Chapter 24
FISH

Kaoru Suzumori

Abstract The establishment of fluorescence in situ hybridization (FISH) techniques has enabled the detection of DNA copy number changes for the mapping of target DNA sequences [Hopman et al. (Molecular neuroanatomy. Elsevier, 1988)]. This technique has a wide range of applications, such as for gene mapping and the ordering of DNA sequences on chromosomes and as an adjunct to conventional cytogenetics for characterizing chromosomal aberrations [Ferguson-Smith and Yates (Am J Hum Genet 48:178, 1991)]. Probes for FISH analysis may consist of DNA segments, such as α-satellite DNA from the centromeric regions, other repetitive DNA sequences, and unique DNA sequences of chromosomes. Widely used chromosome-specific probes are classified as 'repetitive' (centromeric regions) probes [Cremer et al. (Hum Genet 74:346, 1986)], 'painting' probes, and 'locus-specific' probes according to their complementary location on the chromosome. The use of fluorescence microscopy allows the detection of multiple probes, each labeled with a different color. The advancement of this technology now allows combinational fluorescence with 24 different colors that can be visualized on the same metaphase spread, thereby highlighting each chromosome pair [Schröck et al. (Science 273:494, 1996), Speicher et al. (Nat Genet 12:368, 1996)].

This chapter focuses on FISH analysis for fetal aneuploidies, meiotic segregation modes in men with constitutional chromosomal abnormalities, and the prenatal diagnosis of carriers of a complex constitutional chromosome abnormality using spectral karyotyping.

Keywords FISH · Robertsonian translocation · Reciprocal translocation · Meiotic segregation · Spectral karyotyping

K. Suzumori (✉)
Fetal Life Science Center Ltd., Nagoya City University Graduate School of Medical Science, Nagoya, Japan
e-mail: k.suzumori@flsc.jp

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24.1 Rapid Prenatal Diagnosis of Aneuploidies by FISH

The frequency of chromosomal abnormalities has been estimated to be 0.65–0.84% in surveys of newborn babies [1–4], and approximately 80–95% of these abnormalities are common aneuploidies in chromosomes 13, 18, 21, X, or Y [5, 6]. Of note, the risk of these common aneuploidies is correlated with maternal age, and the total frequency of these aneuploidies was 2.26% among pregnancies with a maternal age of over 35 years [7]. Since the 1980s, indications for a prenatal diagnosis of pregnancies at risk of common aneuploidies have included advanced maternal age, abnormal maternal serum screening results, abnormal ultrasound findings in the fetus, and a family history of chromosomal abnormalities. For the past 50 years, G-banding chromosome analysis using cultured amniotic fluid cells has been a key procedure for prenatal diagnosis. Prenatal cytogenetic diagnosis has improved due to the use of metaphase harvesting and a significant reduction in test turnaround times, and final test results can now be obtained within 10–12 days. When cytogenetic results indicate a serious chromosomal abnormality, even a short wait for results can increase the emotional burden on the patient and/or physician.

Rapid detection for aneuploidy in chromosomes 13, 18, 21, X, and Y from interphase amniotic fluid cells is possible with the use of directly labeled, multicolored, commercially available DNA probes. The probes comprise two sets: one set contains 13 unique sequences at 13q14.2 (green), the region 21q22.13 (orange), and the second set contains α-satellite centromere probes for chromosomes X (green), Y (orange), and 18 (blue). Such commercially available probe kits include Cytocell (Oxford Gene Technology, Cambridge, UK) and AneuVysion (AbbotT/Vysis, Downers Grove, IL), among others. Figure 24.1 shows FISH results from four pregnant women with normal female fetus (a), trisomy 21 male (b), trisomy 13 male (c), and trisomy 18 female (d). With probe set 1 (left cell), a normal cell should show two green and two orange (2G, 2O), and with probe set 2 (right cell), a female cell should show two green and two blue signals (2G, 2B) (Fig. 24.1a). While male cells with trisomy 21 should show two green and three orange signals (2G, 3O) with probe set 1, and one green, one orange, and two blue signals with probe set 2 (1G, 1O, 2B) (Fig. 24.1b). With probe sets 1 and 2, male cells with trisomy 13 should show three green and two orange signals (3G, 2O) and two blue, one green, and one orange signals (2B, 1G, 1O) (Fig. 24.1c). However, cells having an extra chromosome 18 should show two green and two orange signals (2G, 2O) and two green and three blue signals in female samples (2G, 3B) (Fig. 24.1d).

Standards for evaluating samples need to be instituted and followed. It has been suggested that a minimum of 50 interphase nuclei with defined hybridization signals should be enumerated for each chromosome and that 80% of cells should show two signals to be considered disomic, while 70% of cells should show three signals to be considered trisomic.

