Criteria to evaluate patterns of segmental and complete aneuploidies in preimplantation genetic testing for aneuploidy results suggestive of an inherited balanced translocation or inversion

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Objective: To define criteria for determining when preimplantation genetic testing for aneuploidy (PGT-A) results are suggestive of a potential balanced chromosomal rearrangement in the egg or sperm source and warrant karyotyping.
Design: Performance evaluation of criteria developed to assess PGT-A results for patterns of imbalances suggestive of a balanced chromosomal rearrangement in the egg or sperm source.
Setting: A single PGT-A laboratory and multiple in vitro fertilization centers.
Patients: Reproductive couples who underwent routine PGT-A testing.
Interventions: Karyotyping of reproductive couples for whom patterns of imbalances observed in PGT-A results suggested a balanced chromosomal rearrangement in the egg or sperm source.
Main Outcome Measures: Correct or incorrect flagging of predicted translocation in either the egg or sperm source based on chromosome analysis.
Results: Proposed criteria correctly predicted a balanced reciprocal translocation in 97% of cases (n = 33), a (13;14) Robertsonian translocation in all cases (n = 3), and an inversion in all cases (n = 2). Other criteria evaluated were determined to be ineffective because of relatively low occurrences that met the criteria and/or low predictive value.
Conclusions: Our results showed that the proposed criteria were effective for evaluating patterns of imbalances observed in PGT-A results suggestive of a potential chromosomal rearrangement in the egg or sperm source. Our proposed criteria can be employed by clinicians in the in vitro fertilization setting in combination with a patient's reproductive history to identify PGT-A patients who are likely carriers of balanced chromosomal rearrangements. (Fertil Steril Rep® 2021;2:72–9. ©2020 by American Society for Reproductive Medicine.)
Key Words: PGT-A results, segmental aneuploidy, translocations, inversions, pattern detection

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Balanced chromosomal rearrangements typically cause no health concerns to carriers, especially if they are identified incidentally (1–5). However, a carrier of a balanced rearrangement can transmit unbalanced rearrangements that can disrupt normal development in their offspring (1–4, 6, 7). Genetic imbalances can be severe enough to prevent early embryonic development or can be compatible with development to term but cause multiple congenital anomalies in liveborns (1, 3, 4, 6, 8).

Preimplantation genetic testing for structural rearrangements (PGT-SR) is performed only when a patient or her

Received August 19, 2020; revised November 19, 2020; accepted December 12, 2020.
A.C.S. has nothing to disclose. T.D. has nothing to disclose. L.S. has nothing to disclose. A.A. has nothing to disclose. C.C. has nothing to disclose. R.K. has nothing to disclose.
Supported by Igenomix, SL.
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Fertil Steril Rep® Vol. 2, No. 1, March 2021 2666-3341

https://doi.org/10.1016/j.xfre.2020.12.003

partner is known to carry a balanced rearrangement, otherwise, routine preimplantation genetic testing for aneuploidy (PGT-A) is performed (9–14). Approximately 1/500 individuals carry a balanced chromosomal rearrangement, and this frequency is approximately 1/100 in the in vitro fertilization (IVF) patient population (5, 15–18). Karyotyping is recommended for patients with recurrent pregnancy loss but is otherwise not routinely performed as part of the standard infertility workup (19–21). Therefore, a patient may undergo IVF with PGT-A without knowing their status as a carrier of a balanced translocation or inversion.

PGT-A can detect aneuploidy, including subchromosomal imbalances greater than approximately 10 MB, in embryos. Most aneuploidy is sporadic, but occasionally, patterns of aneuploidy can be observed through PGT-A suggestive of a balanced structural rearrangement in either the egg or sperm source. Sporadic segmental aneuploidies occur at a considerable frequency in embryos and are most often mitotic in origin (22); however, observing multiple embryos with the same pair of segmental aneuploidy is unlikely to be due to random chance. Similarly, observing multiple embryos with complete aneuploidy of the same chromosome or the same two chromosomes may not underlie the transmission of genetic imbalances from a carrier of a balanced chromosomal rearrangement.

