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Environmental Epigenetics: effects of Endocrine Disruptors on Development of Obesity – Statistical Analysis

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ABSTRACT

Specific epigenetic alternation occurs by direct exposure to toxicants or abnormal nutrition, which influence disease development or physiological phenotypes. Previous study has demonstrated that exposure to endocrine disrupting compounds (EDCs) may contribute to excess adiposity and other features of dysmetabolism, accordingly, EDCs exposure may contribute to development of obesity, and insulin resistance, common precursors of type 2 diabetes and cardiovascular diseases. However, at this time, there is limited human data about contribution of environmental chemicals to the obesity epidemic. As obesity is a major public health problem in Indonesia, it is important to identify common environmental risk factors that may have a role in the development of obesity. Therefore, the objective of this study is to proof whether environmental factors/EDCs affect the development of obesity by using top-down approaches. Use two datasets, expression profile and methylation profile, from NCBI GEO database and apply statistical analysis to investigate the differentially expressed gene, analysis was conducted in R software and GEO2R web tool. Environmental epigenetics help to better understand how endocrine disruptors influences human health and disease, and there are two genes are found to be contribute to the weight gain. Further research is needed to have more confidence result of the analysis.

Keywords: *Epigenetics, obesitas, endocrine disruptor, environmental factor, statatistical analysis*

INTRODUCTION

Environmental epigenetics investigates heritable changes in gene expression and phenotype alterations that lead by environmental factors, the alteration is occurred without changes in DNA sequence. Previous studied show that the majority of environmental factors do not have the ability to alter DNA sequence or promote genetic mutations, however, the environment can dramatically influence epigenetic processes to alter gene expression and development [1;2]. Up until know, a large number of studies have found that specific epigenetic alternation occurs by direct exposure to toxicants or abnormal nutrition (e.g. caloric restriction or high fat diets), which influence disease development or physiological phenotypes [3]. These direct exposures to environmental factors include nutrition, stress, temperatures, pharmaceuticals, synthetic chemicals and environmental toxicants.

Endocrine disruptor is one of the environmental toxicants, is defined as a chemicals/environmental compounds that alter and/or disrupt normal endocrine hormone signalling at the receptor or signal transduction, by mimicking, blocking, and interfering the actions of hormones in the body's endocrine system, also known as Endocrine Disrupting Compounds (EDCs) ('Endocrine-Disrupting Chemicals | Endocrine Society', n.d.). The EDCs is produced by either naturally occurring compounds or man-made EDCs. The most known naturally occurring endocrine disrupting compounds is Phytoestrogens, which can found in soy and other legumes, whereas Bisphenol A (BPA) and Polychlorinated biphenyls (PCBs) are best known as the manmade EDCs. However, people are exposed to EDCs in a myriad of ways; including dermally from the personal care products like lotion for hand and hair; as well as breathe them in; from fragrance

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mixtures; finally, the worst thing is that, people ingest them from food, either naturally contains in the food or products that have been contaminated during processing or from the packaging of the food [1;2].

Obesity is a problem faced by both high- and low- and middle income groups and the prevalence of obesity in all age groups is [4] Previous study has demonstrated that exposure to EDCs may contribute to excess adiposity and other features of dysmetabolism, accordingly, EDCs exposure may contribute to development of obesity, and insulin resistance, common precursors of type 2 diabetes and cardiovascular diseases [5]. However, at this time, there is limited human data about contribution of environmental chemicals to the obesity epidemic, increase in the incidence of obesity has occurred in parallel with increase in the use of plastic and other products containing EDCs. As obesity is a major public health problem in Indonesia and a strong risk factor for cardiovascular disease, diabetes and cancer, it is important to identify common environmental risk factors that may have a role in weight gain and the development of obesity [4]. Therefore, this study is to proof whether environmental factors/EDCs affect the development of obesity by using top-down approaches.

MATERIALS AND METHODS

Transcriptomic data (Expression profil)

Transcriptomic data set of granulosa cell are from NCBI GEO (Gene Expression Omnibus) [http://www.ncbi.nlm.nih.gov/geo/] database. This data set contains RNA samples from granulosa cell of F3-control lineage groups and granulosa cell of F3-vinclozolin lineage groups. Compare the gene that differentially expressed in control groups and vinclozolin lineage groups.

