Published in final edited form as:

Fertil Steril. 2010 July ; 94(2): 781–783.e1. doi:10.1016/j.fertnstert.2009.10.020.

Epigenetics in Polycystic Ovary Syndrome: A Pilot Study of Global DNA Methylation

Ning Xu, M.D., Ph.D.^a, Ricardo Azziz, M.D., M.B.A., M.P.H.^{b,c,d}, and Mark O. Goodarzi, M.D., Ph.D.^{a,b,c,e}

^aDivision of Endocrinology, Diabetes and Metabolism, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

^bDepartment of Obstetrics and Gynecology, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

^cDepartment of Medicine, the David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA

^dDepartment of Obstetrics and Gynecology, the David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA

^eMedical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

Abstract

The percent global methylation of peripheral blood DNA was not significantly altered in 20 PCOS women compared to 20 controls. The study of specific tissues and target genomic regions will be required to further determine whether epigenetic alteration influences the development of PCOS.

Keywords

Epigenetics; DNA methylation; Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is a heterogenic common genetic disorder, and features such as hyperandrogenemia, abnormal insulin resistance/secretion in relatives of PCOS women are heritable. However, the pathophysiology of PCOS is poorly understood. Although positive association results have been reported, no genes have been broadly accepted as causative for PCOS due to lack of replication (1). Environmental factors may play a role in PCOS (2).

Emerging evidence from prenatally androgenized animal models provides new insights into the pathogenesis of PCOS, and suggests that an environmental factor, androgen exposure during *in utero* development, may predispose individuals to this disorder (3). Female mammals (such as rhesus monkeys, sheep, mice or rats) exposed to androgen excess *in utero* develop masculinized phenotypes similar to PCOS as an adult (3,4). This phenomenon has been formulated as the fetal origins hypothesis of PCOS.

Corresponding Author/Reprint Requests: Mark O. Goodarzi, M.D., Ph.D.; Division of Endocrinology, Diabetes and Metabolism, Cedars-Sinai Medical Center, 8700 Beverly Blvd., Room B-131, Los Angeles, CA 90048. 310-423-4774, FAX: 310-423-0440, mark.goodarzi@cshs.org.

Disclosure Statement: N.X., R.A. and M.O.G. have nothing to declare.

Capsule:

In a pilot study, the percent global methylation of peripheral blood DNA was not significantly different between PCOS women and age- and BMI- matched controls.

The mechanism of the fetal origins hypothesis is unknown (5). During development, unfavorable prenatal environmental influences may lead to persistent changes in the epigenome, such as loss of imprinting of insulin-like growth factor 2 (*IGF2*), increased or decreased DNA methylation levels on CpG sites, which result in altered molecular pathways and increase the later risk of adult diseases (6). A rat model exposed to estrogen in the neonatal period was generated to elucidate the potential role of early estrogen exposure in prostate cancer; expression of the phosphodiesterase D4 (*PDE4D4*) gene was increased by hypomethylation (7). In mice, diethylstilbestrol exposure *in utero* resulted in hypermethylation of the homeobox A10 gene (*HOXA10*), and long-term altered *HOXA10* expression (8). Similar to estrogens in rodent models, elevated androgens *in utero* may disturb regular biological signals, and can predispose to disease later in life (9). We and others therefore hypothesize that exposure to excess androgens during early development may possibly reprogram the epigenome, causing persistent DNA methylation modification and irregular gene expression predisposing to PCOS (10).

Epigenetics is the study of heritable changes in gene expression that are not caused by DNA sequence alterations, but are mitotically and transgenerationally heritable. Inappropriate epigenetic reprogramming has been identified as contributing to common diseases with fetal origins such as type 2 diabetes (11), and prostate cancer (12), suggesting it may also contribute to PCOS, given that PCOS is a common disease with both reproductive and metabolic abnormalities (5,10). Additionally, epigenetic alterations have been observed as non-random X-chromosome inactivation in PCOS women, evidence that epigenetics may modulate the effect of the androgen receptor gene located on the X chromosome (13–15). Furthermore, DNA methylation, the principal mechanism of epigenetics, has been reported to play a role in cancer, aging, and complex chronic diseases (16). To investigate the role of epigenetics in PCOS, we conducted a pilot epigenetic study of DNA methylation in PCOS comparing the global methylation percentage between PCOS and matched controls.

Twenty unrelated white patients with PCOS and 20 healthy unrelated white control women were selected from an existing cohort of 335 cases and 198 controls, recruited at the University of Alabama at Birmingham (UAB; n=16) and Cedars-Sinai Medical Center (CSMC; n=24). We selected PCOS subjects who were young and had a BMI less than the median BMI (33.4 kg/m²) of the entire PCOS group to minimize the effects of obesity on epigenetic changes (17). For each PCOS subject a control subject was one-to-one matched for age (+/- 3 years) and BMI (+/- 3 kg/m²). The recruitment strategy (18) and methods of clinical and biochemical characterization (19) of this cohort have been previously described.

