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Analysis of sperm chromosomes in six carriers of rare and common Robertsonian translocations*

Badanie chromosomów plemnikowych u sześciu nosicieli rzadkich i częstych translokacji Robertsonowskich

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Summary

Introduction: Robertsonian translocation (RobT) is the central fusion of the long arms of two acrocentric chromosomes, leading to 45 chromosomes in humans. The most common ones are rob(13;14) and rob(14;21) (91%). Other types of RobT are so-called rare cases. In the general population RobTs occur with a frequency of approximately 0.123%, but among men with reproductive failure this value rises 9-fold. Infertility in RobT carriers is associated with the formation of unbalanced spermatozoa resulting from segregation of the chromosomes involved in trivalent during the meiotic prophase. In spermatozoa of many RobT carriers an increased level of chromosomal aneuploidy is observed.

Materials and Methods: We examined the hyperhaploidy level of chromosomes 7, 9, 18, 21, 22, X and Y in spermatozoa of 6 RobT unrelated carriers: two carriers with rare rob(13;15), one with rare rob(13;22), and three of the common rob(13;14). Results were compared with the control data from a group of 7 fertile men with a normal karyotype. Fluorescent *in situ* hybridization (FISH) was applied.

Results: We found an increased level of sperm aneuploidy regarding at least one of the analyzed chromosomes in each of the carriers, while in rare RobTs interchromosomal effect (ICE) was observed. Meiotic segregation pattern of a rare rob(13;15) carrier revealed the 76% of normal /balanced spermatozoa.

Disucussion: Due to the relatively high population frequency of RobTs, their influence on reproductive failure, hight risk of imbalancement in prenatal diagnosis (7%), and small amount of data for rare RobTs, each newly characterized case is valuable in genetic counseling.

Keywords: **rob(13;15), rob(13;22), rob(13;14), sperm aneuploidy, meiotic segregation pattern, rare Robertsonian translocation, RobT**

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INTRODUCTION

Approximately, 15% of couples in the reproductive age are infertile. About half of infertility cases results from the female side, followed by male reasons of the problem (20–30%), while the combined causes from both partners constitute 20–30% [2]. Beside the known infertility reasons, such as: disturbances in genetic background, changes in hormonal levels, infections, environmental factors or epigenetics, the etiology of many infertility cases remains unclear (idiopathic). Amongst the genetic background of reproductive failure, chromosomal aberrations (both structural and numerical) are observed quite frequently (6/1000 of live births). The most common structural aberrations observed in general population are chromosomal translocations with the frequencies 0.143% for reciprocal (RCT), and 0.123% for Robertsonian (RobT) translocations [24, 51, 52, 54]. In infertile males, those frequencies rise from 5 to 9 times [16, 47]. More than half of the translocation carriers have decreased semen parameters [48, 75]. At the same time, among infertile men with oligozoospermia, RobT is the most frequently detected aberration of autosomal chromosomes [47]. However, the details of its influence on fertility remain unclear – an infertile carrier can be a son or a brother of a fertile carrier of the same aberration within one family.

Robertsonian translocations can be inherited (mostly maternally) or appear *de novo* [60]. RobT arise as a consequence of centric fusion between two long (q) arms of acrocentric chromosomes, leading to formation of one derivative chromosome, followed by the loss of short (p) arms. Thus, centric fusion leads to reduced number of chromosomes in a karyotype of the carrier – 45 instead of 46 in human, but without any phenotypic effects (except infertility/reproductive problems). It was suggested that similar DNA sequences (repetitive or satellite III type) in short (p)arms of acrocentrics may facilitate the formation of RobT. However, detailed molecular mechanisms of their formation still remain unclear [36, 38]. In human, RobTs concern five acrocentric chromosomes: 13, 14, 15, 21 and 22, with short (p) arms built from repetitive satellite DNA and nucleolar organizing regions (NORs; sequences for 18S and 28S rRNA). A derivative chromosome may be dicentric (~90% of all RobT) with two centromeres from both acrocentrics (but only one active, resulting from a centric fusion after breakpoints in (p) arms of both involved chromosomes), or monocentric (observed in only ~10%) with one centromere from one of the involved acrocentrics (centric fusion after breakpoints: one in (p) arm of the first chromosome and the second in (q) arm of the other chromosome involved) [29, 38]. There is 15 different possible RobTs, including 10 heterologous (built from two different chromosomes; 95% of all cases) and 5 homologous (only 5% of all centric fusions, including isochromosomes) rearrangements. The most common heterologous rearrangements are rob(13;14)(q10;q10), and rob(14;21)(q10;q10) observed in approximately 91% of all Robertsonian translocations (Fig. 1B) [49]. It was suggested that a high frequency of those two common RobTs may result from a specific mechanism that involves homological recombination between opposite-directed DNA sequences in chromosomes 13 and 21 vs. 14 [18, 57, 61, 82]. Other types of RobTs are known as rare cases [3, 82, 90].