Previous studies have aimed to characterize the type and frequency of the various modes of segregation observed through PGT from known translocation carriers (9, 23–28). Here, we report on our experience in observing characteristic patterns of aneuploidy in the results of PGT-A, performed for couples who were not known a priori to be carriers of a balanced rearrangement. We developed criteria to evaluate patterns in PGT-A results across multiple embryos. If cases met the criteria, a cytogenetic analysis was recommended for both members of the reproductive couple. The predictive values of the criteria were analyzed, and modified criteria were proposed.

When aneuploidy rates for a PGT-A cycle are higher than expected, and when certain aneuploidies are recurrent, patients and clinicians may wonder if there could be an underlying genetic explanation. We proposed criteria by which PGT-A results suggestive of a potential inherited translocation or inversion can be evaluated in conjunction with clinical reproductive history to determine the need for chromosome analysis in the egg or sperm source.

MATERIALS AND METHODS

This study was deemed exempt from approval by the Advarra Institutional Review Board (Pro00040199).

Development of Criteria

The criteria for flagging potential inherited chromosomal rearrangements were developed based on the expected imbalances from classical meiotic segregation (Fig. 1).

Reciprocal translocation. Three test criteria were developed to flag a potential reciprocal translocation (Table 1). Chromosomes involved in a reciprocal translocation segregate from the meiotic quadrivalent, resulting in balanced gametes, gametes with a pair of segmental imbalances, and gametes with complete aneuploidy (Fig. 1A). These segregation patterns have been observed in embryos through fluorescence in situ hybridization (FISH)-, comparative genomic hybridization by microarray (aCGH)-, single nucleotide polymorphism (SNP)-, and next generation sequencing (NGS)-based PGT (24, 29, 30).

The first criterion for reciprocal translocations requires the same terminal segmental aneuploidy to be observed in ≥ 2 embryos (Table 1, criterion 1a). Classical adjacent or tertiary segregations would predict 2 segmental aneuploidies in the same embryo; however, considering the potential for 1 segmental imbalance to be below the detection limit of PGT-A, it was only required to observe a single segmental aneuploidy in ≥ 2 embryos. An example of PGT-A results meeting criterion 1a is shown in Figure 2A. The second criterion flags cases in which a combination of complete imbalances and segmental imbalances is observed in different embryos (Table 1, criterion 1b). A third criterion flags cases in which patterns of complete aneuploidy only are observed (Table 1, criterion 1c). Criterion 1c requires complete aneuploidy of 1 chromosome to be observed in ≥ 3 embryos, as well as complete aneuploidy of a second chromosome to be observed in ≥ 3 embryos. An imbalance of the first chromosome may be present in the same embryo with an imbalance of the second chromosome. At least 50% of a PGT-A cohort must involve 1 or 2 of these chromosomes.

Robertsonian translocation. Two test criteria were developed to flag a potential Robertsonian translocation. Chromosomes involved in a Robertsonian translocation form a trivalent during meiosis (Fig. 1B). Segregation results in balanced gametes or gametes with complete monosomy or trisomy of one or both of the translocation chromosomes (31).

A Robertsonian translocation was predicted when a high incidence of aneuploidy involving 1 or 2 acrocentric chromosomes was observed. To tease apart aneuploidy due to a Robertsonian translocation from aneuploidy due to random nondisjunction, a threshold incidence of 50% of the PGT-A cohort was implemented. The Robertsonian translocation involving chromosomes 13 and 14, referred to here as the rob(13;14) translocation, occurs at an incidence of 1:1,300 in the general population, whereas all other Robertsonian translocations including acrocentric chromosomes 15, 21, and 22 are more rare (17). Therefore, a lower threshold was established for the rob(13;14) translocation. For rob(13;14) translocations, a minimum number of 3 embryos with an imbalance of chromosomes 13 or 14 was imposed (Table 1, criterion 2a). An example of PGT-A results meeting criterion 2a is shown in Figure 2B. For any other Robertsonian translocation, a minimum number of 4 embryos with an acrocentric imbalance was required (Table 1, criterion 2b).

