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Genome Sequencing Explores Complexity of Chromosomal Abnormalities in Recurrent Miscarriage

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Recurrent miscarriage (RM) affects millions of couples globally, and half of them have no demonstrated etiology. Genome sequencing (GS) is an enhanced and novel cytogenetic tool to define the contribution of chromosomal abnormalities in human diseases. In this study we evaluated its utility in RM-affected couples. We performed low-pass GS retrospectively for 1,090 RM-affected couples, all of whom had routine chromosome analysis. A customized sequencing and interpretation pipeline was developed to identify chromosomal rearrangements and deletions/duplications with confirmation by fluorescence *in situ* hybridization, chromosomal microarray analysis, and PCR studies. Low-pass GS yielded results in 1,077 of 1,090 couples (98.8%) and detected 127 chromosomal abnormalities in 11.7% (126/1,077) of couples; both members of one couple were identified with inversions. Of the 126 couples, 39.7% (50/126) had received former diagnostic results by karyotyping characteristic of normal human male or female karyotypes. Low-pass GS revealed additional chromosomal abnormalities in 50 (4.0%) couples, including eight with balanced translocations and 42 inversions. Follow-up studies of these couples showed a higher miscarriage/fetal-anomaly rate of 5/10 (50%) compared to 21/93 (22.6%) in couples with normal GS, resulting in a relative risk of 2.2 (95% confidence interval, 1.1 to 4.6). In these couples, this protocol significantly increased the diagnostic yield of chromosomal abnormalities per couple (11.7%) in comparison to chromosome analysis (8.0%, chi-square test $p = 0.000751$). In summary, low-pass GS identified underlying chromosomal aberrations in 1 in 9 RM-affected couples, enabling identification of a subgroup of couples with increased risk of subsequent miscarriage who would benefit from a personalized intervention.

Introduction

Recurrent miscarriage (RM) is defined by loss of two or more clinical pregnancies and affects 1%–2% of couples.[1] RM is an important global health issue and carries an underappreciated psychological and financial burden for affected couples. Anatomic factors, antiphospholipid syndrome,[1,2] and endocrinological and chromosomal abnormalities[2] are the most commonly recognized etiologies for RM. However, the etiology of RM in 40%–60% of couples remains idiopathic,[1] resulting in costly testing and remaining a challenge to both counseling and treatment.

Chromosomal abnormalities are the major recognized genetic causes for any miscarriage, accounting for up to 60% of cases;[3] a chromosome abnormality can be found in lymphocyte metaphases in approximately 2%–4% (1 in 50) of couples with RM by routine chromosome analysis,[4,5] which is significantly higher than that reported in the general population (~0.3%).[6] Couples in whom one partner has a balanced translocation or inversion may have an overall miscarriage rate as high as 49%[7] resulting from unbalanced gametes. This depends on the specific chromosomes involved, the type and size of the rearrangements, and sex of the carrier.[8] However, current

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recommendations for management from various professional societies differ largely (Table 1). Emerging studies have demonstrated that genome sequencing (GS)[6,9,10] can be used to delineate breakpoints of balanced translocations/inversions and detect additional CNVs compared to chromosomal microarray analysis (CMA).[6,11,12] We undertook investigation of the role of low-pass GS in deciphering the contribution of chromosome abnormalities in RM in a large cohort of 1,090 RM-affected couples.

Subjects and Methods

Subject Recruitment and Demographics

This study was approved by the Institutional Review Boards of Shandong University and The Chinese University of Hong Kong. RM-affected couples with two or more consecutive clinical pregnancy losses[2,13] were enrolled from RM clinics at these two universities. Couples diagnosed with an established cause (see Supplemental Subjects and Methods)[4] other than chromosomal abnormalities[2] were excluded. During the period from 2004 to 2015, a total of 1,090 consecutive idiopathic RM-affected couples were recruited with informed consent; demographic data are provided in Table S1.

