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GENETICS

Two different microarray technologies for preimplantation genetic diagnosis and screening, due to reciprocal translocation imbalances, demonstrate equivalent euploidy and clinical pregnancy rates

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Abstract
Purpose To compare single nucleotide polymorphism (SNP) and comparative genomic hybridization (aCGH) microarray platforms to evaluate embryos for parental translocation imbalances and aneuploidy.
Methods A retrospective review of preimplantation genetic diagnosis and screening (PGD/PGS) results of 498 embryos from 63 couples undergoing 75 in vitro fertilization cycles due to parental carriers of a reciprocal translocation.
Results There was no significant difference between SNP and aCGH microarrays when comparing the prevalence of embryos that were euploid with no translocation imbalance, euploidy with a parental translocation imbalance or aneuploid with or without the parental chromosome imbalance. The clinical pregnancy rates were also equivalent for SNP (60 %) versus aCGH (65 %) microarrays. Of 498 diagnosed embryos, 45 % (226/498) were chromosomally normal without translocation errors or aneuploidy, 22 % (112/498) were euploid but had a parentally derived unbalanced chromosomal segregant, 8 % (42/498) harbored both a translocation imbalance and aneuploidy and 24 % (118/498) of embryos were genetically balanced for the parental reciprocal translocation but were aneuploid for other chromosomes. The overall clinical pregnancy rate per IVF cycle following SNP or aCGH microarray analysis was 61 % and was higher if the biopsy was done on blastocysts (65 %) versus cleavage stage embryos (59 %), although not statistically significant.
Conclusions SNP or aCGH microarray technologies demonstrate equivalent clinical findings that maximize the pregnancy potential in patients with known parental reciprocal chromosomal translocations.

Keywords Translocation · PGD · PGS · Microarray · IVF · Reciprocal

Capsule Both single nucleotide polymorphism and comparative genomic hybridization microarrays demonstrate an equivalent ability to identify unbalanced parental translocations and the ploidy status within embryos undergoing preimplantation genetic diagnosis/screening and result in equivalent pregnancy outcomes.

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Introduction

Balanced reciprocal chromosomal translocations are a relatively common genetic abnormality that occurs when there is an exchange of terminal segments between different chromosomes [1,2]. The incidence of balanced reciprocal

translocations in genetic surveys of the general population and newborns is approximately 1/800 to 1/1100 and affects 35.5/1000 of couples with recurrent pregnancy loss (RPL) [3,4].

In most cases, a person is unaware that they have a balanced reciprocal translocation because carriers are normally unaffected and their carrier status is not identified unless they undergo a genetic chromosome evaluation. Frequently these evaluations are due to a history of decreased fertility, unexpected RPL, or the birth of a child with a malformations and/or mental retardation. Balanced reciprocal translocations are thought to directly contribute to both infertility and RPL due to the production of genetically unbalanced gametes from the carrier of the balanced reciprocal translocation chromosome [5–8].

The chance that a carrier of a balanced reciprocal translocation will produce a viable chromosomally abnormal embryo ranges from approximately 5–80 %. This depends upon the meiotic segregation pattern of the translocation chromosome in the gametes of the carrier and the location and size of the chromosome imbalance; larger imbalances are likely associated with poor embryo development or embryo lethality [9].

In couples with a history of RPL who also have a known balanced reciprocal translocation in one parent, in vitro fertilization (IVF) coupled with preimplantation genetic diagnosis (PGD) using fluorescence in situ hybridization (FISH) has been used for the purpose of improving pregnancy and live birth rates [10–17]. However, the ability of FISH PGD to improve IVF pregnancy rates has been disappointing with historical clinical pregnancy rates only approximating 40 % [18]. These low pregnancy rates were likely due to the limited chromosomal evaluation that FISH PGD provides. FISH PGD used for reciprocal translocations identifies only those chromosome imbalances of the translocation chromosomes, but it does not evaluate all 23-pairs of chromosomes for the presence of aneuploidy that can occur independent of the known parentally affected chromosomes. Simultaneous aneuploidy screening for all 23-pairs of chromosomes is important because aneuploidy is frequently found in developing embryos [19] and is the most common cause of all first trimester miscarriage [20,21].

