significant gradual increase in methylation was observed for all the above genes in OAC and BO with dysplasia compared to BO: *RGN* (ANOVA $p<0.001$, JT $p=0.001$), *SLC22A18* (ANOVA $p<0.001$, JT test $p<0.001$), *PIGR* (ANOVA $p<0.001$, JT $p<0.001$), *GJA12* (ANOVA $p<0.001$, JT $p<0.001$) and *TCEAL7* (ANOVA $p=0.008$, JT $p=0.001$).

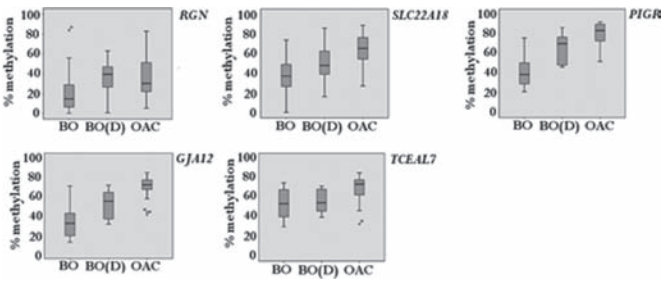

Figure 1 PWE-094

Conclusion High throughput DNA methylation screening using arrays and robust external validation has allowed us to identify novel genes having biomarker potential previously unknown to play a role in Barrett's carcinogenesis. Further validation in an EDRN phase III study is underway.

Competing interests None.
Keywords Barrett's oesophagus, Biomarker, DNA methylation.