Epigenetics: Playing with the Game of Life. University Hospital Halle (Saale).
September 13th – 15th 2019

Influence of the adherence to the Mediterranean diet on the effect of smoking on genomewide methylation among subjects with metabolic syndrome

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Background and aims

Tobacco smoking is an important risk factor for lung cancer, respiratory diseases and cardiovascular diseases, among others. Moreover, smoking can speed up the normal aging process of several tissues increasing the biological age. Changes in methylation due to smoking have been demonstrated at several loci across the genome, particularly in long-term smokers (Figure 1). The most consistent association reported in different populations has been decreased methylation in smokers in comparison with non-smokers at the CpG cg05575921, located in the gene for the aryl hydrocarbon receptor repressor (AHRR) located in chromosome 5 (Figure 2 and **Figure 3**).

Figure 1: Tobacco smoking)

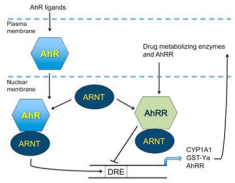


Figure 2: AhRR, a specific competitive repressor of aryl hydrocarbon receptor (AhR) activity based on its ability to dimerize with the AhR nuclear translocator (ARNT) and through direct competition of AhR/ARNT and AhRR/ARNT complexes (Vogel et al, Current Opinion in Toxicology, 2017).

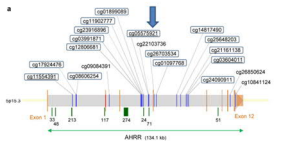


Figure 3: Location of the cg05575921 in the AHRR gene

Several individual studies and meta-analysis in different populations have reported a strong hypomethylation signal in the CpG cg05575921 locus depending on the smoking status . Among these studies, we show specific data in the REGICOR study, a population study carried out in the Mediterranean population (Figure 4), as well as the participants in the MESA study (Figure 5).

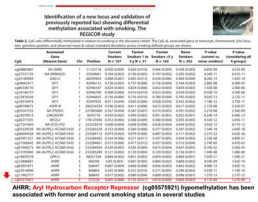


Figure 4: Methylation of the AHRR gene and other genes depending on the smoking status in the REGICOR study (Epigenetics, 2015).

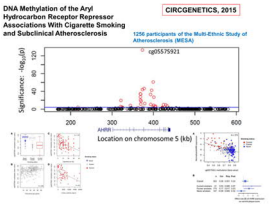
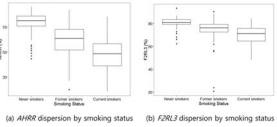


Figure 5: EWAS for methylation status depending on smoking in the MESA participants (Circulation Cardiovascular Genetics, 2015).

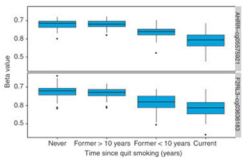
Another consistent association has been found with coagulation factor II (thrombin) receptor-like 3 (F2RL3, cg03636183). Hypomethylation of this gene has been associated with smoking in several studies and in different populations mimicking the AHRR effects (Figure 6 and Figure 7).



(a) *AHRR* dispersion by smoking status (b) *F2RL3* dispersion by smoking status

Figure 6: Hypomethylation of the AHRR and F2RL3 locus by smoking (Lee et al, PlosOne, 2017).

Figure 7: Hypomethylation of the AHRR and F2RL3 locus by smoking (Fasanelli et al, Nat Comunications, 2015).



Despite the numerous studies examining changes in methylation at the genome-wide level depending on the smoking behavior, very few studies have examined the additional influence of the diet in modulating the smoking effects on methylation.
Therefore our aim was to examine whether a higher adherence to a Mediterranean diet (MedDiet) modulated the effect of smoking on genome-wide methylation among older subjects with metabolic syndrome.

Methods

We analyzed 88 participants in the PREDIMED PLUS-Valencia study. The PREDIMED PLUS-Valencia study (located on the eastern Mediterranean coast, Spain) is one of the field centers of the multi-center PREDIMED Plus study, which is an ongoing trial. This RCT was registered at https://doi.org/10.1186/ISRCTN89898870. Eligible participants, were recruited from several primary care health facilities in the Valencia field center, were community-dwelling adults (men, 55–75 years, women, 60–75 years) with a body-mass index (BMI) in the overweight or obesity range ((BMI) ≥ 27 and <40 kg/m2) and had at least three components of the metabolic syndrome. In the Valencia field center, the total number of randomized participants included in the PREDIMED Plus trial was 465. Here we analyzed a subsample of the 88 participants (44 males and 44 females) selected by smoking status and paired by gender. Smoking status was assessed according to the WHO classification (including current smokers, former smokers (including former smokers <1 year, from 1 to 5 years and more than 5 years) and never smokers. These subjects were aged 55 to 75 years, mean age 64+/-5 years, all with metabolic syndrome.