In the early to mid-2000s genetic laboratories began developing new microarray technologies capable of testing all 23-pairs of chromosomes for aneuploidy, while simultaneously testing for structural chromosome aberrations. The two principal microarray platforms available for genetic testing include single nucleotide polymorphism (SNP) and comparative genomic hybridization (aCGH) arrays. The differences between SNP and aCGH arrays are extensive. SNP arrays provide a genotype (i.e. AA, BB or AB) for each marker and are denser than aCGH microarrays. In contrast, aCGH arrays use ratio labeling and are less dense than SNP microarrays. The first successful application of SNP and aCGH microarrays to detect chromosome translocation imbalances due to parental reciprocal translocation carriers and the simultaneous screening for all 23 chromosome pair aneuploidy was reported in 2011 [8,22,23]. While these initial results were promising and showed increased pregnancy rates as compared to FISH, the total numbers of patients evaluated in these studies were limited. Following these initial reports, additional studies [24–26] have further demonstrated success; however the total number of patients analyzed with a balanced reciprocal translocation by PGD and PGS has been limited to 66 cases by aCGH and 130 by SNP microarray analysis. To our knowledge, there are no reports demonstrating successful clinical outcomes supporting the use of two independent microarray technologies.

Here we demonstrate that two different microarray platforms have equivalent ploidy and clinical pregnancy rates and either can be used for PGD due to parental carriers of reciprocal translocations. We report the ongoing clinical pregnancy rates (beyond the first trimester) from 63 couples undergoing 75 IVF cycles on 543 embryos using either a dense SNP or aCGH microarray PGD platform and simultaneous PGS for all 23-pairs of chromosomes due to parental carriers of reciprocal translocations. The results of this study will also provide important insight into the reasons behind the historically low clinical pregnancy rates (only approximating 40 %) when FISH was employed to identify genetic imbalances due to reciprocal translocation carriers [18]. This study shows that diagnosing genomically balanced embryos by 23 chromosome pair SNP or aCGH microarray PGD/PGS is a promising strategy to maximize the pregnancy potential of patients with known parental reciprocal chromosomal translocations.

Materials and methods

Patient population and IVF

We report PGD/PGS data from 543 embryos obtained from 63 couples undergoing 75 IVF cycles in which one partner had a balanced reciprocal translocation (See Table 1 for a complete list of the parental translocations). These patients were treated at 16 different IVF clinical centers. This genetics laboratory functions as a genetics referral center for PGD/PGS testing.

The maternal age ranged from 26 to 42 years (mean 33.5 +/− 4.0) and the paternal age ranged from 26 to 45 years (mean 34.3 +/− 4.2). Of the 63 couples, balanced reciprocal translocations were represented in 30 women and 33 men. In 60 % (38/63) of couples the maternal age was less than 35, 30 % (19/63) were women ages 35 to 39 and 10 % (6/63) were women ≥ 40. Eighty-seven percent (472/543) of biopsies were performed using a laser, 12 % (65/543) were performed using acid tyrodes and 1 % (6/543) by mechanical biopsy. Of the 75 cycles reviewed, 57 % (43/75) of the biopsies were done at the cleavage

Table 1 A listing of the 63 patients' balanced reciprocal translocations evaluated by either SNP or aCGH microarrays