Inversion. A single test criterion was developed to flag a potential inversion. Chromosomes involved in an inversion partially align or form a loop during meiosis (Fig. 1C) (7). When crossover events occur within the inverted segment, unbalanced gametes

result. After fertilization, imbalances would include partial monosomy of 1 terminal segment and partial trisomy of the other terminal segment of the same chromosome. In PGT-A profiles, this imbalance is observed as a unique, 3-step "stair" pattern with a terminal trisomy, interstitial disomy, and a terminal monosomy along the chromosome (Fig. 2C). The inversion criterion required the same "stair" pattern to be observed in ≥ 2 embryos (Table 1, criterion 3).

PGT-A Population

Cases were collected by a single commercial laboratory performing PGT-A by aCGH or NGS at multiple locations in North America performing NGS. PGT-A was requested by various referring physicians and IVF centers. Because PGT-SR is recommended for known carriers of a chromosomal rearrangement, PGT-A is performed only for patients for whom there is no a priori knowledge of a balanced translocation or inversion. Approximately 20,000 PGT-A cases were included in the study; however, because referring providers are not required to provide reproductive or family history when requesting PGT-A, it was not possible to exclude patients with normal karyotypes. Our study population may include patients with normal karyotypes, patients whose reproductive or family history did not indicate karyotyping, as well as patients for whom karyotyping was not performed despite a concerning reproductive or family history. Because of the inability to control for these variables, the current research did not attempt to determine the incidence of unknown chromosomal rearrangement carriers in the general PGT-A population.

PGT Technology

Comparative genomic hybridization by microarray (aCGH). Whole genomic amplification (WGA) of the biopsy samples were performed with the use of the DNA Amplification System (Sureplex; Illumina Inc., San Diego, CA). Labeled WGA was hybridized onto the slides (24sure, Illumina Inc.), scanned at 10 μm using a laser scanner (BlueGnome), and analyzed by algorithm-fixed settings in the software (BlueFuse Multi; Illumina Inc.).

Next Generation Sequencing (NGS). Samples were subjected to cell lysis, WGA, and construction of libraries using the kit for 24 chromosome aneuploidy screening (Ion ReproSeq; Thermo Fisher Scientific, Carlsbad, CA). NGS was performed on the Ion Chef and Ion S5 System instruments (Thermo Fisher Scientific). Data analysis was performed using Ion Reporter software (Thermo Fisher Scientific).

PGT-A was performed using aCGH or NGS. Both technologies screen chromosomes and report aneuploidies and segmental aneuploidies with similar accuracy of 98%. The expected resolution with aCGH and NGS is 6 MB and 10 MB, respectively. The use of different technologies would not be expected to affect the likelihood for a chromosomal rearrangement to be confirmed after criteria have been met. Following completion of PGT-A, the results were evaluated by laboratory personnel for patterns according to the test criteria.

Recommendations for Karyotyping and Follow-up

The referring physician was notified of the suspected maternal or paternal chromosomal rearrangements, and karyotyping was recommended. The results of the karyotypes were requested at least twice. If karyotypes were negative following a prediction of a reciprocal translocation, additional analysis, including reanalysis of the previous karyotype and additional FISH testing, was recommended to assess a cryptic translocation. No additional analysis was pursued if karyotypes were negative following a prediction of a Robertsonian translocation. The predictive values of the criteria were assessed. Nonperforming criteria were eliminated, and new criteria for flagging chromosomal rearrangements were proposed.

RESULTS

A total of 78 cases that met the criteria were flagged as having a potential chromosomal rearrangement. Karyotypes were returned by the referring physician for 41 cases. The remaining 37 cases did not receive clinical follow-up either because of the patient declining the recommendation for karyotyping or from lack of response from the referring physician's office.

Performance for reciprocal translocations. A total of 58 cases were flagged for a potential reciprocal translocation, 55 of which were flagged using criterion 1a (Table 1). The smallest cohort size was 2 embryos, and the average cohort size was 7.5 embryos. Of the cases flagged with criterion 1a in which clinical follow-up was received, reciprocal translocations were confirmed by cytogenetic analysis in 97% of cases (32 of 33). Applying criterion 1b resulted in 3 flagged cases, for which clinical follow-up was provided for 1 case, and a translocation was not observed in either of the reproductive couple. No cases were flagged using criterion 1c.

