Comparative analysis of pyrosequencing and methylation profiling for assessing LINE-1 and Alu methylation in response to low-level benzene exposure

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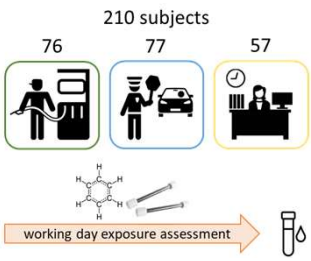
Background

- DNA methylation is an epigenetic mechanism involving the addition of methyl groups to cytosine to form 5-methylcytosine (5mC) that has a key role in gene regulation and chromosomal stability.
- LINE-1 (L1) and Alu methylation has been used as a surrogate marker for global DNA methylation, traditionally assessed via bisulfite-PCR/pyrosequencing. This method interrogates a limited number of CpGs, likely overlooking wider methylation patterns.
- Array-based technologies (e.g. Illumina EPIC assays) profile thousands of loci residing in repetitive elements (RE), offering a detailed though selected view of methylation landscapes.
- Benzene exposure has been linked to L1 and Alu hypomethylation, yet the extent to which different assays capture these changes remains unclear.

Study Design and Aim

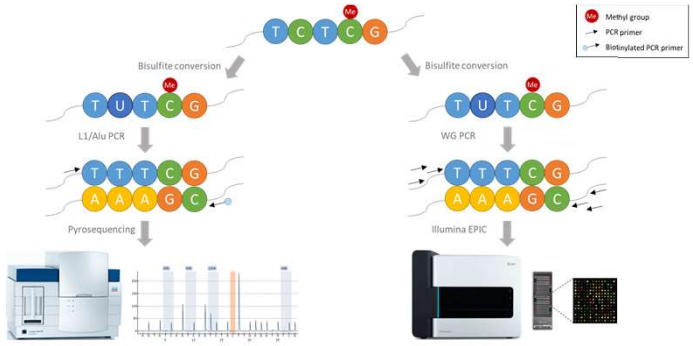


This study aims at comparing mean methylation derived from pyrosequencing and Illumina EPIC assay in adults with low-level occupational benzene exposure, by assessing their concordance in detecting exposure-related changes.

The project **Exposure to low levels of benzene, interindividual biological variability and cancer risk (Grant agreement ID: BMH4983785)** aims at claryifing the role of low-level occupational benzene exposure by integrating individual exposure data, biomarkers of dose and susceptibility, with health outcomes.

Methods

- Peripheral blood DNA was bisulfite-treated and used for L1 and Alu methylation quantification by PCR/pyrosequencing and profiling by the Illumina Infinium MethylationEPIC v1.0 BeadChip.



- L1 and Alu subfamilies analyzed by PCR/pyrosequencing were verified by *in silico* PCR and selected for comparison between methods.
- Raw EPIC assay IDATs were processed to β-values after standard QC. Probes overlapping L1 and Alu elements of interest (RepeatMasker) were used to derive EPIC-based means employed for comparison with pyrosequencing means.
- Methylation distributions were summarized (median + IQR and mean ± SD), and concordance between methods was assessed using correlation and Lin's concordance correlation coefficient (CCC). Analyses were performed using R (v4.5.0).

Results

- L1 and Alu subfamilies identified by *in silico* PCR and selected for further analysis were L1HS, L1PA2 and L1PA3, and AluY and AluS, respectively.

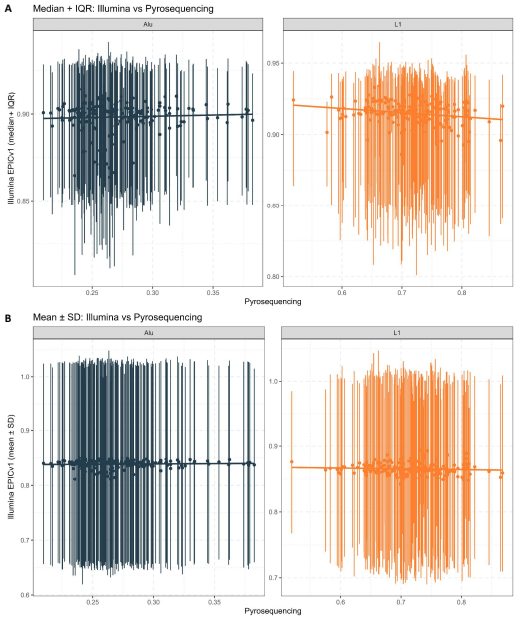


Image 2. **Scatter plots representing EPIC median (*A*) and mean (*B*) methylation with respect to mean pyrosequencing methylation (Alu, *black;* L1, *orange*).**
No correlation between EPIC and pyrosequencing methylation estimates was observed.

- 913 and 15762 EPIC probes corresponding to the selected L1 and Alu subfamilies were used, respectively.
- Lin's CCC (~ $8{,}5 \times 10^{-5}$ for Alu; $-9{,}4 \times 10^{-3}$ for L1), indicated poor overlap between the two methods. Indeed, the two assays do not covary with precision, and present a platform effect that determines poor concordance.

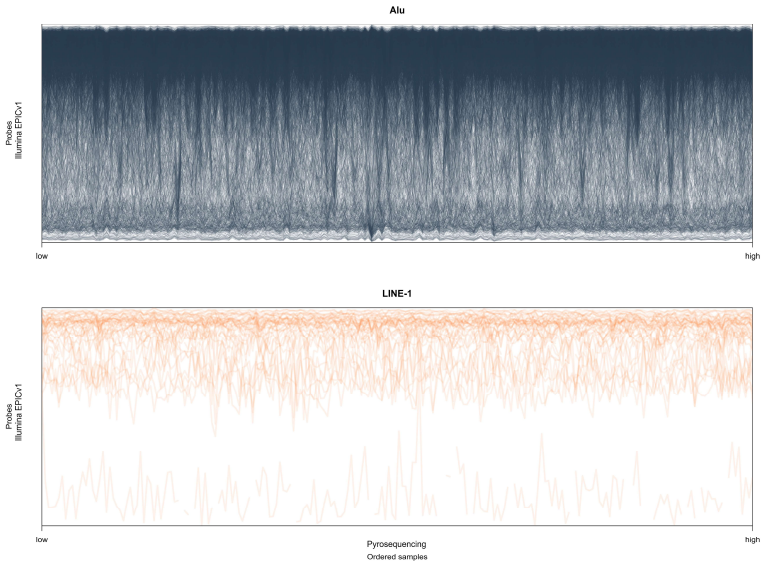


Image 1. **Line plots representing the methylation trend of single EPIC probes across samples ordered by mean pyrosequencing methylation (Alu, *black;* L1, *orange*).**
No overall increased EPIC-derived methylation was observed with the increase in methylation quantified by pyrosequencing.

Conclusions

Our findings show a lack of concordance, both in absolute levels and in trends, between pyrosequencing and EPIC-derived mean methylation estimates for L1 and Alu. This discrepancy highlights the profoundly different aspects of RE methylation captured by these assays, questioning the validity of using them interchangeably or as equivalent proxies for global DNA methylation analysis, notably in exposure studies. While pyrosequencing has proven suitable for the latter, Illumina Infinium MethylationEPIC v1.0 BeadChip allows to uncover subtle methylation changes at the single CpG level across the genome.