SNP			aCGH
46,XX,t (8;12) (q13;q12)	46,XX,t (10;12) (q24;p13)	46,XY,t (1;7) (q41;p15.2)	46,XY,t (10;13) (p11.1;p13)
46,X,t (X;2) (p11.2;q23)	46,XX,t (9;16) (q21;q13)	46,XX,t (4;19) (p19;p13.2)	46,XY,t (1;5) (q21;q31.1)
46,XX,t (4;10) (p15.1;q24.1)	46,XY,t (7;18) (q21;q21.3)	46,XX,t (1;5) (q44;p13)	46,XX,t (4;8) (p16.1;p23)
46,XY,t (4;16) (q21;p13.2)	46,XX,t (8;12) (q13;q12)	46,XX,t (7;8) (q21.2;q21.2)	46,XY,t (3;5) (q23;q13.3)
46,XX,t (10;11) (p12.2;q13.2)	46,XX,t (8;11) (q13.3;p13)	46,XX,t (5;10) (p12;p11.2)	46,XY,t (10;15) (q26.1;q26.1)
46,XY,t (2;5) (p11.2;q35.3)	46,XX,t (4;8) (p16.1;p23)	46,XX,t (9;16) (q21;q13)	46,XY,t (3;4) (p22;p14)
46,XX,t (9;16) (q21;q13)	46,X,t (Y;18) (q11.22;q22.1)	46,XX,t (1;6) (q22;q25)	46,XY,t (1;13) (q31;q22)
46,XY,t (5;11) (q23.2;q24.2)	46,XX,t (5;10) (p15.3;q25.2)	46,XX,t (10;13) (p13;q31)	46,XY,t (4;9) (q21;p13)
46,XX,t (5;8) (q13;q22)	46,XY,t (1;3) (p34;q21)	46,XY,t (6;20) (p21.3q13.1)	46,XX,t (1;18) (p36.1;p11.2)
46,XX,t (10;12) (q24;p13)	46,XX,t (4;10) (p15.1;q24.1)	46,XY,t (1;11) (q21;q13)	46,XY,t (9;19) (p21;p13.2)
46,XX,t (8;11) (q13.3;p13)	46,XY,t (4;20) (q35;p11.23)	46,XY,t (X;1) (p10;p10)	46,XX,t (15;18) (q13;q11.2)
46,XX,t (19;20) (p10;p10)	46,XX,t (9;19) (q12;p13.3)	46,XY,t (2;4) (p13;q33)	46,XY,t (6;13) (p12.3;q31)
46,XX,t (2,6) (q13;p21.3)	46,XY,t (8;13) (q11.22;q21.2)	46,XY,t (2,18) (q14.2;q22)	46,XX,t (2;5) (p11.2;q35.3)
46,XY,t (8;12) (q11.23;q22)	46,XX,t (19;20) (p10;p10)	46, XY, t (2;5) (q21;q31)	46,XX,t (5,9) (q23.3;q21.2)
46,XY,t (2;5) (p11.2;q35.3)	46,XY,t (5;15) (q35.3;q24)	46, XY, t (Y;18) (q11.2;q11.2)	46,XX,t (5;19) (p12;p12)
46,XY,t (9;16) (q12;q11.2)	46, XX, t (5;19) (p12;p12)		46,XX,t (10;22) (p12.2;q12.2)

stage and 43 % (32/75) of cycles were biopsied at the blastocyst stage. Of 543 biopsied embryos, 66 % (356/543) were biopsied on day three of embryo development and 34 % (187/543) were trophectoderm (TE) biopsies from differentiated blastocysts. The mean maternal age separated by microarray platform and timing of embryo biopsy included the following: aCGH at the cleavage stage was 31.6 +/− 1.7, aCGH of the TE was 32.3 +/− 4.5, SNP at the cleavage stage was 34 +/− 5 and SNP of the TE was 34 +/− 3.1. The average number of molecular karyotypes obtained per IVF cycle was 5.9 and the mean number of genetically normal embryos per IVF cycle was 2.6. Of couples undergoing a transfer, the mean number of transferred embryos per cycle was 2. The maternal age ranged from 26 to 42 years (mean 33.5 +/− 4.0) and the paternal age ranged from 26 to 45 years (mean 34.3 +/− 4.2) (See Table 2 for demographic information).

Embryo biopsy, cell culture and microarray analysis

Each individual IVF clinic determined whether the embryo would undergo biopsy at the cleavage stage or the blastocyst stage of development. All embryos were biopsied using either of three embryo biopsy techniques which included ZILOS-tk™ laser (Hamilton Thorne Biosciences Inc., Beverly, MA), acid tyrodes or a mechanical technique. For cleavage stage PGS analysis, one cell was removed from each embryo and for embryos at the blastocyst stage between three to ten cells were removed from the TE on day five of embryonic development. During embryo growth and development between the cleavage stage and day-5 or the blastocyst stage all embryos remained in a standard commercially available media.