Sample Collection and Preparation

At enrollment, a 5 mL peripheral blood sample was collected into a heparinized tube from both partners for diagnostic chromosome analysis. In addition, a 1 mL peripheral blood sample was collected into an EDTA tube for the collection of buffy coat, which was separated and stored at −80°C for future DNA extraction and genetic analysis. Beginning in 2013 when low-pass GS was developed,[14,15] genomic DNA extraction was performed with a DNeasy Blood & Tissue Kit 250 (cat No./ID:69506, QIAGEN) and quality assessed by Qubit dsDNA HS Assay Kit (Invitrogen, Life Technologies) and gel electrophoresis.

Chromosome Analysis

Chromosome analysis was performed at both institutions following standard methods for preparing G-banded metaphases from cultured peripheral blood-derived lymphocytes. Results were reported at a resolution of 400–550 bands (Supplemental Subjects and Methods).

Low-Pass Genome Sequencing

Low-pass GS was performed in batches of 96 samples and according to the workflow outlined in Figure 1 (Supplemental Subjects and Methods and Figures S1–S3). Analysis for each sample was performed without knowledge of any previous cytogenetic results.

Library Construction and Sequencing

For each sample, 1.5 μg genomic DNA was sheared with the HydroShear device (GeneMachines, Digilab) into fragments ranging from 3 to 8 kb.[14] A modified mate-pair library construction approach was applied with ~140 million paired-end read-pairs (~26-bp) generated from a DNA nanoarray-sequencing platform[16] (Complete Genomics; Supplemental Subjects and Methods and Figure S2).

Data Analysis, Interpretation, and Validation

Paired-end reads were aligned to the NCBI human reference genome (hg19).[17] For each sample passing data QC (genome-wide standard deviation of copy-ratios < 0.15, Figure S4),[15] detection of chromosomal rearrangement and CNVs was performed in parallel[14,15] (Supplemental Subjects and Methods and Figure S5) and reported integrally. Chromosomal translocations and inversions (100-kb cutoff[14]) were then validated with polymerase chain reaction (PCR) and Sanger sequencing (Figures 2 and S6).[9,14] Fluorescence *in situ* hybridization (FISH) was performed with resubmitted fresh samples. Structural variants were compared to the Database of Genomic Variants, and genes (RefSeq) disrupted at rearrangement breakpoints were curated using Human Phenotype Ontology and Online Mendelian Inheritance in Man (OMIM).

Recombination rate based on deCODE, Genethon, or Marshfield maps (UCSC Genome Browsers), a dataset of the recombination rates for each chromosome with 1-Mb bin size, was used for inversion (>1-Mb) interpretation with cutoffs of recombination hotspot bin/region in males and females reported[18] (Supplemental Subjects and Methods).

Classification of CNVs followed guidelines from the American College of Medical Genetics and Genomics (ACMG, Supplemental Subjects and Methods)[12,15,19,20] and pathogenic, likely pathogenic CNVs, or variants of uncertain significance (VUS) were validated (Figure S7) using a customized 8x60K Fetal DNA Chip according to the manufacturers' protocols with analysis using CytoGenomics (Agilent Technologies).[15]

Results

We recruited 1,090 couples with RM, representing a total number of 2,180 subjects. GS yielded results in all but 13 samples (2,167/2,180 subjects, 99.4%). The 13 failed samples (representing 13 couples) were collected between 2004 and 2006, and the quality of their DNA was insufficient for data analysis due to degradation presumed from long-term storage (Figure S4). All of these 13 couples had normal karyotyping results. Among the remaining 1,077 couples (98.8%), 127 balanced structural chromosomal abnormalities were detected in 126 couples (11.7%), including 78 subjects with balanced translocations and 48 with inversions (Table 2 and Figure 1). All these 127 events (Tables 3 and S2–S5) were selected for validation by PCR and Sanger sequencing (Figure S6), and all results were confirmed. In addition, FISH conducted in three case subjects with resubmitted peripheral bloods and all were confirmed (Figures 2C, 2G, and S8C). The rate of 11.7% by GS represents a 16.7-fold increase compared to the rate defined by GS in participants in the 1000 Genomes Project (0.7%, ~1 in 146),[21] who were not known to have chromosomal rearrangements and presumed to represent a normal control population.