The first clinical trial of FISH analysis for the detection of common aneuploidies involving chromosomes 13, 18, 21, X, and Y was reported in 1990 by Klinger et al. [8]. The usefulness of interphase FISH analysis for the rapid prenatal diagnosis of

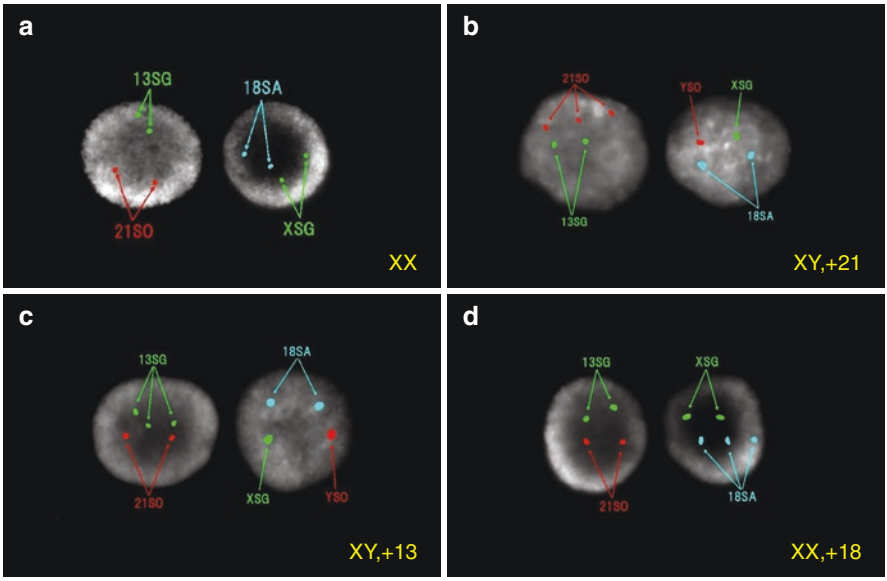

Fig. 24.1 Examples of interphase cell FISH results

aneuploidy has since been confirmed in a number of studies [9–11]. With commercially available FISH prenatal enumeration probe kits, Weremwicz et al. [12] reported an extremely high performance with 94% sensitivity for common aneuploidies and at a 0.1% false positive rate in informative samples. Many papers have demonstrated that inconclusive or informative results are seen in a low percentage of cases, such as those with bloody amniotic fluid or oligohydramnios [13]. In general, this FISH-based procedure cannot detect aneuploidy of nontargeted chromosomes, nor is it currently designed to detect euploid states with other cytogenetic abnormalities, such as translocations, inversions, and marker chromosomes. It has been shown that careful genetic counseling is an important adjunct when ordering FISH testing, and it is essential to explain to patients the limitations of FISH, including its inability to detect all chromosomal abnormalities as well as the possibility of maternal cell contamination, rare technical failures, and uninformative or false negative results in some cases.

Despite its usefulness, care must be taken for the clinical application of FISH assays due to possible pitfalls. The American College of Medical Genetics [14] has issued a policy statement for the clinical application of prenatal interphase FISH assays because of the severe implications of a false positive result. The policy statement called for the reliability, reproducibility, and accuracy of the clinical application of FISH probe sets is to be demonstrated. Prenatal interphase FISH is not a standard procedure and should only be used as an adjunct test with conventional chromosome analysis serving as the primary diagnosis and confirmatory evaluation. Appropriate physician and patient consent should be obtained, and patient management decisions should not be made based on results obtained by FISH alone.

The ACMG recommends the following provisions: (1) Proper informed consent should be obtained following explanations of the purpose, accuracy, potential risks, and limitations of FISH testing; (2) FISH should be used in prenatal interphase cytogenetics only in conjunction with standard cytogenetic analysis; (3) Irreversible therapeutic action should not be initiated on the basis of FISH analysis alone; (4) Providers should confirm the applicability of FISH analysis in prenatal diagnosis after assessing the reproducibility, sensitivity, specificity, and positive and negative predictive values; (5) Appropriate quality assurance/quality control for reagents, as well as techniques in the development of standardized protocols, must be established for FISH analysis.

In conclusion, prenatal FISH detection is valuable for the screening of common aneuploidies, followed by a complete chromosome analysis to confirm anomalies.

24.2 Aneuploidy in Human Spermatozoa: FISH Analysis in Men with Constitutional Chromosomal Abnormalities

Balanced Robertsonian or reciprocal translocations are constitutional chromosomal abnormalities that predispose carriers to the production of chromosomally abnormal gametes. These abnormalities contribute to recurrent abortions of conceptuses with monosomy or trisomy. In general, most autosomal monosomies are eliminated after fertilization, during early pregnancy or in the perinatal period. For this reason, most of them are found in spontaneous abortions [15]. Reproductive failures are closely associated with parental chromosome abnormalities. Male carriers of constitutional chromosome abnormalities may have fertility problems associated with low sperm counts and abnormal sperm morphology. Indeed, among 9207 infertile males reviewed, 0.8% were carriers of a Robertsonian translocation and 0.6% were carriers of a reciprocal translocation [16].