All biopsied cells were either placed in 5 μL of DNA stabilizing buffer (0.2 M KOH) for SNP microarrays or 2.5 μL of 1× phosphate buffered saline for aCGH

Table 2 Demographic data from patients and embryos evaluated by SNP and aCGH microarrays due to a balanced parental reciprocal translocation chromosome

	SNP	aCGH	Combined Total
Number of cycles	58	17	75
Number of patients	47	16	63
Mean maternal age (STD)	34.3 (4.2)	32 (3.3)	33.5 (4.0)
Mean paternal age (STD)	34.4 (4.8)	34 (2.2)	34.3 (4.2)
Maternal translocation carriers	23	7	30
Paternal translocation carriers	24	9	33
Embryos with molecular karyotype	396	102	498
Cleavage stage biopsy	(68 %) 268/396	(57 %) 58/102	(65 %) 326
Trophectoderm biopsy	(32 %) 128/396	(43 %) 44/102	(35 %) 172

microarrays, frozen for transport and sent to the Center for Preimplantation Genetics for microarray PGS testing.

This retrospective review includes two microarray platforms; SNP and aCGH. Only one of the two microarray platforms were used to evaluate the embryonic cells. The platform used for the PGD/PGS analysis was based on the historical availability of the microarray platform within our laboratory. From 2007 to 2011, we employed SNP microarrays and from 2011 to the present aCGH was available. No embryonic cells in this study were evaluated by both technologies simultaneously.

For all microarray analysis, the cell samples from each embryo first underwent cell lysis, DNA extraction and a whole genome amplification (WGA) protocol. For SNP arrays, the cells were lysed using an alkaline denaturation buffer (0.2 M NaOH) followed by a 4 h modified multiple displacement amplification (MDA) protocol using *phi* 29 polymerase to generate template DNA. 4 uL (200 ng) of DNA product then underwent a 13 h WGA amplification protocol again using *phi* 29 polymerase. Each DNA product then underwent enzymatic end-point fragmentation and the resuspended DNA samples were then dispensed onto Human CytoSNP-12 DNA analysis bead chips (Illumnia, San Diego, CA) and allowed to hybridize for 12 h. Each CytoSNP-12 bead chip contained approximately 301,000 SNPs and other genetic markers. Stringency washes were performed to remove un-hybridized and non-specifically bound DNA. The bead chips were dried in a desiccator and scanned using an Illumina iScan Bead Array Reader. Raw data analysis was accomplished using Illumina Genome Studio software. Clinical data was compared to our established embryonic cell normalized data set to remove SNPs with poor or incomplete genotype information.

For aCGH, the cells were lysed and WGA was accomplished using a Klenow fragment and a modified random priming protocol (Blue Gnome, Cambridge, UK). The amplified DNA was then labeled using Cy3 and Cy5 fluorophores, condensed, then hybridized to 24 Sure + array chips at 47 ° C for at least 3 h. Each 24 Sure + array contained approximately 5000 bacterial artificial chromosome (BAC) clones run in duplicate. We then performed stringency washes that removed un-hybridized and non-specifically bound DNA fragments. The microarray chips were then scanned using a TECAN Array Reader (Tecan US, Inc., Morrisville, NC) and raw data analysis was accomplished using Blue Gnome Blue Fuse software. Clinical data was compared to male reference DNA.

Timing of embryo transfer

The timing of biopsy and the transfer of the embryos included in this study were entirely managed by the patients' clinical IVF center. If the embryo underwent a cleavage stage biopsy, it was continued in culture to day five or the blastocyst stage, followed by a fresh single or double embryo transfer. If the biopsy occurred at the blastocyst stage, the embryos were cryopreserved and transferred with a future frozen–thaw cycle.

Statistical analysis

Differences in binomial variables were calculated using the Chi-square or Fisher's Exact tests where appropriate and a p-value of less than 0.05 was considered statistically significant.

Ethical approval

Institutional review board approval was obtained.

Results

In this study we obtained molecular karyotypes from 92 % (498/543) of biopsied embryos. Of the 45 samples with failed DNA amplification, 32 were processed using a SNP DNA stabilizing buffer and 13 samples were placed in molecular grade PBS for aCGH. In embryos with failed DNA amplification, this strongly correlated with poor embryo quality and was likely due to DNA fragmentation. There was no statistical difference between failed DNA amplification from cleavage stage or blastocysts.