Balanced Translocations

Among 78 subjects (78 couples; 7.2%) with balanced translocations identified by GS, 70 rearrangements were also identified by the original karyotyping, while eight were detected by GS alone (Tables 2 and 3). However, GS failed to

Table 1. Genetic Study Recommendations in Different Societies and Communities for Recurrent Miscarriage

Professional Societies	Parental Karyotyping	Study on Products of Conception (POCs)
The American Society of Reproductive Medicine (ASRM)/the American College of Obstetricians & Gynecologists (ACOG)	recommended	not recommended
The European Society of Human Reproduction (ESHRE)	not routinely recommended	not routinely recommended
The National Institute for Health and Care Excellence (NICE)/the Royal College of Obstetricians & Gynaecologists (RCOG)	recommended only when testing of POC reports an unbalanced structural chromosomal abnormality	only recommended in the third and subsequent consecutive miscarriages

detect ten translocations (0.9%) including seven Robertsonian translocations and three translocations with one breakpoint located in 22q11.2 (Table S2).

Among the eight translocations additionally identified by GS, two translocations (Figures 2A–2C and S8) involved a reciprocal exchange between segments below the typical level of resolution limits of karyotyping (e.g., the translocated segments in female subject SD-RSA_11189 were only 2.4 Mb and 3.1 Mb in size; Figure 2A). Contributing to the absence of detection of the remaining six case subjects by the original chromosome analysis was the similar size and banding pattern of the reciprocally exchanged segments, such as in SD-RSA_11359 (Figure S9D).

Although 70 balanced translocations were reported by previous cytogenetic analysis, low-pass GS showed its increased precision in revision of the breakpoints by at least one sub-band in 42 case subjects (60.0%) (Tables S2 and S3) and in the discovery of additional findings of complex chromosomal rearrangements in 11 case subjects (15.7%, Table S4). For example, in subject SD-RSA_20749 (Figures 2D–2G), chromosome analysis indicated an apparently balanced three-way translocation involving chromosomes 1, 2, and 13; low-pass GS revealed a complex rearrangement involving four chromosomes, in which the derivative chromosome 2 harbored an inversion at the breakpoint of the t(2;13) and a translocated segment containing material from both chromosomes 13 and 18. Further, GS identified chromothripsis[22] or chromoplexy-like events[23] in four subjects (Table S4 and Figure S10).

Inversions

GS also elucidated 49 inversions of size ranging from 114.7 kb to 94.5 Mb (Tables 2 and S5 and Figure S6), and in one couple, both partners had the same inv(21) (Supplemental Subjects and Methods and Figures S11 and S12). Overall, GS provided an incidence of inversions of 4.5% per couple (48/1,077) or 2.3% per individual (49/2,154), which is significantly higher than that reported in the 1000 Genomes Project[21] (4/1,166 or 0.3%; chi-square testing $p < 0.0001$).

Among the 49 inversions (in 48 couples), 15 were found to have at least one breakpoint in a recombination hotspot bin/region (Supplemental Subjects and Methods and Table S5),[18] supporting an increased risk for producing duplication-deletion offspring if a cross-over were to occur in the inversion loop during gametogenesis. In addition to these 15 inversions, 4 inversions were found to disrupt an OMIM disease-causing gene (Table S5). Of note, only six inversions were detected by metaphase chromosome analysis with a size ranging from 14.3 to 94.6 Mb (Figures S13A and S13B).

