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The incidence of long heterochromatic polymorphism variants in infants conceived through assisted reproductive technologies

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Andrew Wilson received his BSc in the field of integrated sciences with a focus on molecular genetics in 2010, from the University of British Columbia, Vancouver, Canada. He received his medical degree in 2014 from same university. Under the supervision of Dr Sai Ma, Andrew has examined long heterochromatic variants on chromosomes 1, 9, 16 and Y in children conceived through assisted reproductive technologies.

KEY MESSAGE

Our study investigates the incidence of long heterochromatic polymorphisms on chromosomes 1, 9, 16 and Y, in children conceived using assisted reproductive technologies. Our results suggest that children conceived by assisted reproductive technologies are at no greater risk of inheriting these polymorphisms than children conceived naturally by fertile parents.

A B S T R A C T

Long heterochromatic variants on chromosomes 1, 9, 16 and Y are suspected to be implicated in infertility and early pregnancy loss, but little is known about how these variants are inherited in children conceived by infertile couples through assisted reproductive technologies. In this case–control study, the incidence of these variants was compared between infants conceived using intracytoplasmic sperm injection (ICSI), IVF and natural intercourse by karyotyping lymphocytes from cord blood or peripheral blood. This study included a total of 647 infants, including 189 conceived by ICSI, 177 by IVF, and 281 naturally conceived (NC). Variants were observed in 13.23% of ICSI, 15.82% of IVF and 12.46% of NC infants, showing that the incidence of variants does not appear to be significantly different between infants conceived using assisted reproductive technologies and infants conceived naturally. Because the parents of these infants were not karyotyped, we can only speculate as to whether these variants were directly inherited. This study concludes that infants born from infertile parents using assisted reproductive technologies to achieve pregnancy do not appear to be any more likely than NC infants of fertile parents to possess long heterochromatic variants.

Introduction

For the estimated 8–12% of couples worldwide with infertility (Ombelet et al., 2008), cytogenetic analysis is one of the first routes to investigating whether underlying chromosomal abnormalities may account for bad obstetric outcome and failure to achieve pregnancy. The vast majority of infertility cases examined by G-banded karyotype fail to reveal gross abnormalities and rearrangements, such as inversions or translocations, which could account for failed attempts to conceive. However, mounting evidence suggests that chromosomal regions rich in constitutive heterochromatin may be implicated in certain cases

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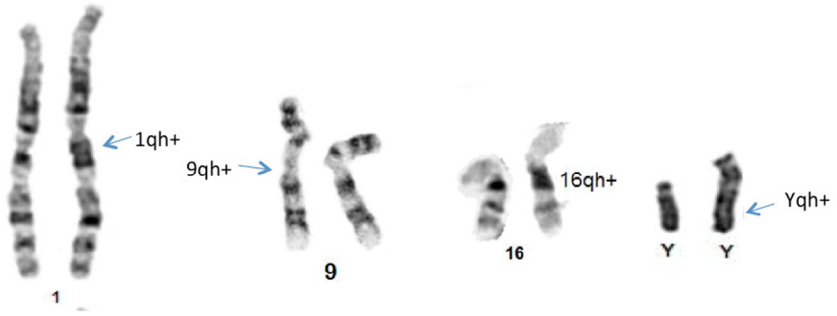

Figure 1 – Long heterochromatic variants of chromosomes 1, 9, 16 and Y. Arrows indicate excess heterochromatin on polymorphic homologue, and the type of polymorphism (long variant) is denoted by 'qh+'.

of unexplained infertility (Madon et al., 2005; Nakamura et al., 2001; Purandare et al., 2011; Šípek et al., 2014).

