Open Access

OBM Genetics

LIDSEN Publishing Inc.

Original Research

Molecular Cytogenetic Characterization of Rare but Repeatedly Observed Translocations

Ana Luísa Teixeira Fontes [1, 2], Niklas Padutsch [1], Stefanie Kankel [1], Thomas Liehr [1, *]

1. Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Jena, Germany; E-Mails: aluisafontes2000@gmail.com; Niklas.Padutsch@med.uni-jena.de; Stefanie.Kankel@med.uni-jena.de; Thomas.Liehr@med.uni-jena.de
2. Coimbra University, Faculty of Medicine, Coimbra, Portugal

* **Correspondence:** Thomas Liehr; E-Mail: Thomas.Liehr@med.uni-jena.de

Academic Editor: Joanne Traeger-Synodinos

Collection: Applications of Fluorescence in Situ Hybridization II.

OBM Genetics
2024, volume 8, issue 2
doi:10.21926/obm.genet.2402240

Received: April 15, 2024
Accepted: May 29, 2024
Published: June 11, 2024

Abstract

Balanced chromosomal rearrangements, including translocations, contribute to infertility, repeated abortions, and/or genetically imbalanced offspring in corresponding carriers. A translocation is usually considered a unique, *de novo,* or familial event. Besides, some translocations have also been shown to develop multiple times with slightly different or even identical breakpoints; for others, founder effects have been suggested. Here, two known recurrent translocations [t(11;22)(q23.3;q11.21) and der(X)t(X; Y)(p22.32;p11.31)] and two possibly at low frequencies repeatedly observable translocation events [t(5;16)(q13.3~14.1;p13.3) and t(Y;12)(q11.23;q12)] were studied. In the here applied molecular cytogenetic setting, it could be confirmed that the translocation t(11;22)(q23.3;q11.21) has its breakpoints in chromosome 11 between 116.585061 and 116.774263 Mb (GRCh37/hg19) and in chromosome 22 between 21.502000 and 21.616240 Mb (GRCh37/hg19). Corresponding suited bacterial artificial chromosome probes are suggested for their unequivocal characterization. For der(X)t(X;Y)(p22.32;p11.31) seen in 46, XX males, it could be confirmed that there is a significant variance in the derivative X-

chromosome' breakpoints and two new breakpoints are reported for one case. Breakpoints could also be narrowed down for two cases, each of a balanced translocation t(5;16)(q13.3~14.1;p13.3) and t(Y;12)(q11.23;q12). For the latter two cases, further studies need to show if these are more often observable rearrangements in infertile. Overall, it seems worthwhile considering translocations as inversions, as possibly regularly observable recurrent chromosomal rearrangements in human (infertile) populations, in which the formation mechanisms are still far from being understood. The contribution of such rearrangements to the genetic variety of the human population has not fully assessed yet.

Keywords
Recurrent translocations; chromosomes 11 and 22; chromosome X and Y; chromosomes 5 and 16; chromosomes Y and 12; infertility

1. Introduction

Structural chromosomal rearrangements can be related to evolutionary events [1] but can also be associated with inborn [2] or acquired diseases [3]; translocation is included in this group of aberrations. Constitutional translocation carriers are typically phenotypically normal but can be associated with reproductive problems [4, 5]. Due to unbalanced segregation of only one of the derivative chromosomes [6] or a meiotic 3:1 segregation [7] during gametogenesis, infertility, early abortions, and/or developmentally impaired and/or otherwise affected offspring may be consequences [8].

Balanced constitutional translocations are usually considered unique events and generated *de novo*, or maximally to segregate within (small) families, due to their described potential adverse effects on fitness. However, recurrent breakpoints have been reported in apparently unrelated individuals, too. This may be observed either due to independent, repeatedly occurring events based on structural peculiarities of the human genome like Robertsonian translocations in acrocentrics [9], translocation t(11;22)(q23.3;q11.21) leading to Emanuel syndrome in offspring [7], or as there was a kind of founder effect like shown for der(Y)t(Y;15)(q12;p11.2) [10]. For infertile male with 46,X, der(X)t(X;Y)(p22.32;p11.2), various breakpoints have been reported [11].