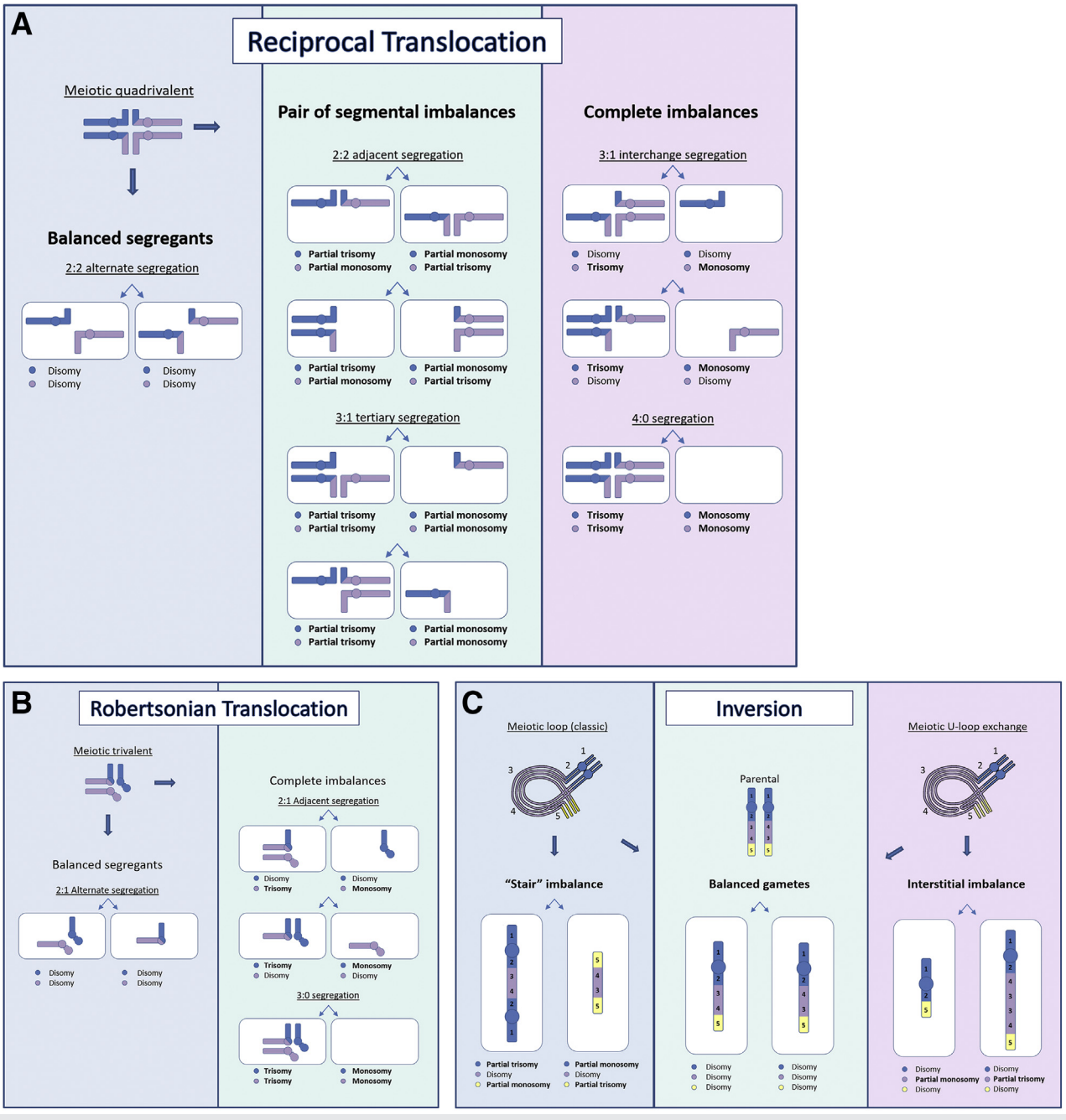
Performance for Robertsonian translocations. A total of 16 cases were flagged using criterion 2 for Robertsonian translocations. The smallest cohort size was 4 embryos, and the average cohort size was 7.4 embryos. Cytogenetic analysis confirmed all 3 of the cases flagged using criterion 2a for a rob(13;14) translocation. Clinical data were not provided for the majority of cases ($n = 11$) that were flagged using criterion 2b for non-rob(13;14) Robertsonian translocations including acrocentric chromosomes 15, 21, and 22. Of the 2 cases in which karyotypes were ordered and results were provided, a Robertsonian translocation was not observed in either of the reproductive couple.