A Robertsonian translocation is a fusion of the long arms of two acrocentric chromosomes 13–15, 21, and 22 after a breakage in the short arms. An individual with what is called a balanced Robertsonian translocation shows only 45 chromosomes, with the translocation chromosomes containing the two complete long arms of the two acrocentric chromosomes involved. The short arm fragments of the translocated chromosomes are lost. Carriers are divided into six groups according to the chromosomes in the translocation: der(13;14), der(14;21), der(13;15), der(14;15), der(13;22), and der(14;22). Logically, during meiosis, pairing and segregation occur through the formation of trivalent in meiosis I. Alternative segregation results in two balanced gametes of either normal chromosomes A and B or derivative der(A;B). The babies with this mode of segregation are usually phenotypically unaffected. In contrast, adjacent segregation modes lead to either sperm nullisomy A or sperm disomy B and produce unbalanced products with monosomy A or trisomy B. The

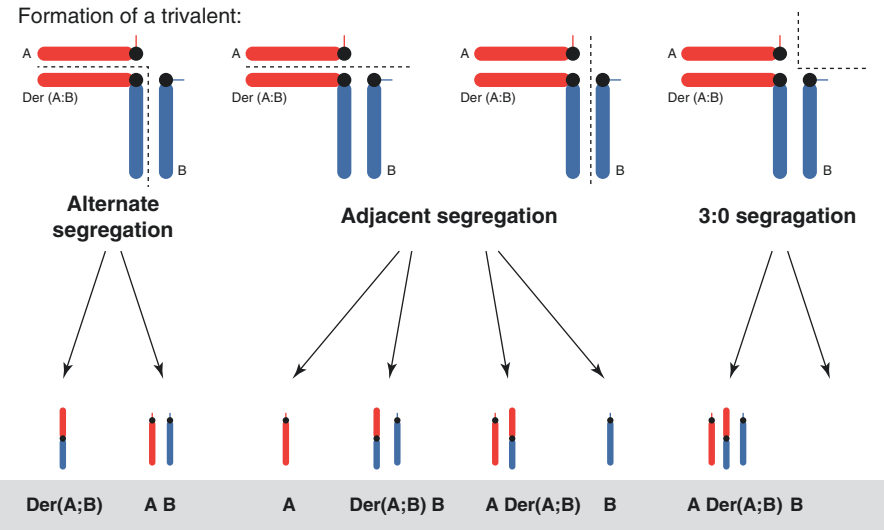

Fig. 24.2 Schematic depiction of trivalent formation and its segregation mode at meiosis in Robertsonian translocation carrier [17]

3:0 mode of segregation leads to sperm double nullisomy or disomy, resulting in unviable monosomy or possibly viable trisomy (Fig. 24.2).

In the late 1980s, investigation of the meiotic segregation of human sperm was made possible by the karyotyping of spermatozoa after the penetration of zona-free golden hamster oocytes [18–20]. However, this test enabled the analysis of only a limited number of spermatozoa. Since the 1990s, FISH has been introduced for the study of the chromosomal content of spermatozoa [21]. Many studies using this technique to estimate meiotic segregation modes in spermatozoa have been published. In the majority of sperm FISH analyses of Robertsonian translocations, dual- or triple-colored FISH approaches have been used, including directly labeled, subtelomeric, locus-specific, centromeric probes for chromosomes involved in the translocation.

There have been several descriptions of meiotic analyses in male Robertsonian translocation carriers. Lamotte et al. [17] reviewed a total aggregated set of 210 patients and analyzed their segregation modes. In the review, spermatozoa from 116 der(13;14), 38 der(14;21), 16 der(13;15), 11 der(14;15), 11 der(14;22), 5 der(13;21), 5 der(13;22), 4 der(21;22), 3 der(15;22), and 1 der(15;21) individuals were described. Dual- and triple-colored FISH analysis using directly labeled subtelomeric and/or locus-specific and/or centromeric probes for chromosomes 13, 14, 15, 21, and 22 was carried out on spermatozoa obtained from translocation carriers. As shown in other literature, the most common Robertsonian translocation is der(13;14), followed by der(14;21). According to the meiotic segregation modes obtained from the compiled Robertsonian translocation carriers, for the alternate segregation mode, it is assumed that translocation carriers have a similar meiotic pattern among