In brief, all cases and controls were not taking hormonal therapy for at least three months prior to enrollment. PCOS was defined by the 1990 National Institutes of Health criteria (20). All subjects had given informed consent, and the study was performed according to the guidelines of the Institutional Review Boards of UAB and Cedars-Sinai Medical Center.

The extracted peripheral leukocyte DNA was quantified using the NanoDrop™ 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE). For each sample, methylation analysis was performed in triplicate and averaged (100 ng DNA each) using a global methylation quantification kit (Methylamp™, Epigenetech, Brooklyn, NY), following manufacturer's instructions. Briefly, the methylated fraction was recognized by 5-methylcytosine antibody and quantified through an ELISA-like reaction. The total amount of methylated DNA is proportional to the optical density (OD). A standard curve was generated by plotting the OD values of a dilution series made from a 100% methylated DNA standard (supplied with the kit), the slope of which was used to calculate the total amount of methylated DNA for each sample. Methylation percentage was obtained by dividing the methylated DNA amount into the initial loaded DNA CpG amount. Purified DNA extracted from MCF-7 human

breast adenocarcinoma cells was provided with the kit and included in each experiment as an internal control. Global methylation percentage values were normalized to this internal control.

Clinical characteristics and methylation percentages of women with PCOS and controls were compared using the Mann-Whitney U test. Clinical and methylation data are expressed as the median (interquartile range). A P-value <0.05 was considered significant.

The clinical characteristics of the subjects are presented in Supplemental Table 1. Overall, the subjects were young (median age 23.0 (4.5) years) and lean (median BMI 23.3 (4.7) kg/m²). Cases and controls differed only in terms of androgen levels and hirsutism score. These subjects had normal insulin sensitivity and insulin secretion. The median global methylation percentages were 6.7% (IQR 5.6%) for PCOS women and 7.1% (IQR 6.2%) for controls, a non-significant difference (P=0.79) (Figure 1).

This is the first epigenetic study to investigate whether global DNA methylation is altered in PCOS patients compared to matched controls. The most accessible and achievable bio-resource is peripheral blood, so we first asked the question of whether the overall methylation percentage is altered in genomic DNA extracted from the peripheral blood in PCOS patients.

A strength of our study was the selection of matched cases and controls. Epigenetic status can be modified by a combination of environment and nutrition; age and BMI may affect methylation patterns. Remarkable differences between older monozygotic twins have been observed in their overall content and genomic methylated DNA and histone acetylation (21). We aimed to match PCOS and controls by age and BMI, and excluded older subjects and extremely obese subjects, avoiding confounding methylation differences arising from these factors.

There are biologic reasons that may explain the negative results of this study. First, epigenetic modification such as methylation on CpG islands is likely to occur at tissue-specific differentially methylated regions (22), although global methylation changes over time have been detected in peripheral leukocyte DNA in a few studies (23,24). Second, our experiment measuring the total methylated DNA fraction across the entire genome is not designed to detect individual methylation alterations at specific sites. Hypomethylation or hypermethylation in specific genomic regions may exist that affect PCOS, but are undetectable by the methodology used in this study (hyper- and hypomethylation would neutralize each other in this analysis). In the absence of knowledge of candidate genes for methylation in PCOS, global DNA methylation analysis was an appropriate first step to study epigenetics; however, our negative results suggest future studies should examine specific candidate genes/regions implicated by other evidence (e.g. expression studies, genetic association studies). Alternatively, site-specific methylation analysis on a genome-wide scale would enable us to systematically discover individual sites differentially methylated in PCOS versus controls.

In part, the small sample size may have been responsible for the negative results. However, a post-hoc power calculation determined that over a thousand subjects would be necessary to establish significance for the observed methylation percentage difference between cases and controls, suggesting that if a difference truly exists, it is likely to be subtle.

In summary, this pilot study found no significant difference in the global methylation of peripheral leukocyte DNA between PCOS and matched controls. Our preliminary study indicates the need to further investigate methylation in key tissues other than peripheral leukocytes, such as human ovaries, adipose tissue or adrenals, and specific target genes or regions. Aberrant DNA methylation patterns could serve as epigenetic biomarkers for early detection of PCOS, and understanding the epigenetic mechanisms involved in PCOS may

provide novel avenues for diagnosis and treatment of this common disorder. Finally, it is also possible that epigenetics does not play a critical role in the development of PCOS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial Support: This study was supported in part by NIH grants R01-HD29364 and K24-HD01346 (to RA), R01-DK79888 (to M.O.G.), and M01-RR00425 (General Clinical Research Center Grant from the NCRR), the Cedars-Sinai Winnick Clinical Scholars Award (to M.O.G) and an endowment from the Helping Hand of Los Angeles, Inc.