Performance for inversions. A total of 4 cases were flagged using criterion 3 for inversions. The smallest cohort size was 5 embryos, and the average cohort size was 7.3. Clinical follow-up was provided for 2 cases, both of which were confirmed predictions. One inversion was found to be pericentric, and the other was paracentric.

Proposed Criteria

The test criteria were modified according to the performance (Table 2). Criterion 1b was removed because of the small number of cases flagged and negative karyotypes. Criterion 1c was

FIGURE 1



Classic segregation patterns in gametes and embryos. During meiosis, chromosomes involved in a rearrangement align in modified structures to allow crossing over and then segregate to daughter cells, resulting in balanced or unbalanced gametes. The resulting genetic complement, following fertilization of the egg and sperm, is indicated for each chromosome below the daughter cell. (**A**) Reciprocal translocations. Balanced gametes can result from alternate 2:2 segregation, segmental imbalances can result from adjacent 2:2 or tertiary 3:1 segregation, and complete imbalances can result from interchange 3:1 or 4:0 segregation. (**B**) Robertsonian translocations. Balanced gametes can result from alternate 2:1 segregation, and imbalances can result from adjacent 2:1 or 3:0 segregation. Imbalances resulting from segregation from a Robertsonian trivalent include complete chromosomal imbalances, rather than segmental imbalances. (**C**) Inversions. Balanced gametes can result from segregation of the parental chromosomes, and imbalances can result in segregation of the recombinant chromosomes. If recombination occurs within the inverted segment of a classic loop, recombinants have a terminal trisomy, followed by an interstitial disomic segment, followed by a terminal segment of monosomy, a pattern referred to here as a "stair" imbalance. Following the U-loop exchange, imbalances involve either an interstitial segment of monosomy or an interstitial segment of trisomy.

Snider. Translocation flagging after PGT-A. Fertil Steril Rep 2020.

FIGURE 2

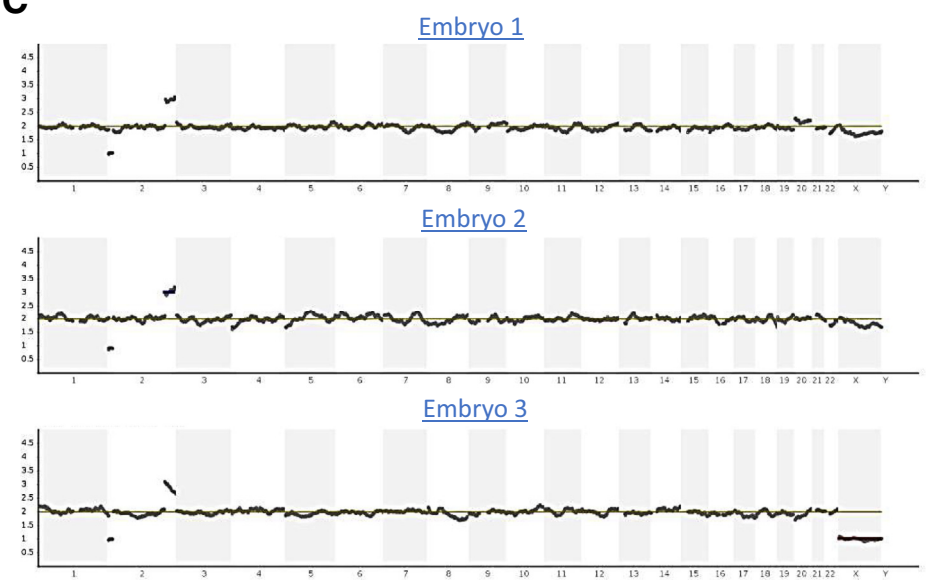
A

Embryo	Results
1	**-2q, +9q**, +22
2	Euploid
3	**-2q, +9q**

B

Embryo	Results
1	**-13**
2	-16
3	-15
4	**+13**
5	Euploid
6	**-13**
7	**+14**

C



Examples of cases flagged for karyotype review. (**A**) Example of PGT-A results meeting criterion 1a for a reciprocal translocation. These results demonstrate a recurring pair of segmental imbalances of chromosomes 2 and 9. (**B**) Example of PGT-A results meeting criterion 2a for 13;14 Robertsonian translocation. These results demonstrate 4 of 7 embryos with complete aneuploidies of acrocentric chromosomes 13 and 14. (**C**) Example "stair" NGS profile meeting criterion 3 for an inversion. A terminal segment of monosomy, an interstitial segment of disomy, and a terminal segment of trisomy is observed for chromosome 2, and this pattern is observed in multiple embryos. NGS = next generation sequencing; PGT-A = preimplantation genetic testing for aneuploidy.