the chromosomes involved [22]. This hypothesis is strongly supported by the similarity in the balanced gamete rate among the different Robertsonian translocation carriers. However, Lamotte et al. [17] demonstrated that the alternate segregation mode is predominant in Robertsonian translocation carriers with 73.45% ± 8.05% balanced spermatozoa (min. 50.92%; max. 89.99%). Their results were consistent among the different types of Robertsonian translocations except for der(13;15), which exhibited lower balanced spermatozoa rates when compared to der(13;14), der(14;21), der(13;21), and der(15;22). The proportion of chromosomally normal (balanced) segregation rates commands two over three, whereas the rate of unbalanced segregation (mostly adjacent, but also extremely rare 3:0 segregations) varies, ranging between 10% and 21%. The adjacent segregation modes result in either monosomic or trisomic gametes. Chromosomal monosomy is not identified in conceptuses, while most trisomic conceptuses abort spontaneously, except for those with trisomy 13, 18, and 21, which can remain viable for several hours to several years or more. In a 3:0 segregation, one gamete receives double disomy, resulting in a zygote with 47 chromosomes. The other corresponding gamete receives double nullisomy, resulting in a zygote with 44 chromosomes. The 3:0 segregation mode is quite a rare event (rate of 0.0–5.0%). In the 3:0 segregation mode, one gamete receives three chromosomes, resulting in a double trisomic zygote. The other corresponding gamete receives no chromosome, resulting in a double monosomic zygote. The conceptus resulting from this segregation is not clinically identified.

Reciprocal translocations are the most common structural chromosome rearrangements in humans, with an incidence of 1 per 1175 newborns [23]. A reciprocal translocation does not change the amount of chromosomal material, and it involves the exchange of chromosome segments between arms of two heterologous chromosomes. Carriers of this type of chromosome translocation involving all chromosomes have been described, and ideal empirical data should be available for each translocation. Usually, empirical data exist only for general categories. In general, reciprocal translocations carry an empiric risk of about 10–15% for abnormal offspring [24]. Prenatal counseling would ideally take into account the segregation modes. FISH analysis in sperm samples obtained from reciprocal translocation carriers has been extensively described and reviewed in the literature. During meiosis I in a reciprocal translocation carrier, a quadrivalent is formed between the translocated chromosomes and their normal homologous (Fig. 24.3). This structure may segregate according to five theoretical modes. Alternate and adjacent-1 segregation modes involve a 2:2 disjunction of homologous centromeres to opposite poles. Instead, when any homologous centromeres migrate to the same pole, the possible segregation modes are adjacent-2 (2:2 disjunction), 3:1 or 4:0 disjunction. There is the widespread assumption that 2:2 alternate segregation leads to the formation of normal or balanced gametes, while the other segregation modes produce unbalanced gametes. The other segregation modes, i.e., adjacent-1, adjacent-2, and 3:1 and 4:0 segregations, produce unbalanced gametes. In adjacent-1 segregation, nonhomologous centromeres segregate together and pass to the same gamete. In adjacent-2 segregation, homologous centromeres pass to the same gamete. Both adjacent-1 segregation and adjacent-2 segregation induce partial disomy or nullisomy in the zygote and result in partial trisomy or monosomy in the embryo. In a