Epigenetic data (Methylation profile)

Epigenetic data sets of differentially methylated DNA sites are from GEO database. The data set use the white adipose tissue (WAT) from 11 obese and 9 non-obese women.

Software

Most computations were done using R studio [https://www.rstudio.com/], for analysis of expression profile and differentially methylated analysis was conducted using GEO2R [https://www.ncbi.nlm.nih.gov/geo/geo2r/]. Both R studio and GEO2R were work using the GEOquery and limma R packages from the Bioconductor project.

Data acquisition

Two of potential data sets was programmatically retrieved from the GEO database using the getGEO function from the R/Bioconductor GEOquery package.

Data pre-processing

Normalization is important for comparison of microarray data sets to reduce the heterogeneity of different datasets caused by different platforms, different gene nomenclature and different control tissues. In this case, for each probe-set intensity values were log2-transformed and normalized to zero mean and unit variance.

Differential expression analysis

Many methods and approaches to find differentially expressed genes (DEGs) exist and this time opted for three different methods; R/Bioconductor limma package and samr package, as well as shrinkage T-statistic that were computed using GEOquery package. In both expression and methylation data set, P-values are corrected for multiple testing using Bonferroni and FDR correction with the cut off of P-value is 0.05, 0.00001 and were selected as the statistically DEGs, respectively.

RESULTS AND DISCUSSION

Analysis of ecpression profile

Statistical analyses suggested that there are some differentially expressed gene in the mouse that exposed with vinclozolin. The summary of statistical analyses were shown in Table 1. In this study, compared the distribution of the differentially expressed gene of the control group and vinclozolin exposed group at the beginning of the work. Of the 50 highly expressed gene in both sample, there is no gene that seems to be the major type (Figure 1). Multiple testing correction also shows that there is no significant gene was found in P=<0.05 with the correction using both Bonferroni and FDR (Figure 2). The SAM plot suggested that eight genes are up-regulated in the vinclozolin exposed group; *Pon1,*

Pold4, two EST's, and rest are no annotation was found (Figure 3). Moreover, there is no result for the LIMMA analysis.

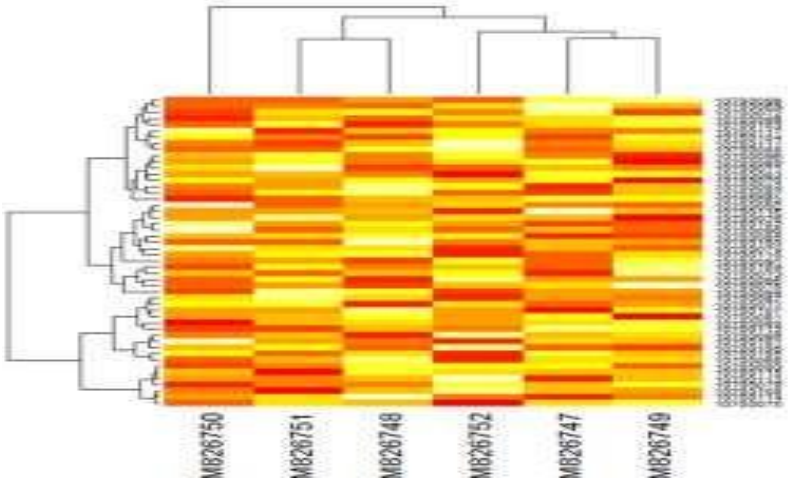

Figure 1. The heatmap of the highly expressed gene in control and vinclozolin exposed

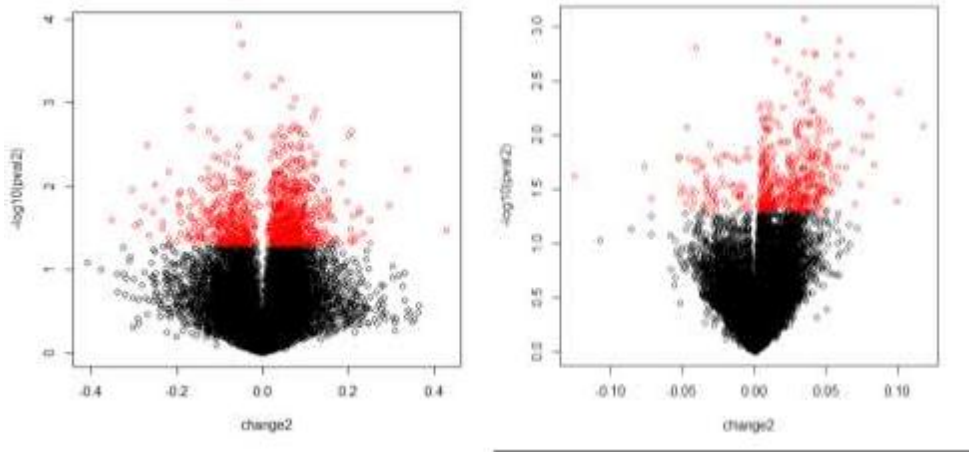

Figure 2. Volcano plot (Left: expression profile; Right: methylation profile)