Copy Number Variants

In total, 2,124 copy-number losses and 4,623 gains were reported by low-pass GS, indicative of 2.0 deletions and 4.2 duplications per couple (Figure S14). Based on ACMG guidelines, we further defined 6 pathogenic or likely pathogenic CNVs and 12 VUS (Tables 2 and S6) ranging from 85.9 kb to 8.1 Mb. Two were located in chromosome X and 16 were in autosomes. All these 18 CNVs, classified as pathogenic, likely pathogenic, or VUS (Tables S6), were selected for validation with CMA, and all were confirmed (Figure S7). A recombination event of the aberrant chromosome segregated to the embryo/fetus would be the mechanism underlining pathogenicity. A female carrier (SD-RSA_10358) was identified with an 8.1-Mb deletion in Xq22.3q23 associated with X-linked Alport syndrome (MIM: 301050), resulting in increased morbidity and mortality in a male fetus. Of note, none was detectable by the original cytogenetic analysis. The extent of the contribution of the detected CNVs to RM remains to be established but our data provide further evidence of the increased detection and precision of GS. In summary, low-pass GS reported a total number of 145 genomic variants, including 78 translocations, 49 inversions (Tables 2 and S2–S5), and 18 CNV classified as pathogenic, likely pathogenic, or VUS (Table S6) in this study, and all were validated.

Secondary Follow-up and Outcome

Fifty couples had additional diagnoses by low-pass GS but with normal karyotypes (8 couples with balanced translocations and 42 with inversions). Ten of these couples subsequently had a spontaneous conception, and five of these couples (50.0%) reported miscarriage or fetal structural anomalies (Tables 3 and S5). The frequency was even more remarkable when compared with 93 RM-affected couples who had both normal GS and karyotype in our study cohort with follow-up pregnancy; among them only 21 (22.6%) couples experienced the same poor