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removed. Criteria for flagging a Robertsonian translocation were restricted to the rob(13;14) translocation, and criterion 2b was removed. No modifications were proposed for inversion criterion 3.

We retroactively evaluated the performance of our predictions by applying the new proposed criteria. Clinical follow-up was received for a total of 37 cases meeting the proposed criteria. Cases that were flagged for a reciprocal translocation were confirmed 97% of the time (n = 33). Cases that were flagged for a rob(13;14) translocation were confirmed 100% of the time (n = 3). Cases that were flagged for an inversion were confirmed 100% of the time (n = 2).

DISCUSSION

A newly identified translocation or inversion can provide an explanation to couples who have experienced infertility or previous pregnancy loss. In addition, identifying a translocation or inversion can benefit patients in their future reproductive journey. Higher resolution aneuploidy screening with PGT-SR may be available following confirmation of the predicted chromosomal rearrangement. More accurate estimates of the chance of aneuploidy in embryos can reframe expectations for upcoming cycles and enable informed decisions about other reproductive options, including the use of gamete donors. In addition, balanced chromosomal rearrangements may be shared among relatives, and this information may be crucial, especially for family members in their reproductive years.

Limitations of the Study

The pattern detection was not automated and required a laboratory technician to manually evaluate each set of PGT-A results to identify patterns meeting the criteria. Cases meeting the criteria may have been missed if a pattern was not readily observed by a technician. Sample sizes were small for rob(13;14) translocations and inversions.

Predictive Values of the Criteria

The predictive values of our test criteria varied between the different types of chromosomal rearrangements. Differences in prevalence and patterns of observed aneuploidies account for much of the differences.

Performance of criterion 1. Reciprocal translocation test criterion 1a that involved the detection of recurrent segmental aneuploidy demonstrated the most success. Imbalances resulting from reciprocal translocations include segmental aneuploidies that are less likely to occur by random chance than complete aneuploidies. Although classical meiotic segregation from a quadrivalent predicts the transmission of a pair of segmental imbalances, PGT-A may detect only one of the segmental imbalances due to resolution limitations. The second segmental imbalance may appear to be a complete imbalance or may appear as disomy ("normal") if one of the segments is very small. Our data demonstrated that, rather than requiring that a pair of segmental imbalances be

TABLE 1

Performance of the test criteria.

Test criteria	Follow-up obtained: Confirmed	Not confirmed	No follow-up	Grand total
Reciprocal translocations	**32**	**2**	**24**	**58**
1a) Segmental imbalances: a terminal, segmental imbalance in ≥2 embryos, involving the same chromosome and the same breakpoint between the embryos	32	1	22	55
1b) Segmental and complete imbalances: 1 segmental imbalance in 1 embryo paired with a complete imbalance of the same chromosome in another embryo and a segmental imbalance of a second chromosome in 1 embryo paired with a complete imbalance of the same second chromosome in another embryo	0	1	2	3
1c) Complete imbalances: a set of ≥3 embryos with monosomy/trisomy of the same chromosome and a second set of ≥3 embryos with monosomy/trisomy of another chromosome. At least 50% of the cohort must include related aneuploidies.	0	0	0	0
Robertsonian translocations	**3**	**2**	**11**	**16**
2a) Rob(13;14): ≥3 embryos with chr13 or chr14 monosomy/trisomy with ≥50% of the cohort affected	3	0	0	3
2b) Any other Robertsonian: ≥4 embryos with acrocentric monosomy/trisomy with ≥50% of the cohort affected	0	2	11	13
Inversions	**2**	**0**	**2**	**4**
3) Two or more embryos with characteristic inversion "stair" pattern in any cohort size. The "stair" pattern involves a terminal segment of monosomy, an interstitial segment of disomy, and a terminal segment of trisomy.	**2**	**0**	**2**	**4**
Grand total	**37**	**4**	**37**	**78**

