Presentation and Treatment of Subfertile Men with Balanced Translocations: The Cleveland Clinic Experience

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Abstract

Introduction: Balanced chromosomal translocations are a relatively common (2–7%) finding among infertile couples. We report clinical features of males with translocations at our institution. ***Materials and Methods:*** Data was collected on men presenting for infertility evaluation between July 2006-March 2010, including presentation, medical history, and infertility treatments. Criteria for genetic evaluation, consisting of karyotype and Y-linked microdeletion assay, included severe oligozoospermia or azoospermia (sperm concentration < 2.5×106/ml) or a history of recurrent miscarriages. ***Results:*** Of the 4,612 patients in our male infertility clinic 306 met criteria for genetic evaluation. Three patients had a balanced translocation, of which 2 had Robertsonian translocations, and 1 had a balanced translocation. One patient had normal bulk semen parameters, normal volume azoospermia, and oligoasthenoteratozoospermia. All patients were offered medical genetics consultation. Potential pregnancy outcomes were evaluated using a predictive software package. One patient had intratubular germ cell neoplasia and underwent orchiectomy; subsequent fertility evaluation has been deferred. The other 2 are considering in-vitro fertilization with pre-implantation genetic evaluation. ***Conclusions:*** Given the low incidence of balanced translocations detected in our population, better clinical indicators other than semen parameters or history of recurrent pregnancy loss are needed to determine screening for this finding.

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Introduction

Chromosomal abnormalities are a significant cause of infertility [1]. A reciprocal translocation is an interchange of chromosomal material between specific chromosomes [2]. These are balanced when the exchange does not result in loss of genetic material [3], and unbalanced when genetic material is gained and/or lost. The incidence of balanced autosomal translocations in infertile men has been reported between 1.6 and 6.65% [1, 4–7].

Robertsonian translocations are the most common type of balanced translocation [7], with an incidence of 1.2 per 1000 live newborns [8–10]. A Robertsonian translocation involves translocation between 2 acrocentric human chromosomes (chromosomes 13, 14, 15, 21, or 22) [8], in which the 2 long (q) arms of each chromosome join to form a new chromosome and the 2 short (p) arms are lost. The resulting karyotype yields 45 chromosomes, but is considered balanced given the short arms contain repetitive genetic material. Seventy-three percent of Robertso-

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nian translocations occur between chromosomes 13 and 14, with 10% between chromosomes 14 and 21 [11].

Balanced reciprocal translocation carriers typically have a normal phenotype if the chromosome complement is truly balanced; the conceptus, however, may have an unbalanced karyotype depending on meiotic segregation leading to repeated spontaneous abortions and thus an infertility evaluation [8]. We report a small series of infertile patients with balanced translocations and review the current literature on balanced reciprocal translocations, with a focus on Robertsonian translocations.

Materials and Methods

We performed an IRB-approved chart review on patients presenting with male factor subfertility between July 2006 and March 2010. Genetic testing, consisting of karyotype and a Y-linked microdeletion assay, was obtained in men with azoospermia, severe oligozoospermia (sperm concentration $< 2.5 \times 10^6$/ml), or a history of recurrent (≥ 2) miscarriages. We reviewed the number of karyotype analyses and Y chromosome microdeletions performed in the Cleveland Clinic central laboratory system to identify the total number tested. Charts were reviewed for presentation, past medical history, family history, fertility treatments, and medical genetics counseling.

Genetics counseling is recommended to all patients with abnormal genetic testing. Our medical genetics counselors utilize a novel computer program, developed by Dr. Carolyn Trunca, at the Genetics Center, to predict offspring karyotype and miscarriage risk in couples with balanced translocations interested in conception (www.thegeneticscenter.com/transrsk.htm). This model is based on retrospective data from over 900 translocation families and estimates risk based on patient gender, infertility presentation, and the translocation discovered. This risk analysis has not been peer reviewed or published.

Results

Of 4,612 infertile males, 306 (6.6%) underwent genetic testing during the 4-year period. Three patients (0.07% of the total and 0.98% of those undergoing genetic evaluation) were found to have balanced translocations: 2 with a Robertsonian translocation, and 1 with a balanced translocation. Their individual cases are discussed.