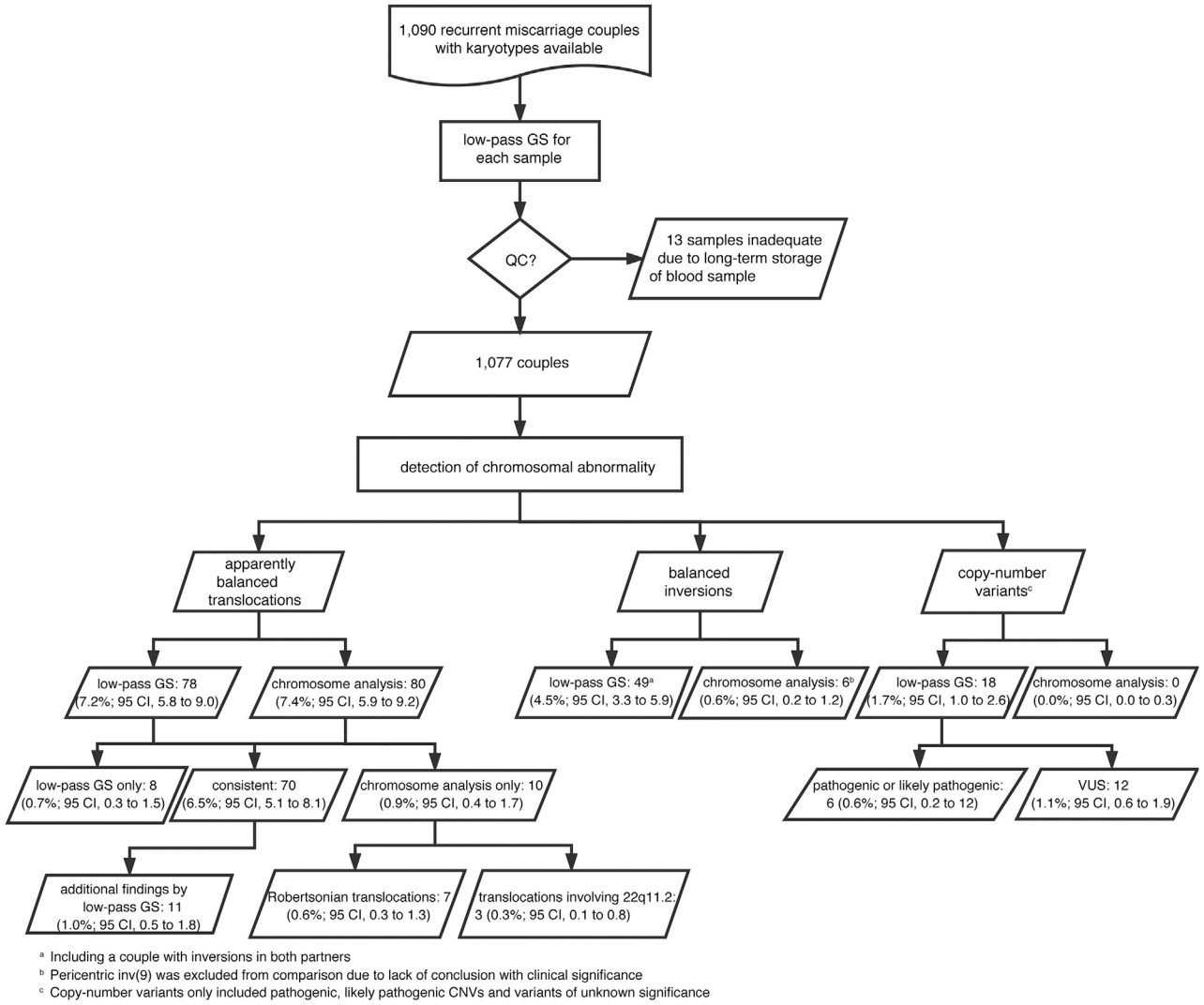

Figure 1. Flowchart of Low-Pass GS in Detection of Chromosomal Abnormalities in 1,090 Recurrent Miscarriage-Affected Couples
Detailed methods and results are described in the main text and the diagnostic rate of chromosomal abnormalities was calculated based on the number of couples.

outcomes, thus resulting in an odds ratio (OR) of 3.4 (95% confidence interval: 1.1 to 4.6).

Among the 126 couples with an abnormal chromosomal diagnosis by GS, 26 "carrier" couples (maternal age: 28.5 ± 4.4) sought *in vitro* fertilization (IVF) and preimplantation genetic testing (for aneuploidy [PGT-A] and structural rearrangement [PGT-SR]; Supplemental Subjects and Methods[24]) after reproductive counseling. In comparison, IVF and PGT-A were also pursued by 68 RM-affected couples with normal GS and karyotype results (68/975; maternal age: 32.1 ± 4.7). Livebirth and miscarriage rates of the first cycle pregnancy were calculated for the two groups. Among the carrier group, 19 clinical pregnancies (18/26, 69.2%) were achieved resulting in 1 miscarriage (1/18, 5.6%) and 18 livebirths (17/26, 65.4%, Table S7). In contrast, in the non-carrier group, 52 clinical pregnancies (52/68, 76.5%) were observed with an outcome of 17 miscarriages (17/52, 32.7%) and 35 livebirths (35/68, 51.5%). This could suggest that intervention by means of IVF and PGT in couples with a chromosomal diagnosis by GS can result in a significantly lower miscarriage rate (OR: 0.1, 95% CI, 0.0 to 0.9, absolute risk reduction 27.2%, Fisher exact test $p = 0.0283$) in their subsequent first IVF cycle.

Discussion

In this study, low-pass GS enables identification of RM-related chromosomal abnormalities at higher diagnostic yield and resolution independent of routine chromosome analysis. Low-pass GS had a diagnostic yield of 11.7% (126/1,077) in couples with RM. Previous large-scale studies on RM-affected couples found balanced chromosomal abnormalities (inversions and reciprocal and Robertsonian translocations) in 4.4% of 11,708 couples with at least two miscarriages[25] and balanced translocations in 3.8% of 10,216 couples.[26] In comparison to chromosome

