

Submitter Name: Rui Luo  
Submitter email: [rlo@memphis.edu](mailto:rlo@memphis.edu)

## **Transgenerational Effect of Smoking During Pregnancy on DNA Methylation of the Newborn**

Rui Luo<sup>1</sup>, Nandini Mukherjee<sup>1</sup>, Hasan Arshad<sup>2,3,5</sup>, John W. Holloway<sup>2,4</sup>, Hongmei Zhang<sup>1</sup>,  
Wilfried J.J. Karmaus<sup>1</sup> and Fawaz Mzayek<sup>1</sup>

<sup>1</sup>Division of Epidemiology, Biostatistics, and Environmental Health, School of Public Health, University of Memphis; <sup>2</sup>Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton; <sup>3</sup>The David Hide Asthma and Allergy Research Centre; <sup>4</sup>Human Development and Health, Faculty of Medicine, University of Southampton; <sup>5</sup>NIHR Respiratory Biomedical Research Unit, University Hospital

**Background:** Maternal smoking during pregnancy (MSDP) is related to adverse health outcomes in the children. MSDP-induced DNA methylation (DNA-m) alterations have been suggested to play a role in these effects. However, whether these effects remain across multiple generations is not known. We investigated the association of MSDP with DNA-m in the blood of the grandchildren across four exposure groups formed by grandmother/mother gestational smoking (yes/yes, yes/no, no/yes, and no/no), using data from the Isle of Wight study.

**Methods:** Mother-daughter pairs (n=750) were recruited at birth of the daughters (1989) and followed up until some daughters became pregnant and gave birth (2011-2015). DNA-m at CpG (cytosine-phosphate-guanine) sites was measured in 187 of the grandchildren using Illumina Infinium MethylationEPIC BeadChip kit (Illumina, Inc., San Diego, CA). MSDP was assessed using self-report. We compared DNA-m levels of 59 MSDP-related CpGs, known from the literature, across the four exposure groups.

**Results:** Grandmaternal MSDP was associated with maternal MSDP (OR = 2.6; 95% CI = 1.7, 4.2). Five CpGs were differentially methylated across the four exposure groups (cg12803068(MYO1G); cg04180046(MYO1G); cg00213123(CYP1A1); cg19089201(MYO1G), and cg05575921(AHRR),  $p < 0.001$  for all). To assess the effect of grandmother gestational smoking, grandchildren DNA-m was compared between the no/yes and yes/yes groups, stratified by the gender of the grandchild. Only three CpGs (cg12803068( $p=0.07$ ); cg04180046( $p=0.07$ ); cg05575921( $p=0.06$ )) were differentially methylated in boys with borderline significance.

**Conclusion:** The results suggest a transgenerational effect of MSDP on DNA-m profile of the grandchildren.