Case 1
A 29-year-old Caucasian male presented with primary infertility. He had a previous diagnosis of dyslexia and a left inguinal hernia repair with concomitant hydrocelectomy at 3 years of age. Notably, his family history is significant for: 1) a maternal cousin with trisomy 13; 2)

another maternal cousin with a balanced 13;14 Robertsonian translocation, and 3) a general history of recurrent pregnancy losses and newborn deaths in maternal relatives.

The patient had an atrophic left testicle with a volume of 4 ml and a contralateral testicular volume of 14 ml. He was well virilized. Semen analysis demonstrated normal volume azoospermia. He had low testosterone levels (200.6 ng/dl, normal 220–1000 ng/dl), mildly elevated prolactin (16.4 ng/ml, normal 2.0–14.0 ng/ml), mildly elevated follicular stimulating hormone (10.4 mU/ml, normal 1–10 mU/ml), and normal luteinizing hormone (3 mU/ml, normal 1.0–7.0 mU/ml). Genetic analysis demonstrated a balanced Robertsonian translocation 45,XY,t(13;14) (q10;q10).

The patient underwent a microscopic testicular sperm extraction (TESE) and biopsy. Pathology demonstrated Sertoli-cell only syndrome in his left testicle and intratubular germ cell neoplasia (ITGCN) in his right testicle. Oncologic TESE was performed on the right testicle prior to radical orchiectomy, but no viable sperm were identified. Final right testicular pathology confirmed ITGCN.

The patient has also been recently diagnosed with Osler-Weber-Rendu disease after further work-up for his ITGCN and prolactinemia found a low grade brain glioma and multiple cerebral and pulmonary arteriovenous malformations. This diagnosis is believed due to a spontaneous genetic event, unrelated to his Robertsonian translocation carrier status.

The couple was referred for genetic counseling regarding the patient's 13;14 Robertsonian translocation. Based on chromosomal segregation patterns, the couple has a 1:3 chance of conceiving offspring with the appropriate amount of genetic material – either being chromosomally normal (46,XX or XY) or being a balanced Robertsonian translocation carrier. Based on cytogenetic analysis, sperm from a 13;14 translocation carrier have an 80% chance of being balanced due to selective advantage, resulting in either a genetically normal gamete or a balance translocation carrier [2, 9]. The Robertsonian translocation potentially affects the success of reproductive technologies such as in vitro fertilization and intracytoplasmic sperm injection (ICSI) due to meiotic segregational abnormalities caused by possessing a Robertsonian translocation [2, 9]. The patient has deferred further fertility work-up for now.

Case 2
A 32-year-old Caucasian male presented with his wife for recurrent miscarriages (3 in 3 years). The patient was

well-virilized with normal volume testicles (~20 ml). Genetic testing revealed a Robertsonian translocation 45,XY,t(13;14) (q10;q10). The patient's other medical and family history was non-contributory.

The patient had a semen analysis with normal bulk semen parameters. He and his wife have been offered genetic counseling; however the couple has not yet pursued this option.

Case 3

A 30-year-old Middle Eastern male presented with his wife for secondary infertility after 3 spontaneous miscarriages. The karyotype of one lost conceptus revealed a 7;9 translocation. The patient's past medical and family history were non-contributory. The patient was well-virilized with normal volume testicles (~20 ml). Semen analysis demonstrated normal volume oligoasthenoteratozoospermia with a sperm concentration of < 2.5 ×106/ml. Karyotype analysis revealed 46,XY,t(7;9)(q31.2;p22).

The couple was referred for genetic counseling. Due to the limited data in the literature on this specific genetic abnormality, a translocation specific risk estimate for miscarriage and risk of unbalanced live-born utilizing the genetic software program was performed. The risk of miscarriage was projected to be 18%, slightly above the 10–15% miscarriage risk in the general population. The risk of a live-born offspring with an unbalanced chromosomal complement was estimated at 6–9%. The couple has been counseled regarding in vitro fertilization with PGD or conceiving naturally with prenatal diagnostic techniques to follow early in the pregnancy.

Discussion

Overview

Genetic factors, like specific chromosomal structural abnormalities and specific genetic conditions (i.e. CFTR gene of cystic fibrosis, Y chromosome microdeletions) are known to be associated with infertility [12]. In a large study of 376 couples evaluated for infertility, repeated spontaneous abortions, or malformed or stillborn offspring, chromosomal aberrations were found in 9% of patients [1].