Constitutive heterochromatin refers to the subsets of the genome which are perpetually in their most highly condensed, transcriptionally repressed state. These areas are generally gene-poor, and consist of highly repetitive tandem sequences. Locations rich in constitutive heterochromatin, such as the subtelomeric and pericentromeric regions, tend to be conserved between individuals of the same species (Grewal and Jia, 2007). Since the introduction of chromosomal staining techniques such as G-banding and C-banding, it has been observed that human pericentromeric heterochromatin is highly heteromorphic, even between members of distinct ethnic populations (Bhasin, 2005). In humans, the subcentromeric regions of chromosomes 1, 9 and 16, as well as the distal q arm of chromosome Y, can vary hugely in size between individuals (Craig-Holmes and Shaw, 1971) (**Figure 1**), and in most cases such variants are stable, and are inherited from one generation to the next (Bhasin, 2005). These heteromorphisms are widely regarded as normal variants (Wyandt and Tonk, 2011), although some studies have implied that more pronounced variants may be involved in impaired sperm production (Yakin et al., 2005), recurrent pregnancy loss (Buretic-Tomijanovic et al., 1997) and general failure to achieve pregnancy (Guo et al., 2012).

The mechanisms through which pronounced heteromorphisms could contribute to infertility are still unclear, but the greater prevalence of these variants among infertile males versus infertile females (Akbaş et al., 2012; Cortés-Gutiérrez et al., 2001) suggests their influence may manifest during the process of spermatogenesis. Impaired synapsis and reduced number of crossovers during meiosis I are associated with abnormal sperm production in azoospermic and oligozoospermic men (Ferguson et al., 2007, 2009; Sarrate et al., 2014). The presence of an abnormally long heteromorphism results in the looping out and asynapsis of this portion of the chromosome during metaphase I, as observed in studies of infertile carriers of these variants (García-Peiró et al., 2011; Sarrate et al., 2014; Solari et al., 1991). This phenomenon could be at least partially responsible for defective spermatogenesis in some males presenting with infertility, although the presence of these variants alone is not significantly associated with abnormal semen parameters (Yakin et al., 2005).

Despite numerous investigations into the incidence of heterochromatic variants among infertile individuals and the general population, no known studies to date have attempted to compare the incidence of these variants between children conceived through different types of assisted reproductive technology, such as IVF and intracytoplasmic sperm injection (ICSI), and children conceived through natural intercourse. A study of this nature could substantiate the evidence in support of the influence of polymorphic variants on infertility, via spermatogenesis. If these variants are overrepresented in infertile males as the literature suggests, one would expect a higher rate of variants in ICSI offspring compared with those from IVF, although it is common for ICSI to be used in cases of both male and female factor infertility. Infertile men who opt for ICSI tend to have more severe oligo- or azoospermic phenotypes, requiring spermatozoa to be isolated from the testicular tissue, bypassing the selection process against spermatocytes with impaired synapsis at meiosis I. IVF, requiring the use of ejaculate spermatozoa, may result in offspring with fewer heteromorphic variants, as these spermatozoa have been vetted through normal spermatogenic progression. In this study, we will investigate whether populations of children conceived by IVF, ICSI or through natural intercourse differ with regards to pronounced heterochromatic variants on chromosomes 1, 9, 16 and Y.

Materials and methods

Participants

A total of 647 cord blood and peripheral blood samples were collected for this case-control association study. Cord blood samples were from ICSI, IVF and naturally conceived (NC) term pregnancies delivered between 2002 and 2015, and peripheral blood was drawn from one infant >1 year of age for whom cord blood was not collected at the time of delivery. Patients were recruited from the UBC Centre for Reproductive Health and other fertility centres and hospitals in the Greater Vancouver area. Ethical approval was obtained from the University of British Columbia Ethics Board (reference number H13-02161) and written consent to use cord blood or peripheral blood for the purposes of this study was given by the mother.

The inclusion criteria for the assisted reproductive technology group (ICSI and IVF) were pregnancies resulting in live birth, conceived by either IVF or ICSI. For the NC control group, we included pregnancies resulting in live births that were conceived spontaneously to mothers after <1 year of trying, excluding any pregnancies with complications such as pre-eclampsia, gestational diabetes or adverse perinatal outcomes. The ICSI group included couples with both male, female and unknown sources of infertility. Maternal age of participants ranged from 15 to 46 years, gestational age ranged

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