Presence of Robertsonian karyotype determines the production of genetically unbalanced gametes. In RobT carriers, a trivalent pachytene figure is being formed during meiosis from chromosomes involved in a rearrangement and determines 8 different types of segregants, produced in three distinct segregation patterns: alternate, adjacent and 3:0 (Fig. 2). Only the alternate segregation leads to genetically normal (46 chromosomes) and balanced gametes (45 chromosomes including one derivative chromosome from two acrocentrics). All other types of segregants are genetically unbalanced and may lead to miscarriages or an offspring with abnormal karyotype [21]. Results of meiotic segregation pattern in male carriers of RobT, were published in at least 212 cases, so far. Majority of data concerned the two common Robertsonian translocations: der(13;14) (116 cases) and der(14;21) (38 cases) with mean value of about 20% of genetically unbalanced gametes. Other data are related to rare RobT cases, also with a mean value of about 20% of genetically unbalanced gametes [3, 4, 5, 10, 12, 13, 14, 16, 22, 23, 25, 26, 31, 32, 37, 40, 41, 43, 44, 45, 46, 50, 52, 53, 55, 56, 57, 62, 65, 66, 67, 68, 70, 71, 72, 73, 79, 80, 85, 86, 87, 89]. However, a range of results concerning the frequency of genetically unbalanced gametes in all analyzed RobT cases is much wider: from 0.2 to 49.1% [41, 89].

Closely associated with the carrying of RobT is the problem of the increased frequency of spermatozoa with aneuploidy of chromosomes that are not involved in the rearrangement. The so-called "interchromosomal effect" (ICE) was analyzed inabout 30 cases of different rare and 90 cases of common RobTs [1, 4, 5, 8, 9, 11, 17, 19, 23, 26, 28, 31, 37, 39, 43, 44, 53, 57, 63, 64, 69, 72, 74, 77, 78, 81, 85, 86]. An increased frequency of aneuploid gametes can arise from a disrupted disjunction of chromosomes during meiosis and is one of the documented causes of reproductive failures [81]. The elevated level of aneuploidy of at least one of the chromosomes analyzed was observed in more than half of RobT carriers [1, 31, 80].

The published data regarding both the aneuploidy levels and the meiotic segregation patterns in carriers of various heterological RobTs are still insufficient and they do not meet the criteria for meta-analyzes [27]. Considering the frequency of RobT occurence and its influence on reproductive failure, research on new RobTs cases, especially of rare ones, is vital and important for genetic counseling. In this work, we examined the level of hyperhaploidy