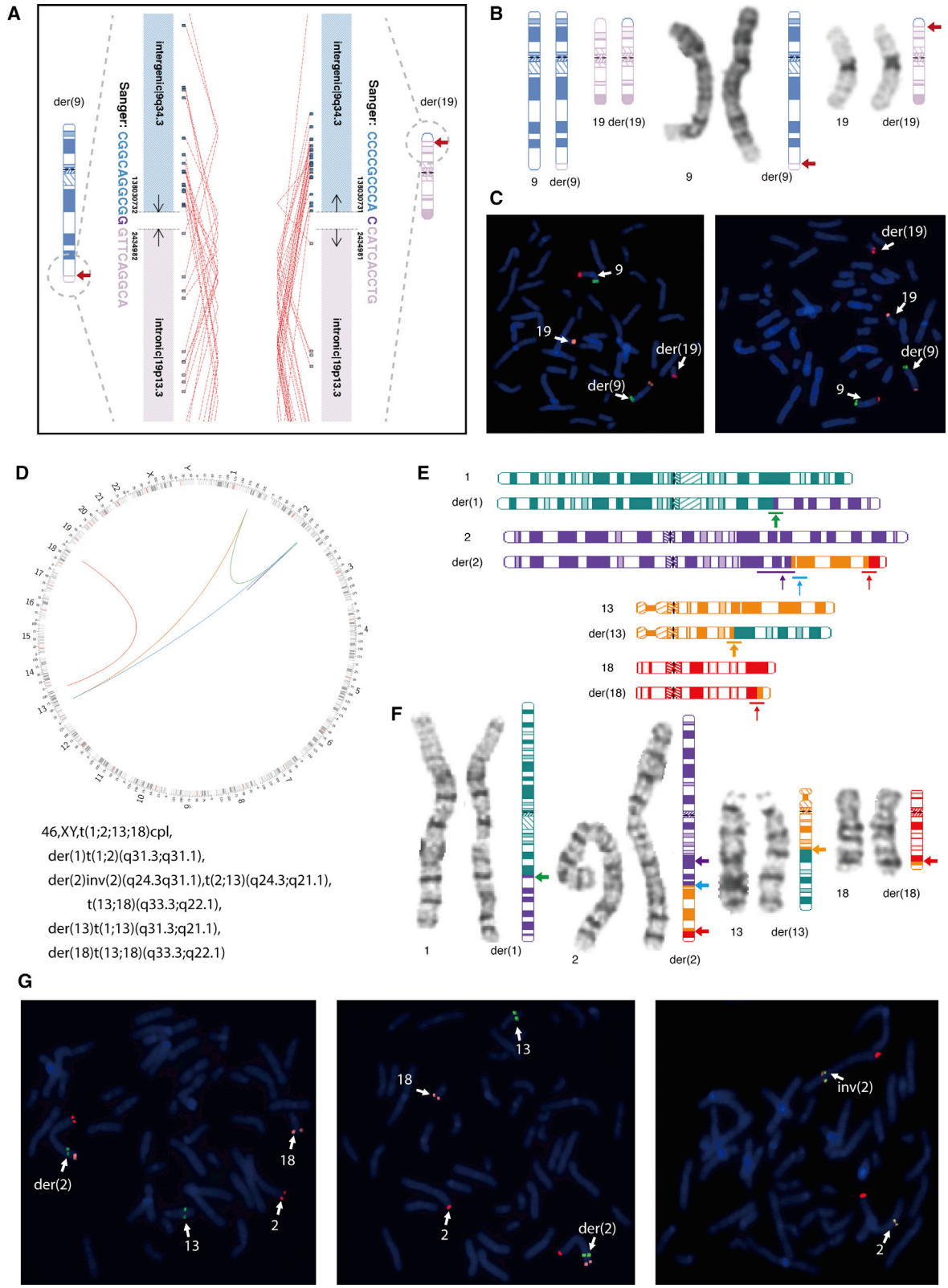

Figure 2. Cryptic Balanced Translocations Detected by Low-Pass Genome Sequencing (GS).
(A) Low-pass GS revealed a balanced translocation 46,XX,t(9;19)(q34.3;p13.3) in subject SD-RSA_11189. Genomic coordinates of breakpoints are indicated next to each derivative chromosome, while the genomic orientation of each chromosomal segment is shown with black arrows. Genic and intergenic regions (RefSeq) of the breakpoints are labeled in each derivative chromosome, and corresponding ideograms for the der(9) and der(19) are depicted. Sequence pairs were independently aligned to the human genome and each chimeric pair of reads is indicated with a red dotted line. Two independent sets of chimeric read pairs support the composition of the two

(legend continued on next page)

analysis, low-pass GS significantly increased the diagnostic yield of chromosomal abnormalities (chi-square test $p = 0.000751$) and established a diagnosis in an additional 50 (4.6%) couples with prior normal chromosome analyses.