The molecular cytogenetic laboratory in Jena (Germany) analyzed during the last >25 years in routine diagnostics ~1900 cases with chromosomal rearrangements (mainly from Germany) [8]. This dataset was screened for potential candidates of recurrent translocations for the present study. Overall, four translocation events stood out here: t(5;16)(q13.3;p13.3), t(11;22)(q23.3;q11.21), der(X)t(X;Y)(p22.32;p11.2) and t(Y;12)(q11.23;q12). These have been studied here by specifically selected bacterial artificial chromosome (BAC) probes to narrow down their corresponding breakpoints and check if they were identical on the molecular cytogenetic level.

2. Materials and Methods

Analyzed cases were in the majority of the cases originally referred for banding cytogenetic analysis because of a history of infertility or repeated abortions; in three cases, children with Emanuel syndrome were known. There were two cases each with a translocation

t(5;16)(q13.3~14.1;p13.3) or a translocation t(Y;12)(q11.23;q12); 5 cases with a der(X)t(X;Y)(p22.3;p11.3) and 11 cases with a t(11;22)(q23.3;q11.21). One of the latter cases had Emanuel syndrome due to a karyotype 47, XX,+der(22)t(11;22)(q23.3;q11.21) dmat (case 11; Table 1). Cases 1-7, 10-17, and 19 were derived from Germany; cases 8, 9, and 18 from Portugal, and case 20 from Belarus. The cases were sent for molecular cytogenetic analysis in Jena (Germany), where they were initially characterized by multicolor banding (MCB) and/or different locus-specific, bacterial artificial chromosome (BAC) probes, as described for other diagnostic cases [12].

Table 1 List of studied cases with gender and indication for studies.

Case	Gender	Indication
t(5;16)(q13.3~14.1;p13.3)		
breakpoints: 75,697,435-78,167,933 and 588,659-789,507		
1	F	infertile
2	M	infertile
t(11;22)(q23.3;q11.21)		
breakpoints: 116,536,280-117,517,615 and 21,433,073-21,616,240		
3	F	Studied woman has a child with Emanuel syndrome
4	F	infertile
5	M	infertile
6	F	infertile
7	F	Studied woman has a child with Emanuel syndrome
8	F	infertile
9	F	infertile
not enough material to clarify whether this variant is present or not		
10	F	infertile
11	F	Emanuel syndrome; mother has translocation t(11;22)
12	F	infertile
der(X)t(X;Y)(p22.32;p11.31)		
breakpoints: 8,054,265-9,329,091 and 6,206,231-6,692,454		
13	M	infertile
not enough material to clarify whether this variant is present or not		
14-18	M	infertile
t(Y;12)(q11.23;q12)		
breakpoints: 28,388,853-28,800,001 and ~38,000,000-38,022,073		
19	M	infertile
not enough material to clarify if this variant is present or not		
20	M	infertile

For the present study, BACs available to the authors from BACPAC Chori (https://bacpacresources.org/) listed in Table 2 were applied in the frame of an expansion of diagnostics to narrow down the breakpoints in the cases where the material was left - this was not possible in cases 10-12, 14-18 and 20. In Table 2, the BACs used are listed together with the characterized breakpoint regions.

Table 2 List of BAC (bacterial artificial chromosome) probes used in this study and results of breakpoint mapping. Breakpoints are highlighted in bold.

cytoband	Localization acc. to GRCh37	BAC probe
Breakpoint in t(5;16)(q13.3;p13.3)		
5q13.2	71,623,857-71,787,690	RP11-115I6
5q13.3	73,780,538-73,931,815	RP11-97L2
5q13.3	75,565,882-**75,697,435**	CTD-2200O3
5q14.1	**78,167,933**-78,248,858	RP11-21K15
5q14.1	79,057,985-79,183,782	CTC-431G16
16p13.3	**588,659-789,507**	CTD-2524J22
16p13.3	1,347,261-1,484,290	CTC-357L21
16p13.3	3,720,076-3,914,571	RP11-619A23
Breakpoint in t(11;22)(q23.3;q11.21)		
11q23.3	115,934,905-116,103,992	RP11-15J11
11q23.3	116,325,596-**116,536,280**	RP11-356E17
11q23.3	**117,517,615**-117,689,361	RP11-35P15
11q23.3	117,963,900-118,157,704	RP11-832A4
22q11.21	21,213,744-21,406,241	RP11-54C2
22q11.21	21,216,527-21,433,067	RP11-1058B20
22q11.21	**21,433,073-21,616,240**	RP11-379N11
22q13.33	21,931,796-22,118,344	RP11-47L18
Breakpoint der(X)t(X;Y)(p22.32;p11.2)		
Xp22.31	7,445,552-7,623,830	RP11-323F16
Xp22.31	7,974,846-**8,054,265**	RP11-692P14
Xp22.31~22.2	**9,329,091**-9,449,776	RP11-126O22
Xp22.31~22.2	9,358,753-9,500,420	RP11-299M10
Xp22.2	9,601,147-9,724,177	RP1-108M6
Yp11.2	4,857,081-5,017,603	RP11-122L9
Yp11.2	6,051,700-**6,206,231**	RP11-35D7
Yp11.2	**6,692,454**-6,859,727	RP11-115H13
Yp11.2	6,817,929-7,014,238	RP11-196J6
Breakpoint in t(Y;12)(q11.23;q12)		
Yq11.23	26,531,842-26,539,305	RP11-424J12
Yq11.23	27,656,954-27,794,030	RP11-497C14
Yq11.23	28,215,812-**28,388,853**	RP11-270H4
Yq12	**28,800,001**-59,373,566	DYZ1
12p11.1~q11	33,300,001-**38,000,000**	D12Z3
12q11	**38,022,073**-38,117,869	RP11-657P13
12q12	40,706,793-40,855,223	RP11-115F18