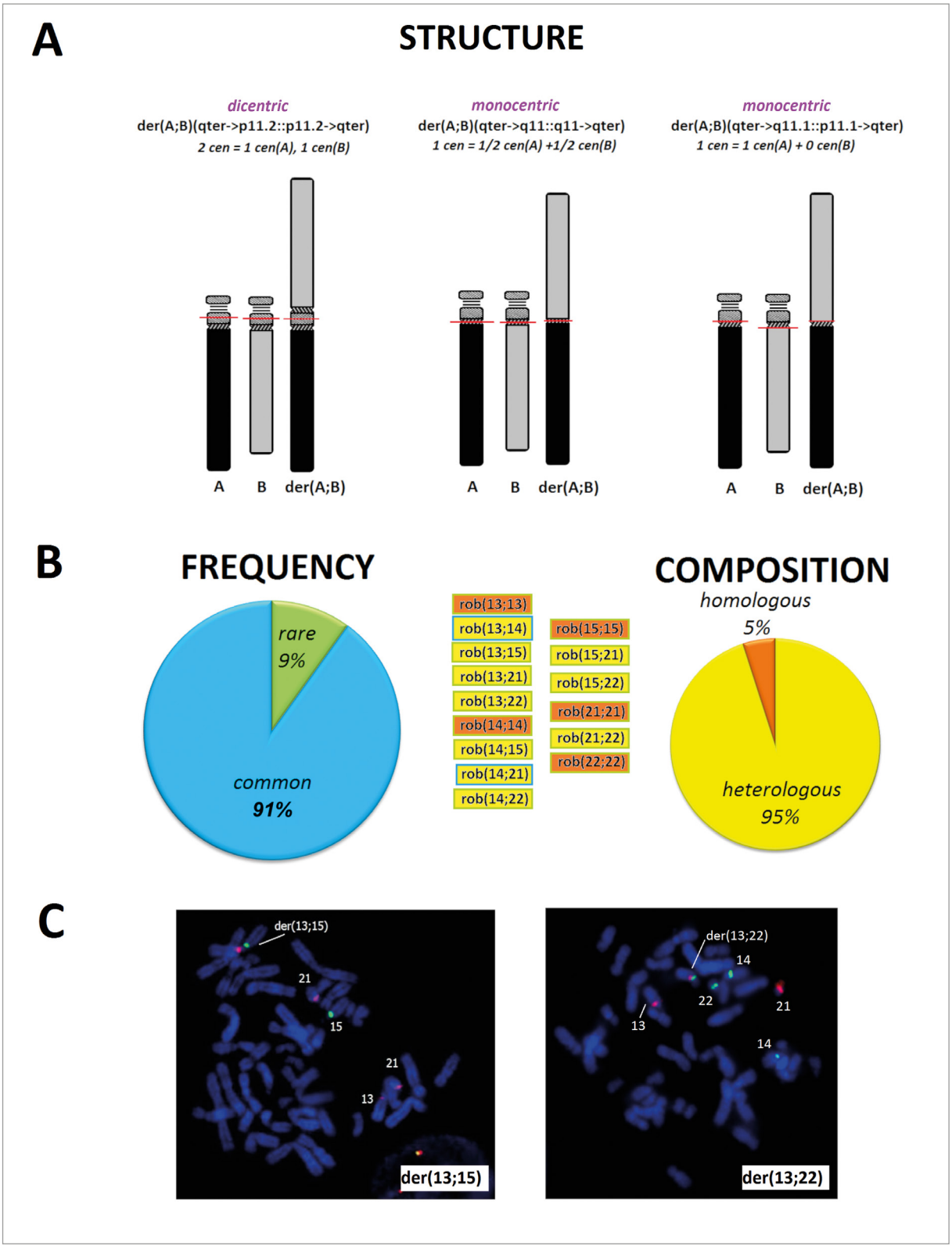


Fig. 1. Characteristics of Robertsonian translocations. A. Three types of rearrangement according to the breakpoints' localization, leading to dicentric (left panel) or monocentric (middle and right panels) or derivative chromosome. B. Frequencies of rare and common rearrangements (marked with green or blue colour), and involvement of homo- or heterologous chromosomes (orange or yellow). C. Characteristics of chromosomes involved in rare Robertsonian translocations analyzed in this study: 45,XY,der(13;15)(q10;q10) mat (FISH with probes: centromere specific 13/21 (red) and 15 (green)); 45,XY,der(13;22)(q10;q10)mat (FISH with probes: centromere specific 13/21 (red) and 14/22 (green)). Microscope used: Olympus BX41, oil-immersed objective 100×, fluorescent filter-set: FITC/TexasRed/Triple/DAPI; software: ISIS (FISH) (MetaSystems, Germany)

