A case-control study identifying chromosomal polymorphic variations as forms of epigenetic alterations associated with the infertility phenotype

Sheroy Minocherhomji, M.S.,[a,b] *Arundhati S. Athalye, M.S.,*[a] *Prochi F. Madon, Ph.D.,*[a] *Dhananjay Kulkarni, M.S.,*[a] *Shonali A. Uttamchandani, B.S.,*[a] *and Firuza R. Parikh, M.D.*[a]

[a] Department of Assisted Reproduction and Genetics, and [b] Psychiatry Research Program, Jaslok Hospital and Research Centre, Mumbai, India

Objective: To study the association of chromosomal polymorphic variations with infertility and subfertility.
Design: A comparative case-controlled association study using cytogenetic techniques to compare the frequency of chromosomal variations in infertile individuals versus fertile controls.
Setting: Department of Infertility Management and Assisted Reproduction, Jaslok Hospital and Research Centre, Mumbai, India.
Patient(s): 760 infertile individuals and 555 fertile controls.
Intervention(s): ICSI, IUI, karyotyping, inverted 4′,6-diamidino-2-phenylindole (DAPI), CBG banding.
Main Outcome Measure(s): Frequency of chromosomal polymorphic variations in infertile individuals undergoing infertility treatment versus fertile individuals.
Result(s): A highly statistically significant increase in the frequency of total chromosomal variants in infertile women (28.31% vs. 15.16%) and infertile men (58.68% vs. 32.55%) was observed. The frequency of 9qh+ was statistically significantly increased in women with primary infertility (16.22% vs. 6.41%) and in men with severe male factor infertility (14.69% vs. 4.25%). A highly statistically significant increase in the frequency of Yqh+ was observed in men whose wives had a bad obstetric history (30.20% vs. 12.74%).
Conclusion(s): The statistically significantly higher incidence of heterochromatic variations found in infertile individuals stresses on the need to evaluate their role in infertility and subfertility. Potential epigenetic, genetic, and chromosomal modifications could be associated with certain complex disorders such as infertility and bad obstetric history. (Fertil Steril® 2009;92:88–95. ©2009 by American Society for Reproductive Medicine.)

Key Words: Chromosomal variations, 9qh+, Yqh+, infertility, bad obstetric history, recurrent spontaneous abortions, male factor infertility, epigenetics

Polymorphic variations, particularly in the heterochromatic region of chromosomes 1, 9, 16, Y and the nucleolar organizing region (NOR) of acrocentric chromosomes are known to occur in the general population (1, 2). However, higher frequencies of these variants have recently been reported in infertile and subfertile individuals compared with population cytogenetic data obtained mainly from newborn screening surveys (3), though a review of literature showed opposing views both for and against the association of chromosome polymorphisms in clinical conditions such as reproductive failure (4). Chromosomal variations associated with male infertility, including structural or numerical chromosomal abnormalities and quantitative or positional modifications of the constitutive heterochromatin, have been shown to affect male gamete formation and function (5). A higher incidence of these variants in the infertile male population is attributed to polymorphic variations on the Y-chromosome, contributing to male infertility or subfertility possibly due to the silencing effect of these heterochromatic variations on otherwise normally expressed genes. However, very few data are available on the frequency of polymorphic chromosome variations in adult fertile individuals for comparison (6).

Recent studies have shown the occurrence of structural and numerical chromosomal abnormalities and chromosomal polymorphic variants in patients undergoing in vitro fertilization (IVF) and other assisted reproduction procedures such as intracytoplasmic sperm injection (ICSI) (7–10). Variants have also been shown to occur in one or both partners of couples with recurrent spontaneous abortions (RSA), bad obstetric history (BOH), and idiopathic infertility (11–13).

Received January 4, 2008; revised April 30, 2008; accepted May 15, 2008; published online August 11, 2008.
S.M. has nothing to disclose. A.S.A. has nothing to disclose. P.F.M. has nothing to disclose. D.K. has nothing to disclose. S.A.U. has nothing to disclose. F.R.P. has nothing to disclose.
Supported and funded by research project grants RP 398 and 317 of Jaslok Hospital and Research Centre Mumbai, India.
Reprint requests: Firuza R. Parikh, M.D., Department of Assisted Reproduction and Genetics, Jaslok Hospital and Research Centre, 15, Dr. G. Deshmukh Marg, Mumbai-400 026, India (FAX: +91-22-6657-3133; E-mail: frparikh@gmail.com).

0015-0282/09/$36.00
doi:10.1016/j.fertnstert.2008.05.071

Repeated sequences of noncoding DNA located a few megabases apart from each other in some heterochromatic regions of chromosomes have been shown to promote unequal recombination of homologues during cell division, responsible for inducing chromosomal aberrations such as inversions, deletions, or extensions (14). Hence, detailed studies on heterochromatin using higher resolution technologies would prove useful to identify genetic and/or epigenetic mechanisms associated with the manifestations of clinical disorders such as infertility (15).