Note: Cases in which the referring provider was notified of the suspected chromosomal rearrangement were considered "flagged." Cases in which no response was received or the patient declined karyotyping were considered to be "lost to follow-up." Cases were counted as "confirmed" when karyotypes were provided confirming the suspected chromosomal rearrangement. Cases were counted as "not confirmed" when cytogenetic analysis failed to identify the suspected translocation.

Snider. Translocation flagging after PGT-A. Fertil Steril Rep 2020.

observed in ≥2 embryos, identifying the same *single* segmental imbalance in ≥2 embryos was sufficient to predict the presence of a balanced translocation in either the egg or sperm source. Cases that were flagged if there was a classic pair of segmental imbalances in ≥2 embryos were predictive in all cases.

Cases in which the PGT-A results were flagged using criterion 1a, but the karyotypes were returned as negative, were worthy of additional scrutiny. In one compelling case in which the karyotypes were originally returned as normal, the suspected translocation was communicated to the cytogenetics laboratory, and reanalysis confirmed a cryptic rearrangement in the patient. The cytogenetics laboratory must be notified of the suspected translocation, and FISH testing may be necessary for some very cryptic rearrangements.

Performance of criterion 2. Imbalances resulting from Robertsonian translocations are complete aneuploidies. However, complete aneuploidies observed with PGT-A are typically spontaneous, especially among women of advanced maternal age. Despite the prevalence of Robertsonian translocations in the general population, very few cases were flagged relative to criterion 1. This is most likely due to known carriers pursuing PGT-SR, small cohort sizes, and the challenges in differentiating between Robertsonian imbalances and sporadic aneuploidy. Although only a few cases were flagged for the rob(13;14) translocation, karyotyping confirmed the translocation in each case. For each of the cases flagged for a Robertsonian translocation other than the rob(13;14), the karyotypes were negative. Therefore, we proposed criteria useful for flagging the more prevalent rob(13;14) translocation and suggested that the results of PGT-A were not able to illuminate any other, less prevalent, Robertsonian translocation. A patient's reproductive and family histories remain the most powerful tool for identifying Robertsonian translocation carriers.

Performance of criterion 3. The characteristic "stair" inversion pattern is rarely observed and is not expected to be due to random chance, especially when observed in more than 1 embryo. Although our proposed criterion picked up only 4 cases, and only 2 of which received clinical follow-up, it is expected that cases flagged using the inversion criterion will be accurate predictions.

Clinical Utility

Identifying a balanced chromosomal rearrangement can alter the expected euploidy rate following PGT and reframe the chance of reproductive success using autologous gametes.

PGT-SR may be appropriate for future IVF cycles. PGT-SR is the validated platform for unbalanced rearrangement detection for translocation and inversion carriers. Breakpoints of the rearrangement confirmed in the egg or sperm source must be determined by high-resolution, G-banding karyotype. The breakpoints of segmental imbalances determined by PGT-A are not precise and should not be used to extrapolate the breakpoints of a presumed parental balanced translocation or inversion.

Rerunning PGT-SR can be considered for additional reassurance in cases in which the structural rearrangement is confirmed. PGT-A routinely detects complete aneuploidies

TABLE 2

Proposed criteria for flagging a potential chromosomal rearrangement.

1) Reciprocal translocations	**Predictive value**
A terminal, segmental imbalance in ≥2 embryos, involving the same chromosome and the same breakpoint	97% (n = 33)
2) Rob(13;14) translocation	
Three or more embryos with complete aneuploidy of chr13 or chr14 with ≥50% of the cohort affected	100% (n = 3)
3) Inversions	
Two or more embryos with characteristic inversion "stair" pattern in any cohort size	100% (n = 2)

Note: These criteria are proposed to be applied to the results of a preimplantation genetic testing for aneuploidy cohort to determine the likelihood of a balanced rearrangement in the egg or sperm source. If cases meet the criteria, it is recommended to perform high-resolution karyotyping, followed by FISH analysis in the case of a possible cryptic translocation. The clinical history of the reproductive couple should also be taken into consideration.