BAC probes were applied with homemade whole chromosome painting (wcp) probes [13] in different combinations in three to four-color FISH-probe sets. As previously described, BAC- and wcp-DNA was amplified and labelled by degenerate oligonucleotide primed polymerase chain

reaction (DOP-PCR) [13]. For centromere 12 and Yq12, commercial satellite DNA probes D12Z3 and DYZ1 from Abbott/Vysis (Wiesbaden, Germany) were applied. FISH was done according to standard procedures [12]. Results were evaluated on a Zeiss-Axioplan microscope equipped with ISIS software (MetaSystems, Altlussheim, Germany), and at least 10 metaphases were analyzed per case and probe set.

Study approval statement: The studied cases were accessed in the frame of routine diagnostics. Accordingly, no specific study approval by an ethical committee was necessary.

Consent to participate statement: Ethical approval was not required for this study in accordance with local/national guidelines.

3. Results

The four translocations studied in overall 20 cases are listed in Table 1 and Table 2:

- For the translocation t(5;16)(q13.3~14.1;p13.3) breakpoints could be narrowed down between breakpoint flanking probes CTD-2200O3 and RP11-21K15 (acc. to GRCh37/h19, chr5: 75.697435-78.167933 Mb) and within breakpoint spanning BAC CTD-2524J22 (chr16: 0.588659-0.789507 Mb; Figure 1A). Both carriers were living in the northeastern part of Germany.
- The six carriers of a translocation t(11;22)(q23.3;q11.21) and the Emanuel syndrome patient with +der(22)t(11;22)(q23.3;q11.21) all had the identical breakpoints which can be characterized by BACs RP11-356E17 and RP11-35P15 (chr11: 116.536280-117.517615 Mb) and RP11-379N11 (chr22: 21.433073-21.616240 - Figure 1B). The studied persons were living all over Germany, and two were from Portugal.
- Only one of the six males with GTG-banding-karyotype 46,XX could be characterized further. Here the involved breakpoints of the der(X)t(X;Y)(p22.32;p11.31) were between RP11-692P14 and RP11-126O22 (chrX: 8.054265-9.329091) and RP11-35D7 and RP11-115H13 (chrY: 6.206231-6.692454 - Figure 1C). Corresponding patients were distributed all over Germany, and one was derived from Portugal.
- The translocation t(Y;12)(q11.23;q12) was observed in 2 cases - only in one case from northeast coast region of Germany breakpoints could be further narrowed down to between RP11-270H4 and DYZ1 (chrY: 28,388,853-28,800,001) and D12Z3 and RP11-657P13 (chr12: 38,000,000-38,022,073 - Figure 1D). The second case (from Belarus) seemed to have the same breakpoints; however, only the probes DYZ1 and D12Z3 could be applied.