Balanced translocations, Robertsonian or reciprocal, are the most common chromosomal structural rearrangements identified [12]. A Japanese study found an increased frequency of balanced chromosomal rearrangements in patients with spontaneous abortions as compared to the general population [4]. In 54 couples with chromosomal

anomalies, there was a 90% abortion rate with only 18 live-born neonates (10%) out of 181 pregnancies. This diagnosis is important in identifying couples with translocations that would benefit from prenatal technologies such as PGD and early amniocentesis [5].

While most Robertsonian translocation are inherited from a parent, up to 40% can be de novo [13]. We found that 1 of our 2 Robertsonian translocations had a clear maternal pattern of inheritance. De novo translocations can occur due to rearrangements in meiosis [14], however, 1 study found that spermatozoal irradiation induced some reciprocal translocations that could result in maturation arrest and resulting infertility [15].

Pathogenesis of Infertility with a
Balanced Translocation

Chromosomal translocations may affect fertility due disruption of meiosis [7]; segregation during meiosis results in gametes with duplication or deficiency of chromosome segments (aneusomy or trisomy) [9, 12]. Some studies indicate that aberrant chromosomal pairing during meiosis in balanced translocation carriers may interfere with certain genetic processes and cause germ cell arrest [16, 17].

The functionality of genes at specific breakpoints may be altered as well, perhaps with a specific role in spermatogenesis. This may cause defective spermatogenesis resulting in the abnormalities seen on semen analyses [18]. One study found 100 break events in 90 different chromosomal regions, preferentially in GTG-light bands, with associated unstable or fragile sites and areas of segmental duplications in patients with repeated spontaneous abortions, unexplained infertility, or children with congenital malformations [12]. Specific genes have been found at the breakpoints in balanced translocations that may function in spermatogenesis and sperm maturation, such as zinc finger proteins 76 and 165, glutathione peroxidase 5, testis abundant finger protein, and casein kinase 2 beta [18].

Another theory is the idea of 'position effect,' where flanking genes may be important in gene activation leading to a specific phenotype. When a balanced translocation is present, these flanking regions can be moved and interrupt pairings between the gene itself and its surrounding environment, affecting ultimate function [19].

Semen Analysis of Balanced Translocations

Given the variety of affects genetic abnormalities can have on spermatogenesis, it is not uncommon to see abnormal semen analysis parameters associated with bal-

anced translocations. Abnormal values have been reported in > 80% of patients [20] and consist primarily of oligospermia [6, 7]. When compared to other chromosomal abnormalities, the mean sperm concentration in Robertsonian translocation carriers has been found to be $4.2 \pm 1.1 \times 10^6$/ml versus $8.0 \pm 2.2 \times 10^6$/ml in men with other chromosomal abnormalities and Yq microdeletion [2].

There has been renewed interest in investigating the meiotic chromosomal segregation in male balanced translocation carriers in order to determine the impact on fertility. Chandley et al. [21] found a reduced proportion of spermatids and spermatozoa to spermatogonia and spermatocytes on testicular biopsy in subfertile men with balanced translocations. They hypothesized that germ cells of balanced translocation carriers cannot proceed to meiosis, resulting in the apparent infertility. Perrin et al. [22] examined spermatogonia meiotic segregation and DNA fragmentation in those with genetic abnormalities and found chromosomally unbalanced spermatozoa present 55% and 14% of the time in patients with a balanced reciprocal translocation and a Robertsonian translocation, respectively. DNA fragmentation was significantly more common in carriers of these structural chromosomal abnormalities compared to those with normal genetics. They concluded that infertility in this patient population was related to the chromosomal abnormalities and high rate of DNA fragmentation, a marker of apoptosis and oxidative damage.

Brugnon et al. [23] evaluated meiotic segregation of sperm in translocation carriers, looking at markers of apoptosis. They utilized the annexin V binding assay and found an increased number of sperm with externalized phosphatidylserine, an early event in apoptosis in somatic cells, in those with reciprocal translocation (p ≤ 0.007) and those with Robertsonian translocation (p ≤ 0.006) as compared to normal controls. DNA fragmentation was also higher in translocation carriers (p < 0.0001); while sperm concentrations and motility were lower as compared to the control group. They concluded that apoptosis may explain chromosomal translocation carrier infertility, with a checkpoint in spermatogenesis ensuring the production of functional gametes. However, a correlation between phosphatidylserine externalization and DNA fragmentation was not identified, suggesting that these anomalies occur at different phases of apoptosis.