Anthropometric variables were determined by trained staff and follow the PREDIMED Plus operations protocol detailed in the study Web site (http://www.predimedplus.com/). Weight and height were measured with calibrated scales and a wall-mounted stadiometer, respectively. BMI was calculated as the weight in kilograms divided by the height in meters squared. Leisure-time physical activity was assessed using the validated REGICOR questionnaire including questions to collect information on the type of activity, frequency (number of days), and duration.

A 17-item screening questionnaire was used for assessing adherence to an energy-restricted Mediterranean diet. Figure 8 shows the detailed questions included in the 17-item screening questionnaire. Two categories: low (less than 10 points in the 17-item score) and high (> =10 points)] for adherence to Mediterranean diet were considered.

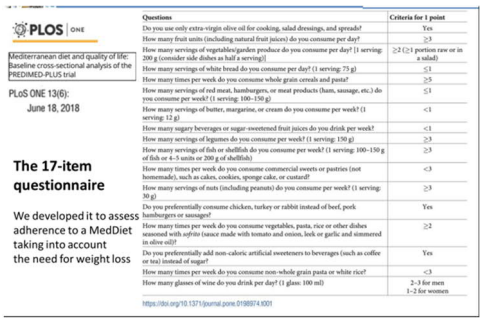


Figure 8: Questions included in the 17-item questionnaire for adherence to Mediterranean diet

DNA isolation and epigenome-wide methylation analyses

DNA was isolated from blood. We performed genome-wide DNA methylation (EWAS) using the Illumina Infinium 850K MethylationEPIC array. Differential methylation (M-values) was statistically analyzed with Partek Genomic Suite using ANCOVA models adjusted for potential confounders including batch effect, age, BMI, diabetes and leukocyte counts, among others. Analyses in the whole population and stratified by the Mediterranean diet adherence level were carried out. Interaction terms were also tested. Quality control was carried out..

Figure 9: Methylation analysis

Results

Mean age of the participants was 64.6+/-5 years, without statistically significant differences between men and women (P=0.236).

Mean BMI was 32.1+/-3.5 kg/m2 with no statistically significant differences between men and women. Mediterranean diet adherence did not differed by sex, being a mean of 8.4+/-3 points in the 17-item score screener.

There were 11% of current smokers, 3.3% former smokers of less than 1 year, 5.5% former smokers 1-5 years, 32% former smokers >5 years and 47% never smokers.

In the EWAS carried out in the whole population, tobacco smoking was associated with methylation of several genes (even after adjustment for batch effect, sex, age, body mass index, diabetes, and leukocyte counts). The top ranked CpG being: cg05575921-AHRR (P=1.57E-09); cg21566642 (P=4.12E-09); cg17739917-RARA (P=7.73E-08); cg01940273 (P=2.68E-07) and cg03636183-F2RL3 (P=1.34E-06).

We observed a statistically significant hypomethylation of the well-known maker of tobacco smoking, the cg05575921-AHRR in current smokers in comparison with never smokers and even with former smokers.

Likely, we observed a similar methylation of the other well-known marker, the cg03636183-F2RL3 locus. On the other hand, adherence to MedDiet [two categories: low (less than 10 points in the 17-item score) and high (> =10 points)] was associated with differential methylation of several genes and some heterogeneity by smoking was detected.

Moreover, in the stratified analyses of the smoking effect on DNA methylation depending on the MedDiet adherence group, several differences were detected. Thus, in subjects with low adherence to MedDiet, the top-ranked CpG associated with tobacco smoking were cg05575921-AHRR (P=8.91E-07) and cg17739917-RARA (P=2.48E-06).

However, in subjects with high MedDiet adherence, some of the op-ranked SNPs differed, indicating an additional modulation by the MedDiet. Specifically, in subjects with high adherence to the MedDiet, the cg05575921-AHRR was not among the 500 top-ranked SNPs, decreasing differences in methylation (P=0.0058).

Conclusions

In conclusion, our results suggest that MedDiet could be an additional modulator of the smoking effects on DNA-methylation. These are preliminary results and more work has to be done increasing sample size and focusing on the different foods of the Mediterranean diet in addition to the global food pattern measured by the 17-item score.

Acknowledgements

This study was partially funded, by the Spanish Ministry of Health (Instituto de Salud Carlos III) and the Ministerio de Economía y Competitividad-Fondo Europeo de Desarrollo Regional (FEDER, "A way of making Europe") (grants CIBER 06/03, PRX17/00500, SAF2016–80532-R) and Premio Rei Jaume I (Valencia, Spain); the University Jaume I (grants P1–1B2013–54, COGRUP/2016/06 and UJI-B2018-69); the Fundació La Marató de TV3 (grant 538/U/2016); and the Generalitat Valenciana (grant PROMETEO2017/017).