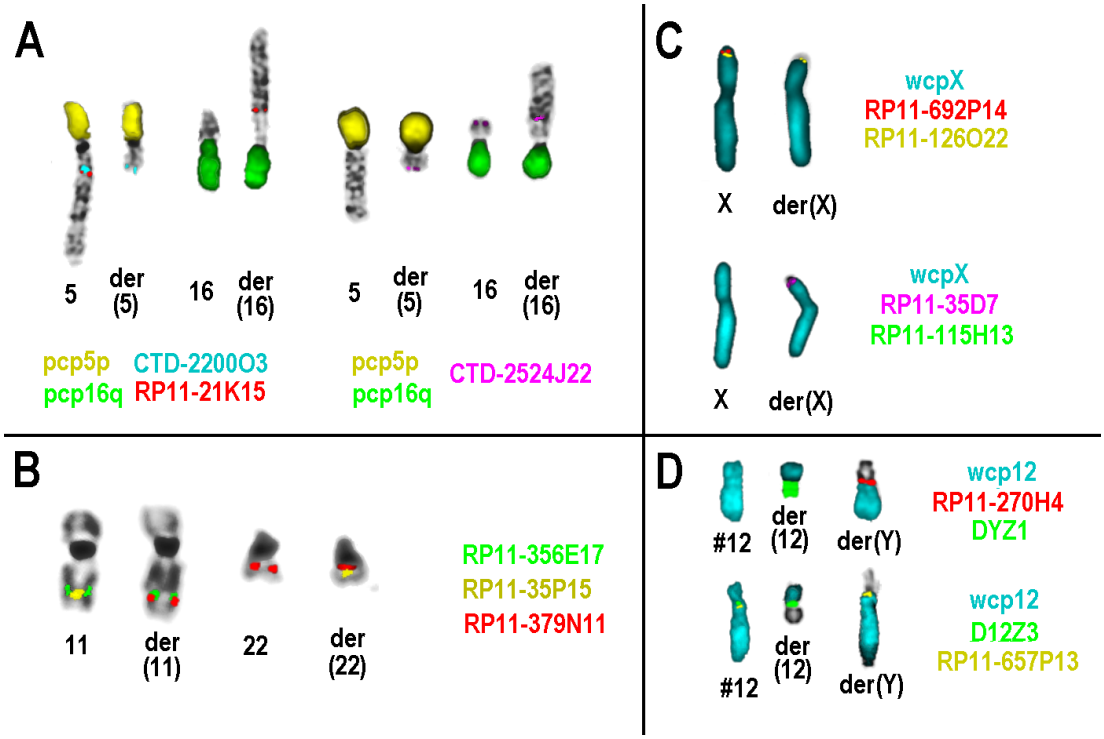

Figure 1 Typical results of one case each for breakpoints determined in this study. (A) In case 1 from Table 1 the breakpoints are shown in two FISH-experiments; in both tests partial chromosome paints (pcps) for short arm of chromosome 5 (5p-yellow) and long arm of chromosome 16 (16q-green) were used. BACs CTD-2200O3 (blue) and RP11-21K15 (red) were breakpoint flanking for chromosome 5 breakpoint (left side), and BAC CTD-2524J22 (pink) was breakpoint spanning for that on chromosome 16. (B) Breakpoint for translocation t(11;22)(q23.3;q11.21) can be characterized using breakpoint flanking BACs RP11-356E17 (green) and RP11-35P15 (yellow) on chromosome 11 and breakpoint spanning RP11-379N11 (red) for chromosome 22; here shown for case 4. (C) In case 13 the breakpoints in Xp22.32 and Yp11.31 were accessed in two seperat FISH-experiments between BACs RP11-692P14 (red) and RP11-126O22 (yellow - upper part) and RP11-35D7 (pink) and RP11-115H13 (green-lower part). The X-chromosome's whole chromosome paint (wcp) was used in both tests as a blue labelled probe. In upper part both BACs give signals on normal X-chromosome, and only the signal for RP11-126O2 is visible on the der(X). Vice versa, for Y-chromosomal probes only RP11-35D7 gives a signal on der(X). (D) The breakpoints in translocation t(Y;12)(q11.23;q12) in case 19 were localized in two FISH tests where wcp12 (blue) was used in both probe sets. In the upper part of this figure, the Y-chromosomal break was narrowed down between RP11-270H4 and DYZ1. Below, the break in 12q12 was shown to localize between D12Z3 and RP11-657P13.