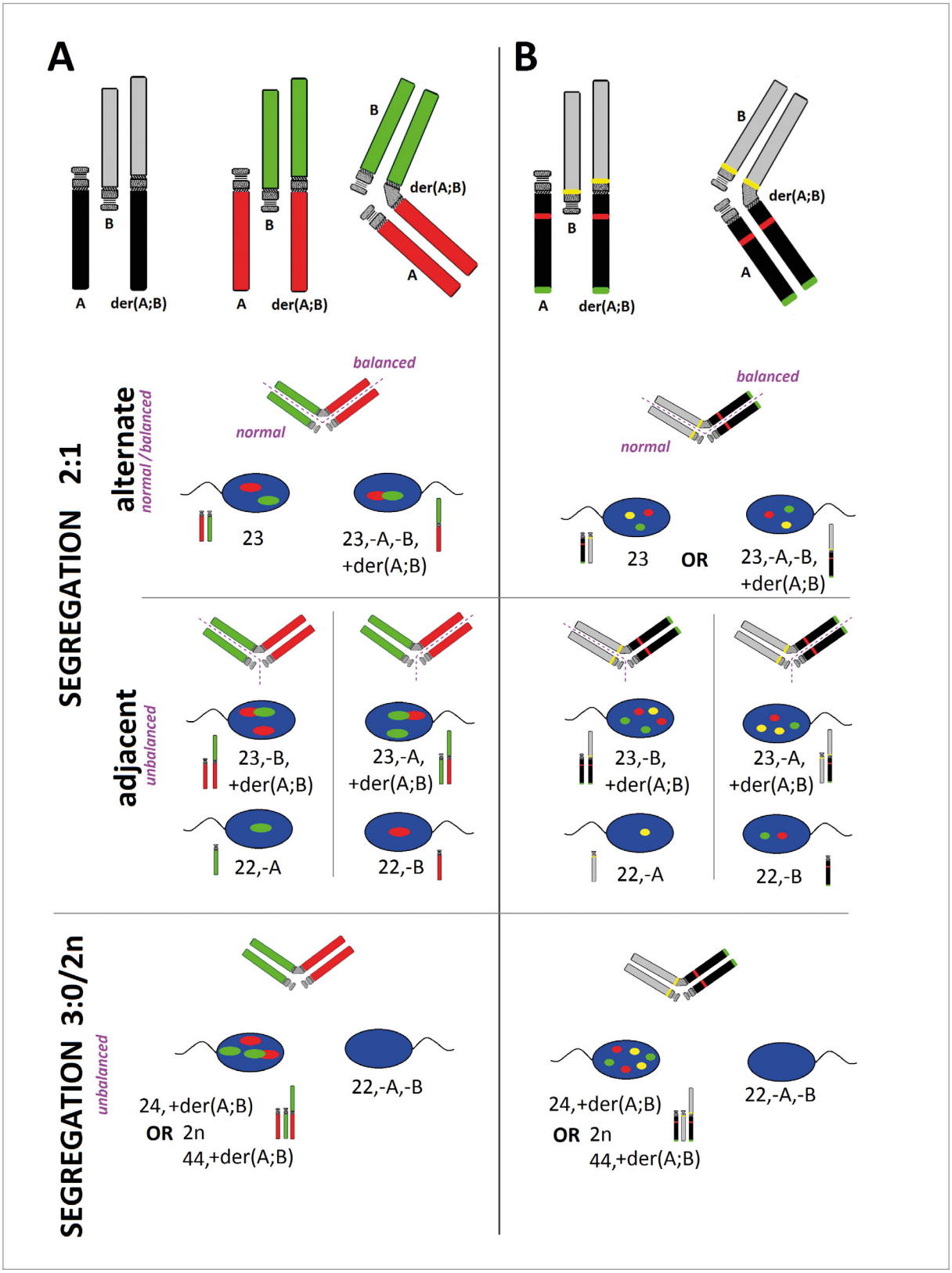


Fig. 2. Schematic representation of meiotic segregation pattern of chromosomes observed in spermatozoa of Robertsonian translocation carriers, including trivalent configuration of chromosomes involved in rearrangements. Two different suggestions of FISH staining were presented: A. Whole chromosome painting (wcp) probes, that allow to differentiate normal and balanced gametes separately (different FISH phenotype; alternate segregants). B. Combination of probes specific for centromeres, subtelomeres, and chromosomal band (mostly used), but resulting in identical FISH phenotype for normal and balanced spermatozoa (alternate segregants)

Table 1. Semen assessment of the six Robertsonian translocation carriers (R1-R6)

No.	Karyotype	Spermiogram (according to WHO, 2010 [88])	Reproductive history
R1	45,XY,rob(13;15)(q10;q10)mat	OAT** data unavailable	2 early miscarriages
R2*	45,XY,rob(13;15)(q10;q10)mat	OA***: concentration: 3.2; motility: <30; normal forms: 9	lack of conception
R3*	45,XY,rob(13;22)(q10;q10)mat	OA: concentration 2.0; motility : 26; normal forms >14	lack of conception
R4*	45,XY,rob(13;14)(q10;q10)	OA: concentration: 8.3; motility: 24; normal forms: 5.5	lack of conception
R5	45,XY,rob(13;14)(q10;q10)	OA: concentration: 0.7; motility: 24; normal forms: 0	lack of conception
R6	45,XY,rob(13;14)(q10;q10)	OA: concentration: 0.5; motility: 17; normal forms: 0	twins after ICSI
WHO, 2010 [88]	46,XY	Concentration (10^6/ml): ≥15; Progressive motility (%): ≥32: Normal forms (%): ≥ 4	

*Semen assessment and meiotic segregation patterns of R2, R3 and R4 carriers were published previously [87]. **OAT – oligoasthenoteratozoospermia; ***OA – oligoasthenozoospermia

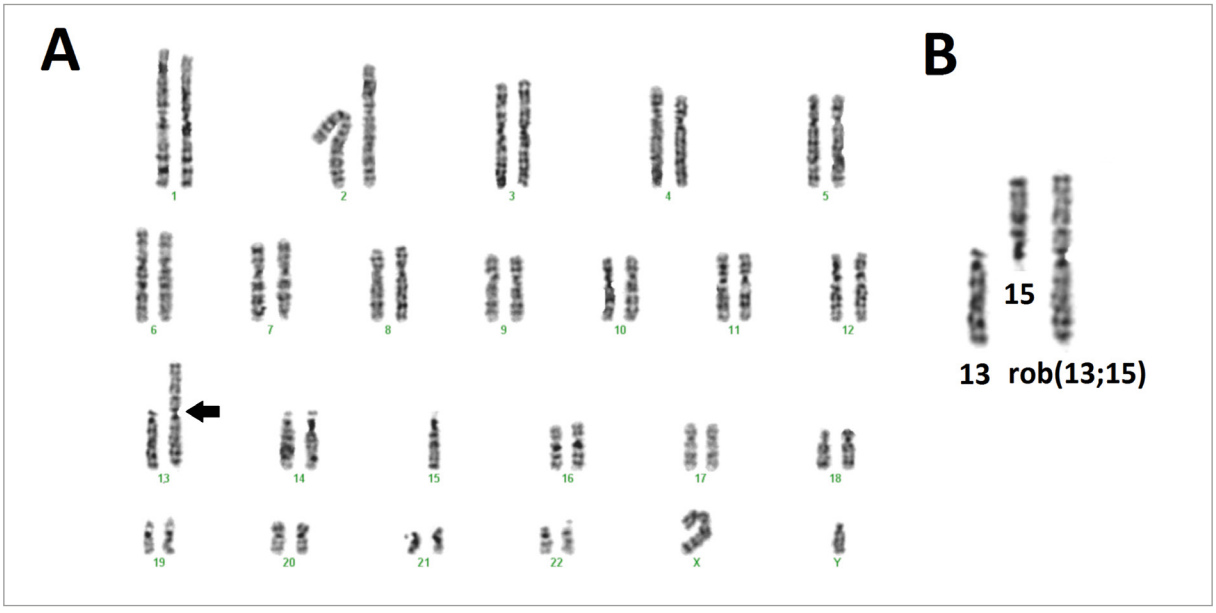


Fig. 3. GTG staining results for 45,XY,der(13;15)(q10;q10)mat carrier (R1). Microscope used: Olympus BX41, oil-immersed objective 100×, software: Ikaros (GTG) (Meta Systems, Germany). A. Whole metaphase plate. B. Chromosomes involved in the rearrangement

of chromosomes 7, 9, 18, 21, 22, X and Y in sperm cells of 6 RobT carriers: two unrelated carriers with rare rob(13;15), one with rare rob(13;22), and three carriers of the most common rob(13;14). The results were compared with the control data obtained froma group of 7 fertile men with normal karyotype. Additionally, for one of the carriers of rare rob(13;15) we offered an analysis of meiotic segregation pattern. For the other carriers, the meiotic segregation results were presented earlier [87].