A subsequent study by Brugnon et al. [24] examined the biochemical and ultrastructural characteristics of apoptotic sperm in oligoasthenoteratozoospermic Robertsonian translocation carriers compared to a control group of fertile donors with normal semen parameters. They again found that carriers had lower sperm concentration (11 vs. 72×10^6/ml, p = 0.002), decreased forward motility (32 vs. 55%, p = 0.006), lower normal morphology (4 vs. 31%; p = 0.006), and higher round cell concentration (4 vs. 0.5×10^6/ml, p < 0.05). Robertsonian sperm also had a higher proportion of activated caspases, involved in apoptosis (42–57% vs. 10–26%), a higher rate of DNA fragmentation (26 vs. 13%), and a higher percentage of immature sperm on electron microscopy (28 vs. 10%) and apoptotic sperm (24.5 vs. 18.5%). Further, fertile male donors overall were found to have a higher percentage of spermatozoa void of ultrastructural defects (49 vs. 27%) compared to the spermatozoa of male Robertsonian translocation carriers. This further led to the conclusion that Robertsonian translocations result in impaired spermatogenesis, possibly predicting the success of assisted reproductive technology (ART).

Given meiotic segregation appears to be unbalanced in the minority of cases [8, 9], perhaps the high incidence of apoptosis in chromosomal translocation carriers is actually part of a selective process to ensure that only balanced spermatozoa are able to survive.

Management

Carriers of balanced reciprocal translocations may require ART with in vitro fertilization and ICSI depending on their sperm counts [2, 25]. One of the benefits of ICSI is the ability for preimplantation genetic diagnosis to allow for a < 50% risk of an unbalanced offspring [26]. Fluorescence in-situ hybridization can also be used in combination with PGD to analyze the chromosomal arrangement of the offspring [26]. PGD then allows the selective transfer of a 'normal' or balanced embryo [27].

Lim et al. [29] evaluated the efficacy and outcome of PGD in couples with known chromosomal translocations and found a 28.6% success in clinical outcome after PGD in couples with a balanced translocation and reduction in spontaneous abortion rate with PGD from 95.8 to 16.7%. They used PGD to determine the risk of chromosomal imbalances in preimplantation embryos of reciprocal translocation carriers in order to properly counsel patients and found a decrease in the spontaneous abortion rate from 95.2 to 17.6% of pregnancies with PGD resulting in the delivery of 20 liveborn infants. Further, patients with acrocentric chromosomes (more reflective of a Robertsonian translocation) were found to have increased meiotic and mitotic instability which could lead to an increase in the development of abnormal gametes [27].

Proper counseling is crucial for couples with balanced translocation interested in fertility as to their appropriate reproductive options. Couples need to be counseled that even the best ART is not a failsafe to guarantee reproduction since Robertsonian translocation carriers may have a greater than two- to four- fold increased risk for multiple ART failures when compared to those with Y linked microdeletions (> 4 failed attempts as compared to 1–2 in those simply with Yq microdeletion) [2]. Therefore, we find that our genetics counselors are an indispensable part of the infertility evaluation.

Conclusion

The incidence of balanced translocation in our population of subfertile patients undergoing genetic evaluation is 0.98%. Given this incidence is lower than the 2– 7% reported in the literature, chromosome translocation carriers may be missed in some of our patients presenting for work-up for infertility. This leads us to question whether semen parameters and/or a history of recurrent pregnancy loss are adequate as the only indications for chromosomal screening in this population.

There are currently no absolute means of determining which patients with infertility require a genetics evaluation. The American College of Obstetricians and Gynecologists recommend a peripheral blood karyotype on both partners for recurrent pregnancy loss [30]. We propose that improved clinical indicators are needed to identify infertile men that require genetic screening for the presence of structural chromosomal abnormalities. Further identification of structural chromosome rearrangements in patients presenting with male infertility can lead to better reproductive outcomes for the patient.

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