Snider. Translocation flagging after PGT-A. Fertil Steril Rep 2020.

such as those that would result from Robertsonian translocations. Segmental aneuploidies resulting from reciprocal translocations and inversions detected in one embryo should have also been detected, if present, in other embryos. However, it is possible that segmental imbalances may have been missed in embryos from individuals carrying a small, balanced reciprocal translocation or inversion. Karyotyping to confirm the breakpoints and size of potential imbalances is necessary to determine the need to rerun samples.

Even if rerunning the samples is deemed unnecessary (e.g., Robertsonian translocations for which the resulting imbalances would be complete aneuploidies), a PGT-SR report documenting the translocation or inversion can facilitate continuity of care because the patient's medical records are transferred to their prenatal care provider. The identification of a rob(13;14) translocation might have the additional benefit of guiding prenatal counseling about uniparental disomy studies.

Lastly, balanced translocations can be shared among family members. Knowing this information could be beneficial to relatives, especially those in their reproductive years.

Considerations for the Proposed Criteria

Despite the high performance of the proposed flagging criteria, there are several limitations in identifying suggestive patterns from PGT-A results that are likely to result in low sensitivity. The reproductive and family history of the patient must be considered when evaluating the indication for karyotyping. PGT-A should not be viewed as a tool for detecting chromosomal rearrangements or performed in lieu of karyotyping for a patient or partner with a concerning family or reproductive history.

Cohort size. Patterns are less likely to emerge in a small PGT-A cohort. For example, a pattern could not be apparent if a patient has only a single embryo. Random, age-related aneuploidy is difficult to differentiate from complete imbalances resulting from the transmission of an unbalanced Robertsonian translocation. The cohort size must be large enough to observe a significant increase in the incidence of acrocentric aneuploidies, and women of advanced maternal age are less likely to achieve this number.

Inability to detect balanced rearrangements. Carriers of balanced rearrangements may transmit the unbalanced rearrangement, balanced rearrangement, or normal (nonderivative) chromosomes to embryos. PGT-A is unable to differentiate between euploid embryos with the nonderivative chromosomes and euploid embryos with the balanced rearrangement. Pattern detection, therefore, relies on the transmission of unbalanced rearrangements.

Translocation/inversion size. Reciprocal translocations and inversions involve breakpoints that define segments of varying sizes. The size of the resulting segmental aneuploidies in embryos is determined by the location of the breakpoints. If the breakpoint of a translocation is very close to the telomere, the resulting segment would be small, below the detection limit of PGT-A. If both breakpoints of a translocation are very terminal, PGT-A would not detect any segmental imbalance, and the inherited translocation would not be flagged. Similarly, if both breakpoints of an inversion were very terminal, the resulting small segmental imbalances would not be detected.

Maternal age. Complete aneuploidy can result spontaneously, especially in older women. Complete aneuploidy resulting from spontaneous nondisjunction cannot be differentiated from aneuploidy resulting from a reciprocal or Robertsonian translocation. Flagging of Robertsonian translocations will likely have a lower predictive value in cases where the woman is of advanced maternal age. Fortunately, maternal age is not expected to affect the predictive value of the reciprocal translocation criterion because segmental aneuploidy is not correlated with increasing maternal age.

In conclusion, we propose criteria that are highly effective for predicting balanced reciprocal translocations, rob (13;14) translocations, and inversions in the egg or sperm source from PGT-A cases in which the rearrangement was previously unknown. The criteria proposed herein should be used in conjunction with other reproductive and family history factors. Such factors include maternal age, PGT-A results between multiple cohorts and/or between multiple reference laboratories, previous pregnancies with aneuploidy, miscarriage, biochemical pregnancies, and failed implantation. Clinicians should still order karyotyping for a patient or partner before a PGT-A cycle if there is reason to suspect a chromosomal rearrangement based on the reproductive and/or family history. PGT-A results are not diagnostic for an inherited balanced chromosomal rearrangement and karyotyping must be performed to confirm a balanced rearrangement in an individual. Our data underscore the importance of follow-up testing and counseling by a certified genetic counselor when PGT-A predicts a balanced chromosomal rearrangement.

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