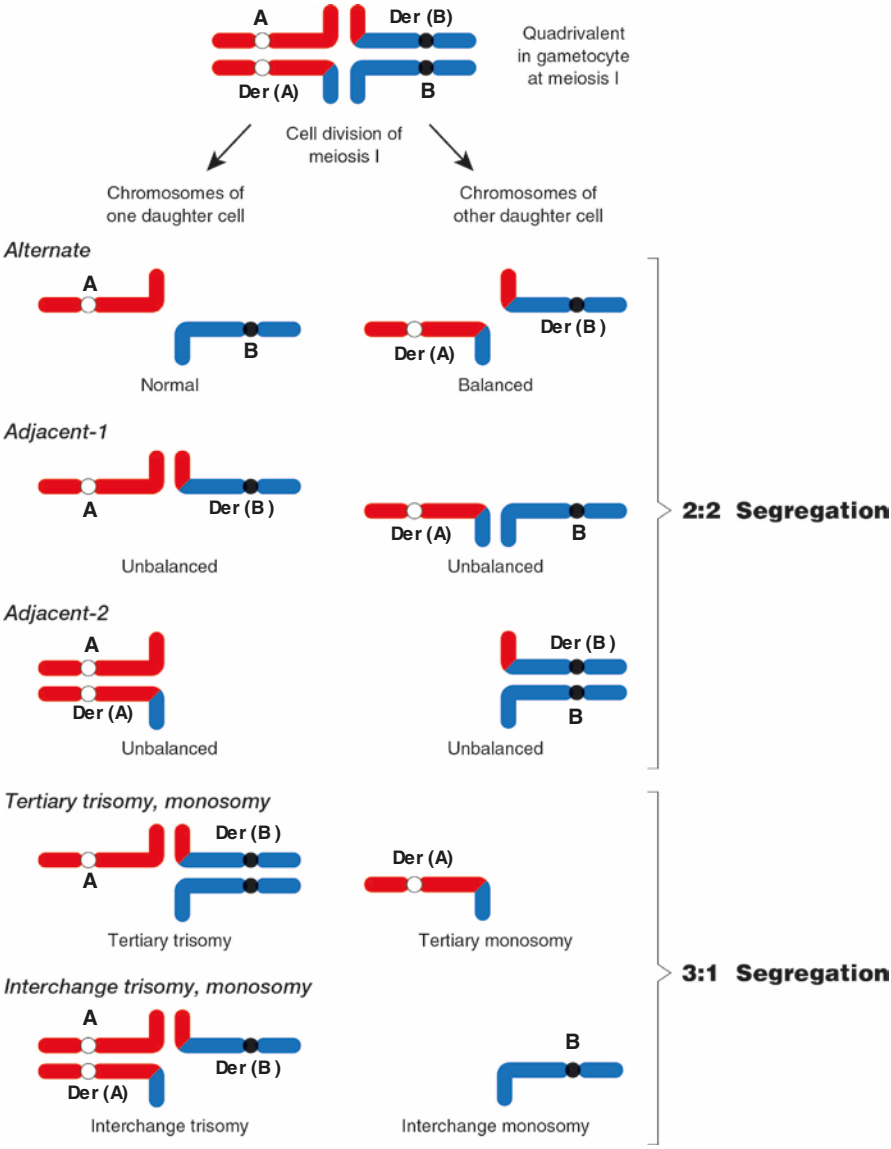

Fig. 24.3 Schematic depiction of quadrivalent formation and its segregation mode at meiosis in reciprocal translocation carrier. A, Chromosome A; B, Chromosome B; der(A), derivative chromosome A; and der(B), derivative B [25]. The 2:2 alternate segregation leads to the formation of normal or balanced gametes, while the 2:2 adjacent-1 or -2 segregations and 3:1 segregation modes produce an unbalanced content

3:1 segregation, one gamete receives two homologous chromosomes (disomy), resulting in a zygote with 47 chromosomes. The other corresponding gamete receives no chromosome (nullisomy), resulting in a zygote with 45 chromosomes. The 4:0 segregation mode produces a gamete with 21 chromosomes and one with 25 chromosomes, but it is quite a rare event.

In meiosis of reciprocal translocation carriers, four chromosomes must pair in reciprocal translocation heterozygotes, and the resulting segregations have a higher frequency of unbalanced chromosomes than Robertsonian translocations. There have been a number of studies on segregation patterns. Zhang et al. [26] summarized numerous previous studies and revealed that alternative segregation was the most frequent mode of segregation (42.71%), followed by adjacent-1, adjacent-2, and 3:1 segregations. Adjacent-1 segregation was observed in 31.13% of spermatozoa, adjacent-2 segregation was observed in 7.87% of spermatozoa, and 3:1 segregation was observed in 4.63% of spermatozoa. In addition, 4:0 and numerical anomalies, presumed to be interchromosomal effects, were observed in 13.66% of spermatozoa. Different studies on meiotic segregation patterns of sperm from reciprocal translocation carriers have revealed variability in the segregation modes, and there is a wide range of unbalanced gamete frequencies, ranging from 18.7% to 91.0%, among patients (Nishikawa et al. 2007) [27–30]. Figure 24.4 shows an

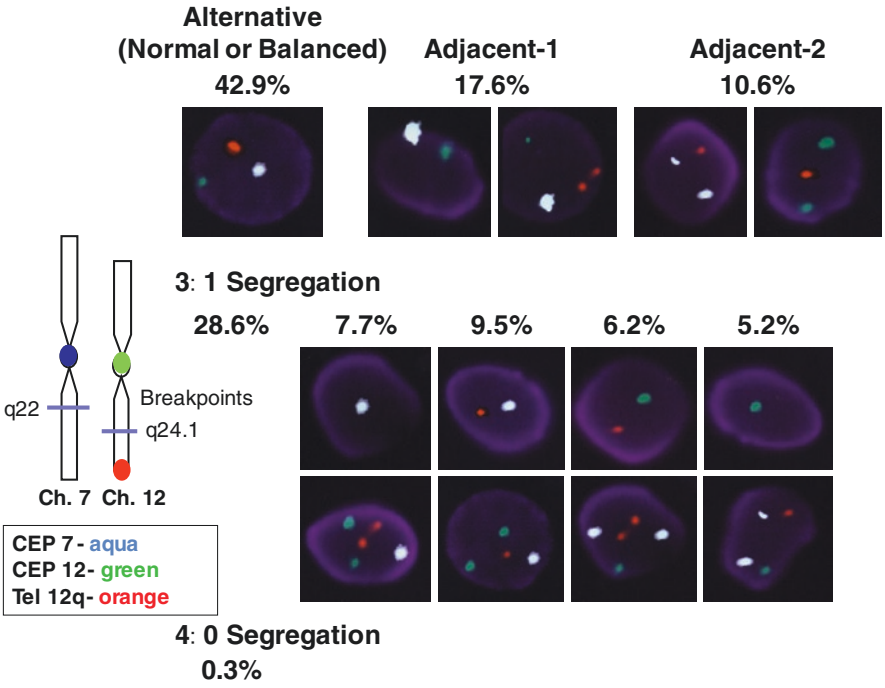

Fig. 24.4 Probes used in the FISH segregation analysis are CEP(centromere) 7 (aqua), CEP 12 (orange), and Tel(telomere) 12q (orange). Pictures show sperm resulting from alternate, adjacent-1, adjacent-2, 3:1, and 4:0 segregations with different signal patterns

example of meiotic segregation analysis on a case with t(7;12)(q22;q24.1) (Nishikawa et al. 2007).