4. Discussion

Here two frequent known and two new, at low frequency repeatedly observed translocation events were studied. The translocation t(11;22)(q23.3;q11.21) is the most frequent recurrent translocation in humans after Robertsonian translocations [7]. It can lead, due to a meiotic 3:1 segregation to the formation of a small supernumerary marker chromosome, a

+der(22)t(11;22)(q23.3;q11.21). Breakpoints of the rearrangement have been narrowed down by molecular genetics before as GRCh37/hg19 chr11: 116.585061-116.774263 Mb and chr22: 21.502000-21.767000 [14]. Here these breakpoints were confirmed by molecular cytogenetics, and three BAC-probes suited for unequivocal characterization of this translocation were identified. Also it was shown that translocation carriers from Germany and Portugal have identical breakpoints when a translocation t(11;22)(q23.3;q11.21) develops. This fits well to the statement from Kurahashi and Emanuel from 2001: "The breakpoint of the t(11;22) has been identified within palindromic AT-rich repeats on chromosomes 11 and 22, suggesting that hairpin/cruciform structures mediate double-strand breaks leading to the translocation" [15].

For carriers of a karyotype 46, XX. ish der(X)t(X;Y)(p22.32;p11.31) it is known that such males are not fertile, as they lack AZF factor and cannot build motile sperm [11]. Accordingly, if no in vitro fertilization is applied, most such cases still are formed de novo. In contrast to translocation t(11;22)(q23.3;q11.21), the der(X)t(X;Y)(p22.3;p11.3) has multiple breakpoints and no such unique hotspot [11]. According to Capron et al. (2022) [11], the here characterized breakpoints in a case of 46,XX males from Germany have not been reported before.

Translocation t(5;16)(q13.3~14.1;p13.3) has been seen in two cases from northeast Germany. This rearrangement was possibly seen previously in Sweden ([6], case 6) but not characterized in detail. It has to be seen if more such cases will be observed and if all of them are derived from that region or found elsewhere. Yet it is unclear if these carriers of molecular cytogenetically identical translocation are far relatives, maybe by chance findings of a more significant (small) subpopulation with this rearrangement or formed several times due to a specific genomic architecture, like in the case of t(11;22)(q23.3;q11.21) [7].

For translocation t(Y;12)(q11.23;q12) is valid what was stated before for the translocation t(5;16)(q13.3~14.1;p13.3) - the rearrangement was seen yet in only two carriers, which were living in Germany and Belarus, respectively. Even though a common ancestor cannot be excluded, it might be more likely that this kind of rearrangement could be formed at low frequency but repeatedly. The latter could be due to the closeness of the breaks to the satellite DNA sequences DYZ1 and D12Z3.

Overall, the present study provided further insights into two known {t(11;22)(q23.3;q11.21) and der(X)t(X; Y)(p22.32;p11.31)} translocations repeatedly seen in infertile. Besides, two possible new repeatedly observed translocations in infertile {t(Y;12)(q11.23;q12) and t(5;16)(q13.3~14.1;p13.3)} are highlighted. The breakpoint characterization was done by molecular cytogenetics, as cytogenetically worked-up material was at hand. At the same time, it would have been impossible to request additional DNA samples of the corresponding patients to study breakpoints by approaches like sequencing [16] or optical genomic mapping (OGM) [17]. Besides, possibly heterochromatic breakpoints, like in the case of translocation t(Y;12)(q11.23;q12), are not accessible by standard sequencing approaches or OGM yet. In addition, the resolution of sequencing approaches, such as in [16], is given as 56 to 1300 base pairs.

5. Conclusions

Overall, our data suggest it is worth considering that not only inversions but also translocations comprise regularly observable recurrent chromosomal rearrangements in the human (infertile) population. Thus, it is not unlikely that more identical balanced aberrations, including translocations,

which have yet to be recognized, will be detected in unrelated individuals with infertility. In the end, this means that it is still possible and necessary to collect and analyze seemingly unique translocations in infertile in more detail in case they show up more than once. The contribution of such rearrangements to the genetic variety of the human population is not yet fully assessed.

Acknowledgments

Cases were provided by Dr. M. Lemmens (Aachen, Germany), Dr. B. Belitz (Berlin, Germany), Dr. M. Stumm (Berlin, Germany), Dr. I.M. Carreira (Coimbra, Portugal), Dr. F. Süss (Cottbus, Germany), Dr. B. Schulze (Hannover, Germany), Dr. P. Javaher (Hannover, Germany), Dr. R. Niemann (Hürth, Germany), Dr. I. Schreyer (Jena, Germany), Dr. A. Polityko (Minsk, Belarus), Dr. U. Hehr (Regensburg, Germany).

