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ARTICLE

Meiotic segregation analysis of embryos from reciprocal translocation carriers in PGD cycles

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Abstract Meiotic segregation patterns of 278 embryos from 41 preimplantation genetic diagnosis cycles of 34 reciprocal translocation carriers were analysed to investigate whether some characteristics of reciprocal translocation, including terminal breakpoints, acrocentric chromosome or carrier gender, are related to meiotic segregation patterns. The incidence of normal/balanced karyotypes in translocations with terminal breakpoints was significantly lower than those without terminal breakpoints (6.5% versus 14.4%, $P = 0.005$). The incidences of adjacent-1 (21.0% versus 29.6%), adjacent-2 (16.1% versus 11.1%) and 3:1 (41.9% versus 30.6%) segregation were not statistically significantly different in translocations with terminal breakpoints versus those without. Translocation with acrocentric chromosomes showed a significantly lower rate of 2:2 segregation (39.2% versus 60.2%, $P = 0.001$) and a higher rate of 3:1 segregation (43.1% versus 27.3%, $P = 0.005$) than those without acrocentric chromosomes. The incidence of 2:2 segregation was significantly higher in male than in female carriers (58.2% versus 45.0%, $P = 0.019$). This study suggested that reciprocal translocation involving terminal breakpoints resulted in a lower rate of normal/balanced karyotype in preimplantation embryos. Some characteristics of reciprocal translocation, such as terminal breakpoints, acrocentric chromosome and carrier gender, are related to the segregation patterns.

KEYWORDS: fluorescence in-situ hybridization, meiotic segregation, preimplantation genetic diagnosis, reciprocal translocation

Introduction

Reciprocal translocations are the most common chromosome abnormalities, found in 0.14% of the neonatal population (Nielsen and Wohlert, 1991). Typically they result from exchange of two terminal segments from different chromosomes. During meiosis I, translocated chromosomes and their normal homologues can form quadrivalents and segregate by alternate, adjacent-I, adjacent-II, 3:1 or 4:0 modes. Genetically normal/balanced gametes are produced by alternate or adjacent-1 segregation following an odd number of crossovers in the interstitial segment between the centromere and the breakpoint, in which translocated chromosomes move to one pole and the normal homologues to

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the other. Other segregation modes would lead to the production of chromosomally unbalanced gametes. Thus, carriers of reciprocal translocations may suffer from reproductive problems, such as birth of chromosomally abnormal children, recurrent spontaneous abortion or infertility.

Preimplantation genetic diagnosis (PGD) has been offered to carriers of reciprocal translocations to reduce the frequency of spontaneous abortions and affected newborns by selecting chromosomally normal/balanced embryos. The pregnancy rate following PGD is closely related to the number of chromosomally normal embryos available for transfer (Munné, 2001). However, different from Robertsonian translocations, the proportion of embryos consistent with alternate segregation in reciprocal translocations is relatively low. In some PGD cycles with reciprocal translocation, embryo transfer has to be cancelled due to the lack of genetically balanced embryos. Thus, it would be important to predict the segregation pattern of a reciprocal translocation and provide genetic counselling to the patient before PGD.

The rate of chromosome abnormalities detected by sperm fluorescence in-situ hybridization (FISH) varies widely, from 18% to 82%, depending on the translocation (Escudero et al., 2003; Munné, 2005), which suggests that each reciprocal translocation has a specific segregation pattern. Thus, it may be difficult to analyse the meiotic behaviour of reciprocal translocation. However, previous studies have found that meiotic behaviour is associated with some specific features of translocation, such as the size of the translocated segments and the presence of centromeres from acrocentric chromosomes in the centre of the cross (Anton et al., 2008; Lim et al., 2008; Munné, 2001). It has been suggested that translocations involving chromosome with terminal breakpoints or acrocentric chromosomes would not form a quadrivalent with close configuration in the first metaphase of meiosis, resulting in higher proportion of unbalanced gametes (Escudero et al., 2003). Moreover, it has been demonstrated that few pregnancies are obtained through PGD when there is a terminal breakpoint in the translocation (Munné, 2001; Munné et al., 2000). Carrier gender has also been reported to have some effect on the segregation products (Ko et al., 2010; Lledo et al., 2010; Ogilvie and Scriven, 2002; Pinton et al., 2009). The present study presents PGD clinical outcomes and evaluates whether some characteristics of translocation are associated with the segregation pattern of embryos.

Materials and methods

Institutional Review Board approval was obtained from the Ethics Committee, Zhejiang University School of Medicine (code number 20090014).

Patients

Thirty-four couples with reciprocal translocations had 41 FISH/PGD cycles from July 2006 through February 2010. The karyotypes of each patient are presented in **Table 1**. The translocations of the patients were ascertained because of spontaneous miscarriage ($n = 19$), routine examination before IVF treatment ($n = 13$) or a child with an unbalanced karyotype ($n = 2$). Twenty-two cycles of 17 couples involved female carriers and 19 cycles from 17 couples involved male carriers.

Embryo cryopreservation and thawing

In most cases (38/41 cycles), fresh embryos were used to make the diagnosis; however, in three FISH/PGD cycles, frozen–thawed embryos were analysed as all viable embryos were frozen in the fresh cycles because of a high risk of ovarian hyperstimulation syndrome. Embryo cryopreservation was performed by a slow-freezing protocol which employed propanediol and sucrose as cryoprotectants (Testart et al., 1986). The embryos were loaded into straws and frozen in a programmable freezer (Planer, Sunbury-on-Thames, UK).

To thaw the embryos, the straws were removed from the liquid nitrogen and maintained at room temperature for 40 s, followed by immersion in a 30 °C water bath for another 40 s. Then the embryos were exposed to a series of solutions with decreasing propanediol concentration. Before the PGD cycle was started, the translocation of each carrier was confirmed by performing FISH on peripheral blood lymphocytes. Informed consent was obtained from all the couples.

Ovarian stimulation and IVF

Ovarian stimulation was performed with FSH (Gonal-F; Serono, Geneva, Switzerland) and human menopausal gonadotrophin (Pergonal; Serono), after pituitary function was down-regulated with gonadotrophin-releasing hormone (GnRH) agonist (GnRH-a, Decapeptyl; Ferring, Lausanne, Switzerland) or antagonist (Centrotide; Serono). Follicular development was monitored using serial vaginal ultrasound and serum oestradiol concentrations. A dose of 10,000 U of human chorionic gonadotrophin (HCG) was administered when two or more follicles reached 18-mm mean diameter. Oocytes were transvaginally retrieved under ultrasound guidance 36 h after triggering ovulation. Retrieved mature oocytes were fertilized by intracytoplasmic sperm injection (ICSI). About 17 h later, the oocytes were checked for pronuclei and polar bodies. Fertilized zygotes were cultured in G1 medium (Vitrolife, Kungsbacka, Sweden) at 37°C in a humidified atmosphere with 6% CO_2.

Blastomere biopsy

To differentiate between products consistent with 4:0 segregation and haploidy or triploidy by FISH, one more probe not involved in the translocated chromosomes should ideally be used; however, this method was not adopted, instead only zygotes with two pronuclei and two polar bodies were further cultured and biopsied on day 3. Thus, few embryos would be haploid or triploid.

Embryo development was evaluated on the morning of day 3. Good-quality embryos with more than five cells were used for PGD. Laser or acid Tyrode's solution was applied to create a hole in the zona pellucida. Then one blastomere was gently aspirated through the hole. The biopsied embryos were transferred to G2 medium (Vitrolife) for further culture.