Meiotic segregation patterns can be influenced by many factors. Published data have indicated that patients with shorter centric segments tended to produce higher numbers of adjacent-2 products, whereas those with shorter translocated segments produced more adjacent-1 products [31]. Additionally, 3:1 segregations required the participation of a small chromosome [31]. Studies on spermatozoa from translocation carriers help to broaden the understanding of the mechanisms of meiotic segregation. They should be integrated into the investigations of infertile men to provide a personalized risk assessment of unbalanced spermatozoa, especially since a correlation was found recently between the percentage of abnormal spermatozoa and that of abnormal embryos [32]. Meiotic segregation analysis facilitates the determination of the reproductive prognosis in male balanced translocation carriers and can be used for appropriate genetic counseling.

24.3 Multicolored Spectral Karyotyping for Complex Chromosomal Rearrangements

Complex chromosomal rearrangements (CCRs) are structural chromosome abnormalities that involve three or more breakpoints located on two or more chromosomes, which makes interpretation difficult. Many studies have reported that carriers of a balanced CCR are prone to infertility and recurrent abortions. CCRs with many breakpoints are usually difficult to clarify. In 1996, Schröck et al. [33] developed a novel approach, termed 'spectral karyotyping' or SKY, based on the hybridization of 24 fluorescence-labeled chromosome painting probes, which allows the simultaneous and differential color display of all chromosomes. This approach was used in the case of a pregnant CCR carrier with a previous abnormal child. The first baby, a female, suffered from cardiovascular abnormalities, including a ventral septal defect and patent ductus arteriosus. The karyotype included a 4q 2.3 trisomy, but further details were unknown. Chromosome analysis of the parents revealed that the mother had a complex chromosomal insertion/translocation between three chromosomes with four breakpoints forming der(4)t(4;16)(q22.2;q22.3), der(13)ins(13;4)(q31.2;q22.2q31.3), and der(16)t(4;16)(q31.3;q22.3) detected by G-banding. These findings of the mother's chromosomes were confirmed using SKY (Fig. 24.5). The couple requested prenatal diagnosis for the second pregnancy [34]. Chorionic villus sampling was performed. Cytogenetic analysis by SKY showed a male balanced carrier, the same as the mother.

In recent years, many studies have revealed that carriers of a balanced CCR are at risk of conceptions with various anomalies and reproductive failures owing to unbalanced arrangements due to either the malsegregation of derivative chromosomes or formation of a recombinant chromosome [35]. Many female carriers with CCRs have been identified after having malformed babies or repeated abortions [36]. Most males with CCRs have been shown to be infertile, and there have been

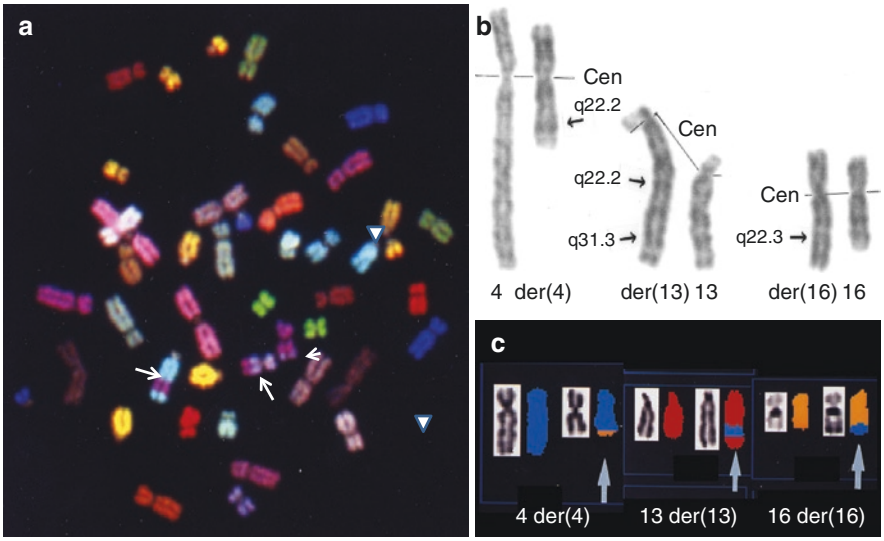

Fig. 24.5 G-banding and spectral karyotyping (SKY) of a case with complex chromosomal rearrangement involving chromosomes 4, 13, and 16. (**a**) Metaphase spread chromosomes stained by SKY (the arrows point to derivative chromosomes), (**b**) G-banded partial karyotypes showing translocation involving chromosomes 4, 13, and 16 and their derivatives (the arrows indicate breakpoints), and (**c**) G-banding and SKY of related chromosomes and their derivatives (the blue arrows indicate translocated chromosomes 4, 13, and 16)