REFERENCES

- Goodarzi MO. Looking for polycystic ovary syndrome genes: rational and best strategy. *Semin Reprod Med* 2008;26:5–13. [PubMed: 18181077]
- Shaw LM, Elton S. Polycystic ovary syndrome: a transgenerational evolutionary adaptation. *BJOG* 2008;115:144–148. [PubMed: 18081596]
- Dumesic DA, Abbott DH, Padmanabhan V. Polycystic ovary syndrome and its developmental origins. *Rev Endocr Metab Disord* 2007;8:127–141. [PubMed: 17659447]
- Abbott DH, Barnett DK, Bruns CM, Dumesic DA. Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? *Hum Reprod Update* 2005;11:357–374. [PubMed: 15941725]
- Xita N, Tsatsoulis A. Review: fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. *J Clin Endocrinol Metab* 2006;91:1660–1666. [PubMed: 16522691]
- Waterland RA. Is epigenetics an important link between early life events and adult disease? *Horm Res* 2009;71 Suppl 1:13–16. [PubMed: 19153498]
- Prins GS, Birch L, Tang WY, Ho SM. Developmental estrogen exposures predispose to prostate carcinogenesis with aging. *Reprod Toxicol* 2007;23:374–382. [PubMed: 17123779]
- Bromer JG, Wu J, Zhou Y, Taylor HS. Hypermethylation of homeobox A10 by in utero diethylstilbestrol exposure: an epigenetic mechanism for altered developmental programming. *Endocrinology* 2009;150:3376–3382. [PubMed: 19299448]
- Homburg R. Androgen circle of polycystic ovary syndrome. *Hum Reprod* 2009;24:1548–1555. [PubMed: 19279033]
- Li Z, Huang H. Epigenetic abnormality: a possible mechanism underlying the fetal origin of polycystic ovary syndrome. *Med Hypotheses* 2008;70:638–642. [PubMed: 17764855]
- Martin-Gronert MS, Ozanne SE. Programming of appetite and type 2 diabetes. *Early Hum Dev* 2005;81:981–988. [PubMed: 16257499]
- Ho SM, Tang WY, Belmonte de Frausto J, Prins GS. Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. *Cancer Res* 2006;66:5624–5632. [PubMed: 16740699]
- Hickey TE, Legro RS, Norman RJ. Epigenetic modification of the X chromosome influences susceptibility to polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006;91:2789–2791. [PubMed: 16636126]
- Shah NA, Antoine HJ, Pall M, Taylor KD, Azziz R, Goodarzi MO. Association of androgen receptor CAG repeat polymorphism and polycystic ovary syndrome. *J Clin Endocrinol Metab* 2008;93:1939–1945. [PubMed: 18303071]
- Hickey T, Chandy A, Norman RJ. The androgen receptor CAG repeat polymorphism and X-chromosome inactivation in Australian Caucasian women with infertility related to polycystic ovary syndrome. *J Clin Endocrinol Metab* 2002;87:161–165. [PubMed: 11788641]
- Feinberg AP. Epigenetics at the epicenter of modern medicine. *JAMA* 2008;299:1345–1350. [PubMed: 18349095]

17. Campion J, Milagro FI, Martinez JA. Individuality and epigenetics in obesity. *Obes Rev* 2009;10:383–392. [PubMed: 19413700]
18. Goodarzi MO, Jones MR, Chen YD, Azziz R. First evidence of genetic association between AKT2 and polycystic ovary syndrome. *Diabetes Care* 2008;31:2284–2287. [PubMed: 18768676]
19. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab* 2004;89:2745–2749. [PubMed: 15181052]
20. Zawadzki, JK.; Dunaif, A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif, A.; Givens, JR.; Haseltine, F.; Merriam, GR., editors. *Polycystic Ovary Syndrome*. Cambridge: Blackwell Scientific Publications; 1992. p. 377-384.
21. Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, et al. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci USA* 2005;102:10604–10609. [PubMed: 16009939]
22. Song F, Mahmood S, Ghosh S, Liang P, Smiraglia DJ, Nagase H, et al. Tissue specific differentially methylated regions (TDMR): Changes in DNA methylation during development. *Genomics* 2009;93:130–139. [PubMed: 18952162]
23. Bjornsson HT, Sigurdsson MI, Fallin MD, Irizarry RA, Aspelund T, Cui H, et al. Intraindividual change over time in DNA methylation with familial clustering. *JAMA* 2008;299:2877–2883. [PubMed: 18577732]
24. Shimabukuro M, Sasaki T, Imamura A, Tsujita T, Fuke C, Umekage T, et al. Global hypomethylation of peripheral leukocyte DNA in male patients with schizophrenia: a potential link between epigenetics and schizophrenia. *J Psychiatr Res* 2007;41:1042–1046. [PubMed: 17049557]

Figure 1.

Percent methylation box plots. Controls are represented in blue, PCOS subjects in red. For each group, the bottom and top bars indicate the 10th and 90th percentiles. The bottom and top of each box indicate the 25th and 75th percentiles, and the line in the middle of each box is the median.