Of the 396 embryos diagnosed by SNP microarrays, 47 % (186/396) were euploid without an inherited unbalanced translocation chromosome, 21 % (84/396) were euploid but had a parentally derived unbalanced segregant, 25 % (98/396) were aneuploid alone and 7 % (28/396) contained a parentally derived translocation imbalance and aneuploidy. In comparison, of the 102 embryos diagnosed by aCGH, 39 % (40/102) were euploid without an inherited unbalanced translocation chromosome, 27 % (28/102) were euploid but had a parentally derived unbalanced segregant, 20 % (20/102) were aneuploid alone and 14 % (14/102) contained a parentally derived translocation imbalance and aneuploidy (Table 3).

We next compared the molecular karyotype results depending upon whether the biopsy occurred at the cleavage stage or the blastocyst stage of development (Table 4). Of the 326 molecular karyotypes obtained from cleavage stage biopsies, 45 % (147/326) were euploid without translocation errors, 17 % (56/326) were euploid with a parentally derived translocation imbalance and 38 % (123/326) of embryos were aneuploid with or without a parental translocation imbalance. In contrast to cleavage stage, embryos biopsied at the blastocyst stage showed that 46 % (79/172) were euploid without parental translocation errors, 33 % (56/172) were euploid with a parentally derived translocation chromosomal error and 22 % (37/172) of the embryos were aneuploid. Of these 37, 14 were aneuploid and also included a parentally derived segregant.

Table 3 Molecular karyotypes of embryos evaluated by SNP or aCGH microarrays categorized by the presence or absence of aneuploidy and/or the parental unbalanced translocation chromosome. Statistical comparisons were performed using chi-square analysis and a *p*-value of greater than 0.05 was considered not significant (NS)

	SNP	aCGH	*p*-value	Total
Molecular karyotypes	396	102		498
Euploid and no translocation imbalance	(47 %) 186	(39 %) 40	NS	(45 %) 226
Aneuploid and no translocation imbalance	(25 %) 98	(20 %) 20	NS	(24 %) 118
Euploid with translocation imbalance	(21 %) 84	(27 %) 28	NS	(23 %) 112
Aneuploid with translocation imbalance	(7 %) 28	(14 %) 14	NS	(8 %) 42

Combining both microarray technologies, there was a significant ($p<0.05$) increase in the prevalence of aneuploid embryos, with or without the translocation imbalance, when biopsied at the cleavage stage (38 %) versus the blastocyst stage (22 %).

Our overall results for SNP and aCGH arrays showed aneuploidy alone without the inheritance of a parental translocation chromosome imbalance was observed in 24 % (119/498) of embryos analyzed. Aneuploidy combined with a parental translocation imbalance was seen in 8 % (41/498) of embryos analyzed. Overall, aneuploidy was seen in 32 % (160/498) of embryos tested.

We next compared the prevalence of aneuploidy according to maternal age and identified a significant increase ($p<0.05$) in embryo aneuploidy (aneuploidy with or without an unbalanced parental translocation chromosome) from women ≥ 40 as compared to women <35; 50 % (20/40) versus 31 % (121/392) respectively.

In comparing the embryos resulting from either the maternal or paternal carrier of the translocation, 216 embryos resulted from couples with a balanced maternal translocation and 282 resulted from couples with a paternal translocation carrier. There was no difference in the prevalence of euploid embryos with a translocation imbalance whether the translocation imbalance was maternally derived (24 %) or paternally derived (21 %). Both 2:2 (adjacent-1 or adjacent-2) segregation and 3:1 segregation pattern translocation error imbalances were observed in embryonic cells.

The average size of the observed chromosomal imbalance in embryos due to the transmission of unbalanced parental translocations was 42.08 megabases (Mb) (range of 9.29-141.17 Mb) for duplication errors and 35.07 Mb (range of 6.76-77.26 Mb) for deletion errors. The sizes of these chromosome imbalances due to parental reciprocal translocations were greater than 6.75 Mb for both microarray platforms. Within our laboratory, the internally conducted validation experiments demonstrated that both platforms were capable of identifying unbalanced translocation segments of 1 Mb or greater.

Of the 75 IVF cycles in this study, 85 % (64/75) of couples had at least one normal embryo that was both euploid and genetically balanced for the parentally inherited translocation chromosome and available for uterine transfer. The overall clinical pregnancy rates following SNP or aCGH analysis were comparable at 60 % (35/58) versus 65 % (11/17) respectively (Table 5). The clinical pregnancy rate per IVF cycle was 65 % (20/31) when the biopsy was done on blastocysts versus 59 % (26/44) when done at the cleavage stage, however the difference was not statistically significant.