several reports of CCRs in male with oligozoospermia [36–38]. According to the literature, 70–75% of CCRs are de novo in origin. They are found in almost equal proportions among phenotypically normal subjects and individuals with phenotypic abnormalities. The de novo CCRs appear to be mostly of paternal origin. This agrees with the epidemiological finding that most prenatally diagnosed balanced CCRs are maternal in origin (70% maternal versus 30% paternal), while the abnormalities found in newborns are of paternal origin [39].

In conclusion, the complexity of chromosomal rearrangements in patients with CCRs plays a role in male factor infertility and affects the spermatogenetic process rather than the number of chromosomes involved or the location of breakpoints. To corroborate this conclusion, further studies with larger sample sizes and advanced techniques, such as array-based comparative genomic hybridization, are required to characterize the breakpoints in detail [35].

References

1. Hopman AHN, Raap AK, Landegent J, et al. Non-radioactive in situ hybridization. In: Van Leeuwen F, Buijs RM, Pool CW, Pach O, editors. Molecular neuroanatomy. New York: Elsevier Science Publication B.V. (Biomedical Division); 1988. p. 43–68.
2. Ferguson-Smith MA. Putting the genetics back into cytogenetics. Am J Hum Genet. 1991;48:178–82.

3. Cremer T, Landegent J, Bruckner A, School HP, et al. Detection of chromosome aberrations in the human interphase nucleus by visualization of specific target DNAs with radioactive and non-radioactive in situ hybridization techniques: diagnosis of trisomy 18 with probe LI.84. Hum Genet. 1986;74:346–52.
4. Schröck E, du Manoir S, Veldman T, et al. Multicolor spectral karyotyping of human chromosomes. Science. 1996;273:494–7.
5. Speicher MR, Ballard SG, Ward DC. Karyotyping human chromosomes by combinatorial multi-colour FISH. Nat Genet. 1996;12:368–75.
6. Friedrich U, Nielsen J. Chromosome studies in 5,049 consecutive newborn children. Clin Genet. 1973;4(4):333–43.
7. Lubs HA, Ruddle FH. Chromosome abnormalities in the population: estimation of rates based on New Haven newborn study. Science. 1970;169(3944):495–7.
8. Nielsen J, Wohlert M. Chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arthus. Hum Genet. 1991;87(1):81–3.
9. Sergovich F, Valentine GH, Chen A, et al. Chromosome aberrations in 2159 consecutive newborn babies. N Engl J Med. 1969;280(16):851–5.
10. Jacobs PA, Melville MM, Ratcliffe SG, Keay AJ, et al. A cytogenetic survey of 11680 newborn infants. Ann Hum Genet. 1974. 1974;37:359–76.
11. Ratcliffe SG, Stewart AL, Melville MM, et al. Chromosome studies on 3500 newborn male infants. Lancet 1970. 1970;1:121–2.
12. Ferguson-Smith MA, Yates JRW. Maternal age specific rates for chromosome aberration and factors influencing them: Report of a collaborative European study of 52965 amniocenteses. Prenat Diagn. 1984;4:5–44.
13. Klinger KW, Dakowski W, et al. Prenatal detection of aneuploidy of 21, 18, 13, X or Y by interphase in situ hybridization. Am J Hum Genet. 1990; Suppl. 47A:224.
14. Baart EB, Martini E, Van Opstal D. Screening for aneuploidies of ten different chromosomes in two rounds of FISH: a short and reliable protocol. Prenat Diagn. 2004;24:955–66.
15. Klinger KW, Landes G, Shook D, Harvey R, Lopez L, et al. Rapid detection of chromosome aneuploidies in uncultured amniocytes by using fluorescence in situ hybridization (FISH). Am J Hum Genet. 1992;51(1):55–65.
16. Ward BE, Gersen SL, Carelli MP, et al. Rapid prenatal diagnosis of chromosomal anueploidies by fluorescene in situ hybridization: clinical experience with 4500 specimens. Am J Hum Genet. 1993;52(5):854–65.
17. Wenemowicz S, Sandstrom DJ, Morten CC, et al. Fluorescence in situ hybridization (FISH) for rapid detection of aneuploidies: experience in 911 prenatal cases. Prenat Diagn. 2001;21:262–9.
18. Winsor EJT, Silver MP, Theve R, et al. Maternal cell contamination in uncultured amniotic fluid. Prenat Diagn. 1996;16:49–54.
19. American College of Medical Genetics. Prenatal interphase fluorescence in situ hybridization (FISH) policy statement. Am J Hum Genet. 1993;53:526–7.
20. Jacobs P, Hassold TJ. The origin of numerical chromosomal abnormalities. Adv Genet. 1995;33:101–3.
21. De Braekeleer M, Dao TN. Cytogenetic studies in human male infertility: a review. Hum Reprod. 1991;6:245–50.
22. Lamotte A, Martinez G, Devillard F, et al. Is sperm FISH analysis still useful for Robertsonina translocation? Meiotic analysis for 23 patients and review of the literature. Basic Clin Androl. 2018;28:2–11.
23. Balkan W, Martin RH. Chromosome segregation into the spermatozoa of two men heterozygous for different reciprocal translocations. Hum Genet. 1983;63:345–8.
24. Martin KH. Analysis of human sperm chromosome complements from a male heterozygous for a reciprocal translocation t(11;22) (q23;q11). Clin Genet. 1984;25:357–61.
25. Rudak F, Jacobs A, Yanagimachi R. Direct analysis of the chromosome constitution of human spermatozoa. Nature. 1978;274:911–3.