Balanced translocations are the most common genetic abnormality identified among RM-affected couples (61.9%, 78/126), confirming previous findings by karyotype.[27,28] Among these translocations, affected chromosomes and the sizes of exchanged segments varied (Tables 3, S3, and S4). We were able to define and discover those cryptic chromosomal rearrangements by low-pass GS due to its agnostic advantage to size and banding pattern of chromosomal segments. In addition, among the 70 cases detected by both low-pass GS and karyotyping, revision of rearranged chromosome bands was made in 60.0% (42/70) of subjects by at least a sub-band with implications for interpretation of the underlying molecular mechanism of the RM. Most importantly, in 15.7% (11/70) of cases, additional findings identified by low-pass GS revealed more complex rearrangements than evident from chromosome analysis alone. Breakpoints and chromosomes involved are unique for each couple, thus eliminating empirical evidence of the segregation patterns in gametes or pregnancies,[8] compared to the known segregation patterns for the Robertsonian translocations involving acrocentric chromosomes (i.e., 13, 14, 15, 21, and 22).[29] Specifically, it has been observed that offspring of a parent with such a complex rearrangement may inherit either a subset or the full set of the chromothripsis-like events from the presumably healthy parent but acquired *de novo* rearrangements leading to copy-number changes resulting in miscarriages, or severe congenital disorders.[30] Therefore, refined information of the chromosomal abnormalities by GS provides carrier couples with individualized estimated miscarriage risk and actionable plan with respect to future pregnancies.[30]

Inversions were the second most common type of chromosomal abnormality (38.1%, 48/126). Only six of these inversion cases were reported by routine karyotyping (Figures 1 and S13) due to the limited resolution and similar banding patterns. Five consecutive miscarriages were reported in the non-consanguineous couple in which both partners carried the same 1.2-Mb inv(21)(q22.11q22.12) located within a recombination hotspot region,[18] supporting that the inv(21) could be associated with RM and worthy of further study.

Low-pass GS revealed a failure to detect 10 (0.9%) translocations with one breakpoint in an extensive length of repetitive sequence (Table S2), recognized for Robertsonian translocations[31] and the recurrent t(11;22)(q23;q11.2),[32] which has been known to be the limitation by current molecular technologies.[14] With our current protocol, low-pass GS may be viewed most appropriately as a complementary tool to conventional karyotyping. Employing both methods, the incidence of balanced translocations and inversions was further increased to 12.6% (136/1,077), revealing the underlying chromosomal aberrations in 1 in 8 couples with RM.

Modifications to the protocol were employed to increase the DNA fragment size to 40 kb, and the results show that further optimization of low-pass GS can improve detection of such translocations mediated by low-copy repeats (Figure S15). For the Robertsonian translocations, although the breakpoint junction regions have been mapped to p11.2 in the acrocentric chromosomes, there is still more than 100 kb of sequence located in the junction region without complete delineation due to the presence of repetitive elements or satellite DNAs.[33] With development of third-generation sequencing methods to provide access to longer DNA sequences (i.e., 100 kb), further modifications would be possible to optimize low-pass GS for their detection. Nonetheless, with the current protocol, an alternative approach is to count copy numbers of