MATERIALS AND METHODS

Patients

The collected group of RobT carriers, in whom sperm aneuploidy analysis was performed, comprised of six carriers with oligoasthenozoospermia or oligoasthenoteratozoospermia (OA or OAT, according to the WHO criteria, 2010 [88]) between 30 and 40 years of age. Carriers were coded as follows: R1 – rare rob(13;15), R2 – rare rob(13;15), R3 – rare rob(13;22), R4 – common rob(13;14), R5 – common rob(13;14) and R6 – common rob(13;14). The patients were diagnozed as Robertsonian carriers by classical cytogenetic karyotyping due to the reproductive failures and abnormal spermiogram. The general semen assessment of R1-R6 carriers is presented in a Table 1. Female partners had normal karyotypes. The aneuploidy evaluation of our control group (consisting of 7 fertile volunteers with normal karyotype between 20 and 25 years of age) was published earlier [58, 59]. For carrier R1 the analysis of meiotic segregation pattern was also carried out. For carriers R2, R3 and R4, the meiotic segregation patterns were previously described [87]. For R5 and R6, i.e. carriers of common rob(13;14), the meiotic segregation patterns were not performed, because the literature data indicate stable similar results for rob(13;14) carriers.

Our research program was approved by Local Bioethical Committee, Poznan University of Medical Sciences, and the R1-R6 carriers were notified of the purpose of the research, and the written informed consent was obtained.

Characteristics of chromosomes in peripheral blood lymphocytes

Peripheral blood lymphocytes were cultured *in vitro* for 72 hours (whole-blood cultures), and then incubated with colcemide (KaryoMAX/PBS, final concentration 100 ng/ml, Gibco, Grand Island, NY, USA), hypothonic solution of 0.075M KCl, and 3 times fixed with a freshly made solution of methanol: acetic acid, 3:1 v/v, -20°C, according to classic cytogenetic methodology [76]. Next, classical karyotyping using GTG banding technique (Trypsin, Lonza, Walkersville, MD, USA; Giemsa staining; Merck, Germany) was performed at metaphase stage [76]. Karyotypes were evaluated for at least 25 metaphase spreads in each case, with the resolution at 400–550 bands [49]. Banding results were documented using Olympus BX41 light microscope (Japan), equipped with objectives: 10× and 100× (an oil immerse objective)and analyzed with Ikaros software (MetaSystems, Germany) (for an example see Fig. 3).

2D-FISH (fluorescence *in situ* hybridization) procedure

For more detailed characteristics of RobT translocations, FISH was preparedaccording to a manufacturer's protocols with the following mixtures of directly labelled red or green FISH probes: (1) whole chromosome painting probes (wcp) for chromosomes: 13 (red) and 14 (green), or 13 (red) and 15 (green), or 13 (red) and 22 (green) (MetaSystems, Germany), each 8.0 µl (for an example see Fig. 4), and (2) α-satellite (centromere-specific) probes for chromosomes: 13/21 *(loci: D13Z1/D21Z1)*, 14/22 (*loci: D14Z1/D22Z*1), and 15 (*locus* D15Z4) (Cytocell, UK), each 2.5 µl, filled with hybridization buffer to a final volume of 10.0 µl (for an example see Fig. 1C). The efficiency of FISH was estimated at approximately 99%. At least 25 metaphase spreads were evaluated to find the mono- or dicentric character of the derivative RobT chromosome. To verify that RobT rearrangement was present in 100% of the cells, and to exclude the probability of mosaicism, at least 1.000 of interphase cells were also analyzed. According to the exclusion criteria of chromosomal mosaicism [33], the 99% confidence level with 1% of mosaicism excluded can be reached when analyzed at least 459 cells.

Sperm chromosomes evaluation

Semen preparation and FISH on spermatozoa were previously described [58, 59]. Briefly, ejaculated semen samples were collected after 3–5 days of sexual abstinence. After liquefaction and seminological analysis, the sperm samples were washed in F-10 medium, fixed with a fresh fixative solution (methanol:acetic acid, 3:1 v/v, -20°C) and stored at -20°C until further use.