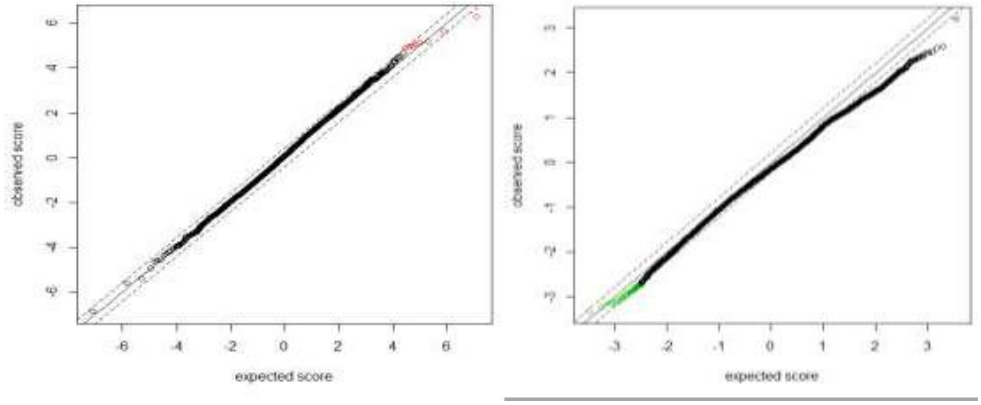

Figure 3. SAM Plot (Left: ecpression profile; Right; methylation profile)

Table 1.Summary of the differentially expression gene

	Expression Profile*	Methylation Profile*
T-test (P = <0.05)	**1058 genes**	**492 genes**
T-test (P = <0.001)	**6 genes**	**1 gene**
	10765102/ NM_001012207/Pigc 10787603/ NM_001107306/ Armc6 10822080/ XR_008091/ - 10880590/ NM_001107986/ Srrm1 10908255/ NM_001048243/ ubl5 10918674/ NM_173146/ Unc13c	09060914 / NM_003005.2/SELP
Penalized SAM	**8 up-regulated genes**	**31 genes**
	10875005/ XR_008241/ - 10700584/ - / EST's 10860867/ NM_032077/Pon1 10909129/ - / - 10701280/ - / - 10700157/ - / - 10712858/ NM_001013195/Pold4 10701505/ - /EST's	09060914 / NM_003005.2/SELP 05873268 / NM_003294.3/ - 08881519/ NM_015433.2 / - 24981018 / NM_015193.3/ - 18885346 / NM_138694.2/ -
Moderated LIMMA	-	-

Methylation profile analysis

Statistical analyses suggested that there is obvious differentially expressed gene in obese woman. The summary of statistical analyses were shown in Table 1. As shown in Table 1, *SELP* is the one that significantly expressed in the all statistical test, except LIMMA. GEO2R analysis supported that the *SELP* gene is most significantly expressed gene in the obese woman (Table 2).

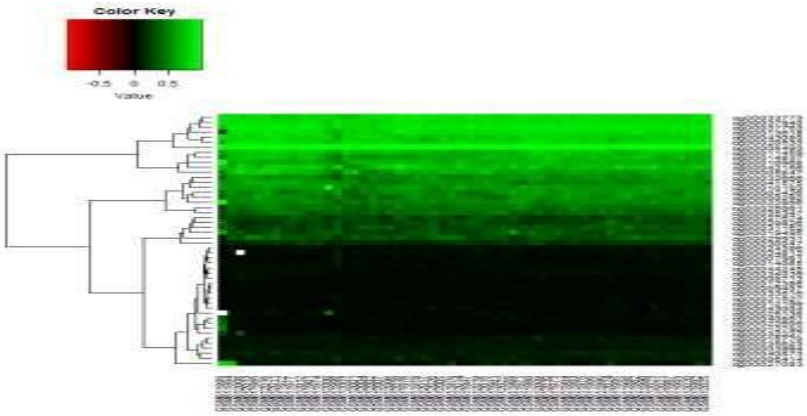

Figure 4. Heat map of the 50 hight expression gene in obese and non-obese woman

Epigenetic data (Methylation profile)