Author Contributions

ALTF, NP and SK made a substantial and significant contribution to the work reporte in the design of the work, the acquisition, analysis, and interpretation of data. TL did study conception and design of the work, and final analysis and interpretation of data; also, he drafted the article. All authors revised the paper critically for important intellectual content, reviewed and agreed on all versions of the article from submission to final publication and agreed to be accountable for the content of the article and share responsibility for the appropriate resolutions of questions related to the accuracy or completeness of the published work.

Competing Interests

The authors have declared that no competing interests exist.

References

1. Weise A, Gross M, Schmidt S, Reichelt F, Claussen U, Liehr T. New aspects of chromosomal evolution in the gorilla and the orangutan. Int J Mol Med. 2007; 19: 437-443.
2. Liehr T, Kosayakova N, Schröder J, Ziegler M, Kreskowski K, Pohle B, et al. Evidence for correlation of fragile sites and chromosomal breakpoints in carriers of constitutional balanced chromosomal rearrangements. Balkan J Med Genet. 2011; 14: 13-16.
3. Schoder C, Liehr T, Velleuer E, Wilhelm K, Blaurock N, Weise A, et al. New aspects on chromosomal instability: Chromosomal break-points in Fanconi anemia patients co-localize on the molecular level with fragile sites. Int J Oncol. 2010; 36: 307-312.
4. Flannigan R, Schlegel PN. Genetic diagnostics of male infertility in clinical practice. Best Pract Res Clin Obstet Gynaecol. 2017; 44: 26-37.
5. Ogur C, Kahraman S, Griffin DK, Yapan CC, Tufekci MA, Cetinkaya M, et al. PGT for structural chromosomal rearrangements in 300 couples reveals specific risk factors but an interchromosomal effect is unlikely. Reprod Biomed Online. 2023; 46: 713-727.
6. Wan X, Li L, Liu Z, Fan Z, Yu L. Recurrent spontaneous abortion related to balanced translocation of chromosomes: Two case reports. J Med Case Rep. 2021; 15: 270.

7. Kurahashi H, Inagaki H, Ohye T, Kogo H, Tsutsumi M, Kato T, et al. The constitutional t(11;22): Implications for a novel mechanism responsible for gross chromosomal rearrangements. Clin Genet. 2010; 78: 299-309.
8. Liehr T. Constitutional chromosomal breakpoints [Internet]. ChromosOmics-Database; 2024. Available from: https://cs-tl.de/DB/CA/BPs/0-Start.html.
9. Guarracino A, Buonaiuto S, de Lima LG, Potapova T, Rhie A, Koren S, et al. Recombination between heterologous human acrocentric chromosomes. Nature. 2023; 617: 335-343.
10. Fuchs S, Lisfeld J, Kankel S, Person L, Liehr T. The acrocentric part of der(Y)t(Y;acro)(q12;p1?2) contains D15Z1 sequences in the majority of cases. Hum Genome Var. 2021; 8: 32.
11. Capron C, Januel L, Vieville G, Jaillard S, Kuentz P, Salaun G, et al. Evidence for high breakpoint variability in 46,XX,SRY-positive testicular disorder and frequent ARSE deletion that may be associated with short stature. Andrology. 2022; 10: 1625-1631.
12. Weise A, Starke H, Heller A, Tönnies H, Volleth M, Stumm M, et al. Chromosome 2 aberrations in clinical cases characterised by high resolution multicolour banding and region specific FISH probes. J Med Genet. 2002; 39: 434-439.
13. Liehr T, Claussen U. Current developments in human molecular cytogenetic techniques. Curr Mol Med. 2002; 2: 283-297.
14. Hill AS, Foot NJ, Chaplin TL, Young BD. The most frequent constitutional translocation in humans, the t(11;22)(q23;q11) is due to a highly specific Alu-mediated recombination. Hum Mol Genet. 2000; 9: 1525-1532.
15. Kurahashi H, Emanuel BS. Long AT-rich palindromes and the constitutional t(11;22) breakpoint. Hum Mol Genet. 2001; 10: 2605-2617.
16. Aristidou C, Koufaris C, Theodosiou A, Bak M, Mehrjouy MM, Behjati F, et al. Accurate breakpoint mapping in apparently balanced translocation families with discordant phenotypes using whole genome mate-pair sequencing. PLoS One. 2017; 12: e0169935.
17. Zhang S, Pei Z, Lei C, Zhu S, Deng K, Zhou J, et al. Detection of cryptic balanced chromosomal rearrangements using high-resolution optical genome mapping. J Med Genet. 2023; 60: 274-284.