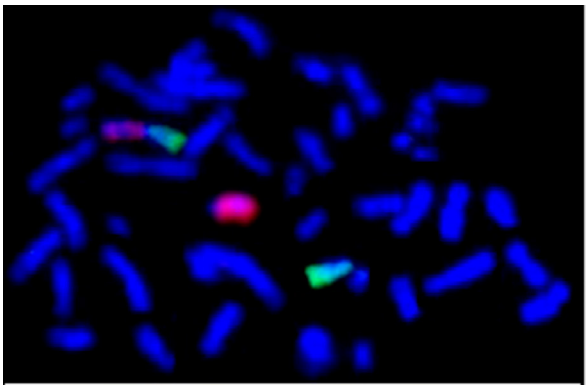


Fig. 4. An example of FISH staining with whole chromosome painting (wcp) probes for 45,XY,der(13;15)(q10;q10) (R4) carrier. Probes used: 13 – red, 14 – green (Cytocell, UK). Microscope used: Olympus BX41, oil-immersed objective 100×, fluorescent filter-set: FITC/Texas Red/Triple/DAPI; software: ISIS (FISH) (Meta Systems, Germany)

FISH on spermatozoa was prepared for sperm aneuploidy analysis of chromosomes 7, 9, 18, X and Y in R1-R6 carriers and for meiotic segregation patternin case of rob(13;15) carrier (R1). The following mixtures of directly labeled red or green FISH probes (Cytocell Technologies Ltd., Cambridge, UK) were applied: (1) for aneuploidy evaluation: D7Z1 (Green), D9Z1 (Red), D18Z1, (mix 1:1 Red:Green = orange), X (locus DXZ1; Green), Y (locus DYZ3; Red), band-specific probe 21q22.13 (green) and 22q12 (red) (Cytocell Technologies Ltd., Cambridge, UK); and (2) for meiotic segregation pattern: wcp for chromosomes 13 (red) and 15 (green) (Meta Systems, Germany), each 8.0 µl. FISH experiments were performed according to the manufacturer's protocol, and the technical details were published elsewhere [82]. Briefly, minor changes concerned: lower volumes of probes used for centromere-specific probes, and simultaneous denaturation of sperm DNA and probes' mixes on microscopic slides (2.5 min., 75°C) in all FISH combinations. Only the spermatozoa with untouched tail after DTT treatment (10 mM DTT, 100 mM Tris-HCL, pH 8.0; 43°C, 4–5 min.) were selected for analyzes. The efficiency of FISH was estimated at approximately 99%.

For sperm aneuploidy rates, at least 1.000 of sperm cells (mean n = 1.478) were counted for each RobT carrier, and for each FISH probes combination. Control aneuploidy evaluation was done for at least 5.000of spermatozoa per male per chromosome, and were published previously [58, 59]. For meiotic segregation pattern in the R1 case, 1.262 spermatozoa were analyzed. Two or three slides were analysed per each patient and FISH experiment. The FISH results were scored using Olympus BX41 fluorescent microscope (Japan) fitted with a 10x objective, a 100x oil immerse objective and a proper filter set (FITC/TexasRed/Triple/DAPI). The acquired images were analyzed using ISIS software (MetaSystems, Germany).

Statistical analysis

For the comparison of individual results of RobT carriers to the results of our control group, a Wilcoxon test was used. A significance level of $p<0.05$ was considered to be statistically significant (Origin Lab, v. 8.5 software).

Table 2. Individual aneuploidy results from the spermatozoa of six Robertsonian translocation carriers (R1–R6) analyzed in the study, and our laboratory mean control value. The results concern only the hyperhaploidy of chromosomes that are not involved in a particular translocation. Grey color indicates results statistically higher than mean control value

No.	Karyotype	Frequency of spermatozoa with disomy of chromosomes (n = 24) [%]:								Frequency of diploid spermatozoa (2n) [%]
		+7	+9	+18	+21	+22	XX	YY	XY	
Rare:										
R1	rob(13;15)	0.54*	0.43*	0.13	0.44*	0.24*	0.13	0.10	0.10	0.44*
R2	rob(13;15)	0.34*	0.35*	0.12	0.29*	0.29*	0.12	0.09	0.12	1.06*
R3	rob(13;22)	0.17	0.17	0.73*	0.31*	–	0.16	0.22*	0.33*	0.84*
Common:										
R4	rob(13;14)	0.13	0.13	0.07	0.08	0.16*	0.30*	0.03*	0.03	0.24*
R5	rob(13;14)	nd	nd	0	0	0	0.32*	0.11	0.11	0
R6	rob(13;14)	0.32*	0.26*	0.12	0	0	0.10	0.10	0.11	0.06
Mean control value [n=7] ± SD#		**0.13 ±0.07**	**0.12 ±0.08**	**0.09 ±0.05**	**0.11 ±0.07**	**0.08 ±0.06**	**0.11 ±0.09**	**0.10 ±0.05**	**0.08 ±0.02**	**0.07 ±0.02**

*statistically significant in comparison to our mean control value [p<0.05]; a Wilcoxon test at the significance level α = 0.05 was used to compare results. nd – not done. #laboratory control results previously published in Olszewska et al., 2013, 2014 [58, 59] *statistically significant in comparison to our mean control value [p<0.05]; a Wilcoxon test at the significance level α = 0.05 was used to compare results. nd – not done. #laboratory control results previously published in Olszewska et al., 2013, 2014 [58, 59]

RESULTS

Characterization of leukocyte chromosomes of R1-R6 carriers

The analysis of karyotypes using the GTG banding technique and 2D-FISH staining showed that RobT carriers (R1-R6) evaluated in this study were non-mosaic and the derivative RobT chromosomes were dicentric (for an example, see Fig. 1C).