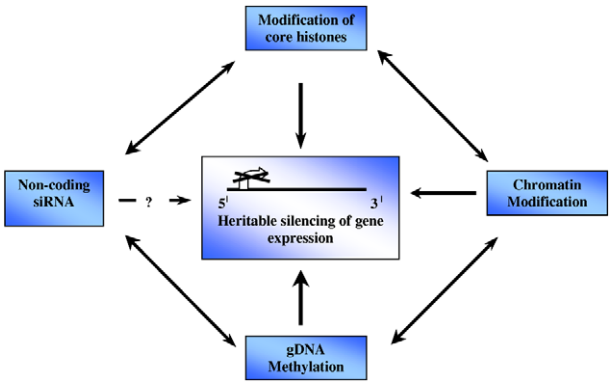
Earlier studies (16) led to a consensus that polymorphic variants did not play an important role in the etiology of recurrent spontaneous abortions. Hennig (17) reconsidered the properties of heterochromatin in the context of gene silencing, position-effect variegation, and X-chromosome inactivation and proposed that the chromatin in heterochromatic regions was generally similar in its molecular composition to that in silenced chromosomal regions. The appearance of heterochromatin therefore mirrors only a specific kind of chromatin packaging, which could be initiated by particular epigenetic signals in DNA such as gene promoter hypermethylation and histone modification, particularly hypermethylation and hypoacetylation of histone tails, comparable to those that are necessary for the inactivation/silencing of genes (17). A higher incidence of chromosomal variants in infertile men could be suggestive of large heterochromatic blocks being responsible for the weakening of chromosome pairing (18), spindle fiber attachment, or down-regulation of normally expressed/active genes, leading to meiotic arrest and infertility.

Heterochromatin is considered to be of two types, facultative and constitutive. Facultative heterochromatin is not rich in satellite DNA and is not very polymorphic. Constitutive heterochromatin is composed of satellite DNA *I, II,* or *III* and is known to be highly polymorphic and unstable. The abundance of satellite DNA in constitutive heterochromatin influences its properties for staining, as the highly condensed heterochromatin renders it strongly chromophilic and inaccessible to DNAse 1 and other restriction enzymes (19). On acrocentric chromosomes, the NOR region on the stalks (pstk) of satellites consists of rRNA, while the short arm (p) and satellites (ps) consist of heterochromatin. Chromatin modification, covalent modifications of histone core proteins, noncoding small interfering RNA (siRNA)–related silencing of gene expression, and reversible methylation of DNA all form part of epigenetic alterations that affect gene expression (20). Each of these mechanisms has been associated with the initiation of each other although the direction of control has not yet been proven (Fig. 1).

Certain heterochromatic regions are now known to be associated with stress stimuli such as heat shock and the assembly of heat shock proteins, which may result in the modification of gene expression (21, 22). Rizzi et al. (23) produced a primary finding of an active heterochromatic region of a chromosome, arising via transcription in the human genome in response to heat shock. Heat shock triggers the assembly of nuclear stress bodies that contain heat shock factor 1 (HSF 1) and a subset of RNA processing factors that are formed on the pericentromeric heterochromatic regions of specific chromosomes including chromosome 9, transcriptional activation of which is representative of an epigenetic status, similar to that of active euchromatic regions. To regulate HSF function, cells transmit growth control and developmental signals and interdigitate cellular physiology (24). Jolly et al. (25) have shown the formation and localization of HSF 1 granules during heat shock on the pericentromeric heterochromatic region 9q11–q12, giving the first example of a transcriptional activator that accumulates transiently and reversibly on a chromosome-specific heterochromatic locus. Heat shock factor 1 binds to a subfamily of DNA satellite III repeats through a direct DNA-protein interlink in the heterochromatin region (25). Though heterochromatic variations have not yet been proven to be responsible for clinical disorders such as infertility and behavioral problems, renewed interest in their possible association with the complexity of the disorder could help to identify an additional etiologic factor with the use of current technology and expertise in molecular biology.

FIGURE 1

Possible association of chromatin modification, covalent modifications of histone core proteins, siRNA, and DNA methylation with silencing of gene expression.



Minocherhomji. Chromosomal variations and infertility. Fertil Steril 2009.

MATERIAL AND METHODS

A total of 1315 individuals were included in this case-control study. Couples or individuals (n = 760) seeking infertility management at our center from April 2005 to October 2007 formed the study group, which consisted of 380 men and 380 women. The assisted reproduction techniques performed mainly included intracytoplasmic sperm injection (ICSI), use of donor gametes, or intrauterine insemination (IUI). The randomly selected age-matched fertile control group (n = 555) consisted of 212 men and 343 women from a similar