Discussion

This study provides data supporting the use of two different microarray technologies to perform PGD/PGS for reciprocal

Table 4 Comparing the timing of the embryo biopsy at the cleavage stage to the blastocyst stage with the presence or absence of aneuploidy and/or the parental unbalanced translocation chromosome. Statistical comparisons were performed using chi-square analysis and a *p*-value of greater than 0.05 was considered not significant (NS)

	Cleavage stage	Blastocyst stage	*p*-value	Total
Molecular karyotypes	326	172		498
Euploid and no translocation imbalance	(45 %) 147	(46 %) 79	NS	(45 %) 226
Aneuploid and no translocation imbalance	(29 %) 96	(13 %) 23	$P<0.001$	(24 %) 119
Euploid with translocation imbalance	(17 %) 56	(33 %) 56	$P<0.001$	(23 %) 112
Aneuploid with translocation imbalance	(8 %) 27	(8 %) 14	NS	(8 %) 41

Table 5 Clinical pregnancy rates per IVF cycle with PGD/PGS by SNP or aCGH microarrays categorized by timing of the embryo biopsy at either cleavage stage or blastocyst stage of development. Statistical comparisons were performed using Fisher's exact analysis and a *p*-value of greater than 0.05 was considered not significant (NS)

Clinical pregnancy rates	SNP (total cycles: 58)	aCGH (total cycles: 17)	*p*-value	Total
Cleavage stage biopsy	21/36 (58 %)	5/8 (63 %)	NS	26/44 (59 %)
Trophectoderm biopsy	14/22 (64 %)	6/9 (67 %)	NS	20/31 (65 %)

translocation carriers. We show that PGD/PGS testing for parental reciprocal translocation carriers using either a SNP or aCGH microarray to simultaneously test for translocation imbalances and aneuploidy for all 23-pairs of chromosomes demonstrate comparable ploidy and clinical pregnancy rates.

We identified aneuploid chromosomes not associated with the parental reciprocal translocation imbalance in 24 % (119/498) of embryos tested. If any of these genetically balanced, but aneuploid embryos were tested by FISH and transferred, the resultant outcome would have been failure to achieve a pregnancy, a miscarriage or the birth of an aneuploid baby. We feel that our clinical pregnancy results, demonstrating an approximate 22 % increase as compared to historical FISH testing, are primarily due to the failure of FISH to identify aneuploidy for chromosomes not associated with the parental translocation. Furthermore, the use of either microarray technology demonstrates higher on-going clinical pregnancy rates versus historical reciprocal translocation FISH data [18] by approximately 22 %; 62 % vs. 40 %.

Our results demonstrate that of 498 embryos diagnosed, 31 % (153/498) were genetically unbalanced for the parental translocation chromosomes. In general, when a couple presents for possible PGD due to a parental reciprocal translocation carrier, the approximate risk of transmission of a translocation chromosome imbalance into gametes and eventual embryos is 50 %. This number varies greatly due to the chromosomes involved in the reciprocal translocation, the location of the breakpoints and the size of the trisomic and/or monosomic genomic segments in the resultant unbalanced chromosomes. In our study, the average size of the paternal or maternal chromosomal imbalance due to the transmission of unbalanced segregant was relatively large and could account for a lower than expected percentage of translocation imbalances seen in the embryos.

In addition, there was a significant increase in aneuploidy in women ≥ 40 versus women <35 years of age. In the general population, aneuploidy is well documented to increase with advancing maternal age. This finding aligns itself with the well-established trend that increased maternal age is associated with increased aneuploidy [27].