Sperm FISH analysis

The results of hyperhaploidy levels of sperm chromosomes 7, 9, 18, 21, 22, X and Y from R1-R6 carriers are shown in Table 2. The results of R1-R6 carriers were compared with the mean control values acquired in our laboratory group [54, 55]. The results (mean) of R1-R6 carriers were significantly higher ($p<0.05$, Wilcoxon test) for all chromosomes analyzed when compared to control values. For each of the carriers we observed an elevated level of hypehaploidy of at least in one of the tested chromosomes. For R1 and R2 carriers, the increased level of disomy concerned chromosomes 7, 9, 21, 22, as well as the presence of diploid spermatozoa. For R3 carrier, the increased level of disomy concerned chromosomes 18, 21, YY, XY, as well as the diploid spermatozoa. Thus, for each of the carriers of rare RobT, an increase of the disomy of chromosome 21 and the diploidy were found. However, in the case of three carriers of common rob(13;14), there was 50% less results with elevated level of aneuploidy. For R4 carrier, the increased level of disomy concerned chromosomes 22, XX, YY, as well as the diploid spermatozoa. For R5 carrier, the increased level of disomy concerned only the XX spermatozoa. For R6 carrier, the increased level of disomy concerned chromosomes 7 and 9. All the listed differences were statistically significant ($p<0.05$).

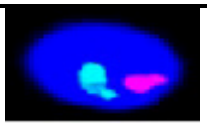
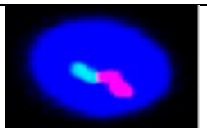
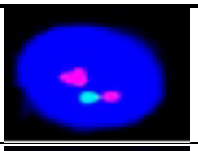
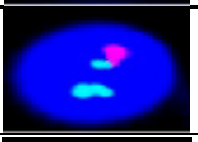
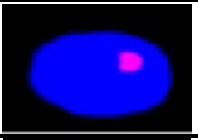
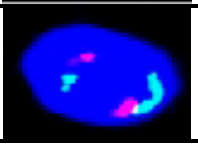
The result of the meiotic segregation patern obtained for the R1 carrier of rare rob(13;15)was presented in Table 3. The frequency of the spermatozoa exhibiting together a normal and balanced chromosomal content was 75.96%. Since we applied whole chromosome painting probes, an observation of normal and genetically balanced spermatozoa separately was possible(schemes and FISH pattern in the Table 3). The proportion of normal and genetically balanced sperm cells (40.26%:35.70%) was about 1.13. The frequencies after adjacent-1 and adjacent-2 segregations exhibited values from 3.76% to 8.08%. The frequency after 3:0/diploid segregation was 1.09%, while 0.11% of the results remained unexplained because of the untypical colour pattern observed (Table 3).

DISCUSSION

In the present study we performed an aneuploidy screening in spermatozoa of three new cases of rare RobT: two rob(13;15) and one rob(13;22), and three cases of common rob(13;14) (Table 2). Also, theanalysis of meiotic segregation pattern in one case of rare rob(13;15) was performed (Table 3).

According to the literature, aneuploidy levels have been recognized in at least 126 RobT carriers, and meiotic segregation pattern in 212 RobT carriers, including rare and common cases [1, 3, 4, 5 8, 9, 10, 11, 12, 13, 14, 17, 19, 22, 23, 25, 26, 28, 31, 32, 37, 39, 40, 41, 43, 44, 45, 46, 50, 53, 55, 56, 57, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 77, 78, 79, 80, 83, 84, 85, 86, 87, 89]. However, these data – although seemingly numerous, are still insufficient and they do not meet

Table 3. The result of meiotic segregation pattern analysis from the spermatozoa of rob(13;15) carrier (R1). Whole chromosome painting (wcp) probes of 2D-FISH were used, that allow to differentiate normal and balanced gametes separately (different FISH phenotype)

Segregation type			Schemes	FISH pattern probes: 13 wcp red; 15 wcp green	Sperm genotype	%	Total %
2:1	Alternate normal/ balanced	BALANCED			23	40.26	**75.96**
					22,–13,–15, +der(13;15	35.70	
	Adjacent-1	UNBALANCED			23,–15, +der(13;15)	8.08	**22.84**
					22, –13	3.76	
	Adjacent-2				23,-13, +der(13;15)	6.37	
					22, –15	4.63	
3:0 and/or 2n					24, +der(13,15)	1.09	**1.09**
					Sum of unbalanced		**23.93**
					Unexplained signals		**0.11**

criteria for meta-analyzes [27]. This is due to the fact that the data are very heterogenous, e.g., they concern carriers with different type of spermiogram abnormalities, most often the number of patients in the studied group is small, and the number of analyzed spermatozoa in each case differ significantly. Due to the relatively high frequency of RobTs carriers among infertile men, it is necessary to obtain more results with a considerably larger group of patients. This is especially important for carriers of rare RobTs because data for individual types of rare RobT are very little [41].