Moreover, distribution of the differentially expressed gene of the obese and non-obese woman suggested that the gene *EPB41L3* seems to be the major type in the non-obese woman whereas gene *CPSF3* for the obese woman (Figure 4). Multiple testing correction also shows that there is no significant gene was found in P=<0.05 with the correction using both Bonferroni and FDR (Figure 2). The SAM plot suggested that 31 genes are down-regulated in the obese group (Figure 3). There is no result for the LIMMA analysis.

Table 2. Result of GEO2R analysis

ID	adj.P.Val	P.Value	F	Accession	RANGE_GB	RANGE_START	RANGE_END	RANGE_STRAND	GB_ACC	ORF
[illegible]										

EDCs or environmental toxicants are recently found to be associated with various epigenetic transgenerational inheritance disease, including obesity. As obesity is a major public health problem in Indonesia and a strong risk factor for cardiovascular disease, diabetes and cancer, it is important to identify common environmental risk factors that may have a role in weight gain and the development of obesity . However, currently, there is limited human data about contribution of environmental chemicals to the obesity epidemic. Identification of the gene that contributed in the development of obesity that supports by expose to the environmental toxicants may help for the further research in development of obesity, especially in Indonesia.

In this work found out that EDCs affects the PON1 activity. PON1 or Paraoxonase-1 is a polymorphic enzyme that is strongly associated with circulating high-density lipoproteins, which protects lipoproteins from oxidative modifications and hence against development of atherosclerosis. Lower serum PON1 arylesterase activity is associated to metabolic alterations related to childhood overweight and onset and/or development of diabetes and CVD later in life.

As shown in the result, SELP is known to be an active regulator in changing body weightS, which can increase the metabolic activity. Previous research found out that the P-sel KO suppressed body weight gain [6]. Most recent study shows that the Deletion of P-selectin correct high fat diet induced metabolic disturbance, liver injury and slow down the progression of NAFLD. On the other hand, lack of the data also affects the significance of the result, less statistical power result is produced in the analysis of expression profile. Thus, further investigation is required to be more statistical powerful analysis with increasing sample. In addition, pathway analysis is also required to proof the result in biological manner.

CONCLUSION

Environmental epigenetics and epigenetic inheritance will help to better understand how endocrine disruptors influences our health and disease. Obesity was proof to be induced by environmental compounds.

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REFERENCES

1. Rissman EF, Adli, M (2014) Minireview: Transgenerational Epigenetic Inheritance: Focus on Endocrine Disrupting Compounds. Endocrinology, 155 (8) 2770–2780. doi.org/10.1210/en.2014-1123.
2. Skinner MK (2014) Endocrine disruptor induction of epigenetic transgenerational inheritance of disease. Molecular and Cellular Endocrinology. 398 (1–2) 4–12. doi.org/10.1016/j.mce.2014.07.019

3. Holland N, Lizarraga D, Huen K (2015) Recent progress in the genetics and epigenetics of paraoxonase: why it is relevant to children's environmental health Current Opinion in Pediatrics 27 (2) 240–247. doi.org/10.1097/MOP.0000000000000192.
4. McCarrey JR, Lehle JD, Raju SS, Wang Y, Nilsson EE, Skinner MK (2016) Tertiary Epimutations - A Novel Aspect of Epigenetic Transgenerational Inheritance Promoting Genome Instability. PLOS ONE. 11 (12) e0168038. doi.org/10.1371/journal.pone.0168038.
5. Schneider DJ, Hardison R, Lopes N, Sobel BE, Brooks MM (2009) Association Between Increased Platelet P-Selectin Expression and Obesity in Patients With Type 2 Diabetes. Diabetes Care. 32 (5) 944–949. https://doi.org/10.2337/dc08- 1308.
6. Martinez JA, Milagro FI, Claycombe KJ, Schalinske KL (2014) Epigenetics in Adipose Tissue, Obesity, Weight Loss, and Diabetes. Advances in Nutrition: An International Review Journal, 5(1) 71–81. doi.org/10.3945/an.113.004705