Our study demonstrated that overall 32 % of embryos were aneuploid. There was significantly more aneuploidy diagnosed at the cleavage stage (38 %) as compared to the blastocyst stage (22 %). This finding is not unexpected as aneuploid embryos are more likely to experience arrested development, preventing this population of embryos from reaching the blastocyst stage [28,29]. Comparing this study's results with the overall reported prevalence of aneuploidy in patients undergoing PGS for clinical indications other than balanced reciprocal translocations ranges between approximately 38–82 % from cleavage stage to 29–69 % from blastocysts [19, 30–36]. Two additional studies that determined ploidy and chromosome imbalances in embryos from couples with balanced reciprocal translocations observed highly varying prevalence's of aneuploidy. Colls et al. evaluated 280 embryos from 30 couples (56 embryos evaluated at the blastocyst stage and 224 at the cleavage stage) and observed 57.6 % to be aneuploid with or without the unbalanced parental translocation [24]. Dramatically contrasting Colls et al., Tan et al. evaluated 499 embryos from 117 couples (all biopsied at the blastocyst stage) and observed an aneuploidy prevalence of 16 % [25]. However, the aneuploidy prevalence from Colls et al. drops to 26.7 % when the results include only TE cells. Additionally, the maternal ages of the two cohorts (TE samples only) from Colls et al. and Tan et al. were very similar at 33.5 and 31 years respectively. From the above referenced studies, it appears that the prevalence of aneuploidy in embryos from carriers of reciprocal translocations may differ than the most commonly reported rates from patients undergoing PGS due to RPL or other clinical indications. Further studies are required to clarify this question.

An important finding that this research contributes is that aCGH and SNP microarrays for the purposes of identifying parental translocation imbalances and aneuploidy are equivalent. Both the SNP microarray and the aCGH chip can identify reciprocal translocation imbalances either by overall density of the SNP chip or sub-telomeric density of aCGH arrays. Furthermore, both arrays determine all 23-chromosome pair aneuploidy equally. However, both platforms have potential strengths and weaknesses. Because SNPs are a genotyping array, they can detect uniparental disomy (UPD) and identify the parental origin of chromosome abnormalities. However, SNP arrays take approximately 30–40 h to complete the analysis and if a TE biopsy is performed, transfer on the following day is impossible. In contrast, aCGH can be completed in approximately 12 h and offers the potential benefit of performing the analysis on TE cells and transferring the

embryo on day 6; all part of a fresh IVF cycle. One limitation of an aCGH array, is the inability to identify 69, XXX because aCGH is a ratio array and 69, XXX looks exactly the same as 46, XX.

One limitation of PGD IVF using SNP or aCGH microarrays is that embryos with balanced translocations cannot be identified. Only genetic imbalances are detected. Therefore, SNP or aCGH microarrays will not be able to provide assurance that the transferred embryo does not harbor a balanced translocation as present in one of the parents. Babies born with a balanced reciprocal translocation, while nearly all are phenotypically normal, also may harbor a future risk of RPL due to translocation imbalances. However, in our experience, most couples choose to accept this diagnostic limitation. Additional limitations of this study include the retrospective design which compares the two modalities and the demographic differences between the patients included. Within our laboratory, SNP microarray was the first platform applied in clinical practice followed by aCGH. As the embryos analyzed were not randomized to one platform or the other, the chronology for setting up the microarray platforms as well as the patient demographics which include: varying parental translocation imbalances, age, timing of the embryo biopsy and other the specific clinical IVF practices at specific infertility center all contribute to the clinical outcomes. Thus this study's findings cannot be considered as definitive evidence for the clinical observations and should be considered limited, although nonetheless important observations.

Cellular mosaicism is well documented to exist in the developing human embryo. An inherent limitation of all studies evaluating the genetic composition of an embryo via biopsy of a single cell or cells is the inability to conclusively identify the chromosomal status of the remaining (non-biopsied) embryonic cells. However, preimplantation genetic testing is the only modality that permits genetic testing while maintaining embryo viability.

As our study included embryos sent from 16 clinical centers, we were unable to complete a reanalysis of cytogenetically abnormal embryos to calculate an error or misdiagnosis rate for this cohort of patients. However, prior to, and continuing monthly, our laboratory completes a rigorous validation and blinded proficiency testing [32,37] with continued quality assurance measures.

Diagnosing viable embryos through whole genome microarray PGD/PGS is a promising strategy to maximize the pregnancy potential of patients with known reciprocal translocations. This molecular genetic assay improves the potential for patients with a balanced reciprocal translocation to ultimately have a healthy child.

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Conflict of interest None of the listed authors have a financial, commercial or corporate conflict of interest.

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