Published aneuploidy results for individual RobT carriers concern the most of chromosomes, but predominant analyzes were concentrated on hyperhaploidy of chromosomes 13, 15, 16, 18, 21, 22, X and Y (and also diploidy). These chromosomes have a special tendency to undergo non-disjunction, and/or these aneuploidies can generate potentially viable aneuploid embryos. So far, it has been known the results of aneuploidy analysis of 18, 20, 21, and sex chromosomes (plus diploidy) in only 6 cases of rare rob(13;15) [9, 23, 43, 57, 63, 83], and of chromosomes 6, 9, 15, 18 and 21 (plus diploidy) in only 4 cases of rare rob(13;22) [5, 11, 26, 86]. All these carriers had abnormal spermiograms, and there were only two (2/9) carriers who showed normal aneuploidy levels. In 79 cases of analyzed common rob(13;14) there were presented the aneuploidy results of 1, 2, 3, 7, 8, 12, 15, 17, 18, 21, 22 and sex chromosomes (plus disomy) (for chromosomes 1 and 15 available data are only from 3 carriers, for chromosomes 2, 3, 12 and 17 only from 2 carriers) [4, 8, 9, 17, 19, 23,

26, 28, 37, 43, 44, 53, 57, 74, 78, 85, 86]. Most of the analyzed cases (> 70%) were characterized by both elevated level of the hyperhaploidy of at least one of the tested chromosomes and abonormal spermiogram. From the data presented in the Table 1 it is clear that all our carriers R1-R6 demonstrated spermatogenic impairment. At the same time, we found the hyperhaploidy of at least one of the tested chromosomes in each of the carriers. Thus, our patients' aneuploidy results were typical for infertile RobTs carriers.

The fact that most of infertile RobT carriers have an altered spermatogenesis, i.e. they have mostly oligoasthenoteratozoospermia (OAT) with varying degree of intensity, has been known since the beginning of the RobTs study [15, 41, 48, 56, 84]. Moreover, unusual ultrastructural sperm anomalies related to immaturity of the cells were observed [8]. Additionally, in approximately half of described RobT carriers, so-called: interchromosomal effect (ICE; increased frequency of spermatozoa with aneuploidy of chromosomes that are not involved in rearrangement) was observed [4, 6, 8, 17, 19, 26, 53, 57, 72, 77]. Increased frequency of aneuploid gametes arises from a disrupted disjunction of chromosomes during meiosis leading to an association of asynaptic chromosomal regions preferentially with the sex chromosomes or with the acrocentric ones [30, 34, 35]. It is known that the presence of aneuploidyis one of the documented causes of decreased semen concentration and reproductive failures [6, 11, 39, 43]. It can be postulated that in RobT carriers the ICE frequency is higher because of the increased probability of association of additional chromosome to the asynaptic regions during formation of the trivalent in prophase I. However, there are also literature data concerning RobT carriers without ICE phenomenon [1, 31, 80]. Also, an increased frequency of spermatozoa with aneuploidy can be observed in males with normal karyotype but those usually demonstrate reproductive failure [30, 34, 35]. Thus, each RobT carrier with ICE presence should be evaluated individually.

It is estimated that in most carriers of different nonhomologous RobTs (a total of 212 analyzed cases), the frequency of genetically normal/balanced segregants is close to 80%, although the results range from 51% to 99% [5, 41]. The highest frequency of spermatozoa after alternate segregation (>50%) and also the proportion of other segregantsis characteristic for all carriers of RobT regardless of the type of RobT [41]. Luciani f [42] suggested that the dominant number of normal and genetically balanced segregants can be the consequence of the fact that in the pachytene the trivalent of pairing chromosomes occurs in the cis configuration. It is also known that in the most carriers of nonhomologous RobT, the number of offspring with normal and balanced karyotypes are similar, which is compliant with theoretical expectations [20]. According to many authors, the above data can indicate a "homogenous segregation behaviour" of Robertsonian translocations independent of the chromosome pairs involved [5, 57, 67, 73].

So far, results of meiotic segregation pattern of rare rob(13;15) have been published only for 16 carriers [12, 13, 23, 41, 51, 52, 57, 62, 66, 68, 70, 87]. About 70% of the rob(13;15) carriers had normal/balanced sperm cellsfrequency above 75%, and our R1 carrier also belongs to this group (75.96% of alternate segregants in Table 3).

It is generally assumed that the reduced fertility in RobT carriers is mainly the consequence of the formation of chromosomally unbalanced sperm cells as a result of the segregation of the chromosomes involved in the meiotic prophase trivalent. The second critical impact on carriers' reproductive ability seems to be closely related with the multifactorial nature of aneuploidy of chromosomes unrelated to those involved in the particular translocation. In some RobT carriersthe relatively low frequency of aneuploidy of some chromosomes can be caused by a meioticcheckpoints triggering apoptosis. At the same time however, the elimination rate of these abnormal cells is not totally efficient, leading to both:a reduction in gamete production and not preventing aneuploidy as such. However, it should be noted that it is hard to clearly assess the influence of RobT on infertility, since there are also fertile carriers among the carriers of the same RobT. This could suggest that sperm production impairment is due to global interactions between the translocated chromosomes and the whole genome, perhaps influencing not shown epigenetic changes. Also, the genetic counselling (including prenatal genetic diagnostics, when necessary) for the RobT carriers, who have reproductive problems has to be implemented to evaluate the individual risk level of the miscarriages and/or a probability of having a healthy offspring.

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