26. Perrin A, Morel F, Douet-Guilbert N, et al. A study of meiotic segregation of chromosomes in spermatozoa of translocation carrier using fluorescent in situ hybridization. Andrologia. 2010;42:27–34.
27. Ogur G, Asse EV, Vegetti W, et al. Chromosomal segregation in spermatozoa of 14 Robertsonian translocation carriers. Mol Hum Reprod. 2006;12(3):209–15.
28. De Braekeleer M, Dao TN. Cytogenetic studies in human spontaneous abortions. In: Hafez ESE, editor. Assisted human reproductive technology. New York: Hemisphere Publication Company; 1991. p. 41–5. Google Scholar.
29. Daniel A, Boue A. Prospective risk in reciprocal translocation heterozygotes at amniocentesis as determined by potential chromosome imbalance size. Data of the European Collaborative Prenatal Diagnosis Centres. Prenat Diagn. 1986;6:315–50.
30. Gardner RJM, Sutherland GR. 4. Autosomal reciprocal translocations. In: Chromosome abnormalities and genetic counseling. Oxford Monograph on Medical Genetics No. 46. Oxford: Oxford University Press; 2004. p. 59–97.
31. Zhang S, Lei C, Wu J, et al. Analysis of segregation patterns of quadrivalent structures and the effect on genome stability during meiosis in reciprocal translocation carriers. Hum Reprod. 2018;33(4):757–67.
32. Ko DS, Cho JW, et al. Clinical outcomes of preimplantation genetic diagnosis (PGD) and analysis of meiotic segregation modes in reciprocal translocation carriers. Am J Med Genet A. 2010;152A(6):1428–33.
33. Lim CK, Cho JW, Song IO, et al. Estimation of chromosomal imbalances in preimplantation embryos from preimplantation genetic diagnosis cycles of reciprocal translocations with or without acrocentric chromosomes. Fert Steril. 2008;90:2144–51.
34. Martin RH. Cytogenetic determinations of male fertility. Hum Reprod Update. 2008;14:379–90.
35. Ye Y, Qian Y, Xu C, et al. Meiotic segregation analysis of embryos from reciprocal translocation carriers in PGD cycles. Repro Biomed Online. 2012;24(1):83–90.
36. Nishikawa N, Sato T, Suzumori N, et al. Meiotic segregation analysis male translocation carriers by using fluorescent in situ hybridization. Inter J Androl. 2007;31:60–6.
37. Fraut T, Mermet MA, et al. Cooperation of selection and meiotic mechanisms in the production of imbalances in reciprocal translocation. Cytogenet Cell Genet. 2000;88:15–21.
38. Morel F, Dout-Guilber T, et al. Meiotic segregation of translocations during male gametogenesis. Int J Androl. 2004;27:200–12.
39. Tanemura M, Suzumori K, Nishikawa N, Ishihara Y. Multicolour spectral karyotyping for complex chromosomal rearrangements in repeated abortion or congenital anomalies. Prenat Diagn. 2001;21:1123–8.
40. Kim JW, Chang EM, Song S-H, et al. Complex chromosomal rearrangements in infertile males: complexity of rearrangement affects spermatogenesis. Fertil Steril. 2011;95(1):349–52.
41. Batista DA, Pai GS, Stetten G. Molecular analysis of a complex chromosomal rearrangement and a review of familial case. Am J Med Genet. 1994;53(3):255–63.
42. Coco R, Rahn MI, Estangain PG, Antonioni G, et al. A constitutional complex chromosome rearrangement involving meiotic arrest in a review of familial male: case report. Hum Reprod. 2004;19:2784–90.
43. Kausch K, Haaf T, Khler J, et al. Complex chromosomal rearrangement in a woman with multiple miscarriage. Am J Med Genet. 1988;31:415–20.
44. Pellestor F, Anahory T, Lefort G, et al. Complex chromosomal rearrangements; origin and meiotic behavior. Hum Reprod Update. 2011;178(4):476–94.