derivative chromosomes. Sanger sequencing results are shown in the same color as the corresponding chromosome, while micro-homology is shown in dark purple in each derivative chromosome.
(B) Ideograms of the balanced translocation are shown on the left (note the banding and size similarities between normal and derivative chromosomes), while the G-banded chromosome is to the right with the corresponding ideogram of the derivative chromosomes for reference. Breakpoint regions are indicated with red arrows.
(C) FISH validation of the translocation is shown. Derivative chromosomes and normal chromosomes are labeled and indicated by arrows. BAC probes RP11-31F19 at 9p24.3 (green), RP11-100C15 at 9q34.3 (red), and RP11-75H6 at 19p13.3 (orange) were used.
(D) Low-pass GS revealed rearrangements involving four different chromosomes in sample SD-RSA_20749. Circos diagram shows complex rearrangements involving chromosomes 1, 2, 13, and 18. Next-generation cytogenetic nomenclature is shown below. Green line indicates t(1;2)(q31.3;q24.3) in der(1), while orange line shows t(1;13)(q31.3;q21.1) in der(13). In der(2), inv(2)(q24.3q31.1) is shown by a purple line, while t(2;13)(q24.3;q21.1) is indicated by a blue line. The red line denotes the balanced translocation t(13;18)(q33.3;q22.1) in both der(2) and der(18).
(E) Ideograms of the chromosomal rearrangements. For der(2), the inv(2)(q24.3q31.1) is shown by a purple arrow, and translocations between chromosomes 2 and 13 and between chromosomes 13 and 18 are shown with arrows in blue and red, respectively. The colored arrows correspond to the chromosome of origin in (D).
(F) High-resolution chromosome analysis shows a three-way chromosome translocation, t(1;2;13)(q31;q24;q21), but cannot readily identify the inv(2)(q24.3q31.1) and translocation between chromosomes 13 and 18. The corresponding ideograms of the exact composition of each derivative chromosome are shown with arrows indicating breakpoint regions. The color of the arrows represents the chromosome of origin shown in (D) and in (E).
(G) FISH confirms the additional rearrangements in der(2) identified by low-pass GS. In the left and middle images, BAC probes RP11-664N22 at 2p25.3 (red), RP11-43P9 at 13q22.2 (green), and RP11-7H17 at 18q23 (orange) were used to validate rearrangements of chromosomes 2, 13, and 18. In the right image, BAC probes RP11-664N22 at 2p25.3 (red), RP11-11N16 at 2q24.3 (orange), and RP11-608P21 at 2q31.1 (green) were used to validate the inv(2)(q24.3q31.1).

Table 2. Comparison of Detection Yields in 1,077 RM-Affected Couples between Low-Pass GS and G-Banded Chromosome Analysis

Chromosomal Abnormalities#		G-Banded Chromosome Analysis[a]	Low-Pass GS[a]	Additional Diagnosis in Couples by Low-Pass GS[a]
Apparently balanced translocation		80[b] (7.4%, 95 CI, 5.9 to 9.2)	78 (7.2%, 95 CI, 5.8 to 9.0)	8 (0.7%, 95 CI, 0.3 to 1.5)
Inversions		6 (0.6%, 95 CI, 0.2 to 1.2)	48 (4.5%, 95 C.I., 3.4 to 5.9)	42 (3.9%, 95 CI, 2.9 to 5.2)
CNVs	Pathogenic or likely pathogenic CNVs	0 (0%, 95 CI, 0 to 0.3)	6 (0.6%, 95 CI, 0.2 to 1.2)	6 (0.6%, 95 CI, 0.2 to 1.2)
	VUS	0 (0%, 95 CI, 0 to 0.3)	12 (1.1%, 95 CI, 0.6 to 1.9)	12 (1.1%, 95 CI, 0.6 to 1.9)
Overall chromosomal structural variants[c]		86[b] (8%, 95 CI, 6.4 to 9.8)	126 (11.7%, 95 CI, 9.9 to 13.8)	50 (4.6%, 95 CI, 3.5 to 6.1)

[a]Calculation was performance based on couples
[b]Including ten samples with balanced translocations that were not detected by low-pass GS (Table S2)
[c]Only translocations and inversions were calculated

rDNA clusters (Figures S16), so as to overcome the limitation of their direct detection by the current protocol. In addition, further improvement of the current protocol, such as increasing read-depth for comprehensive detection of genetic and genomic abnormalities[34–37] including single-nucleotide variants, is warranted in the near future. Nonetheless, with a comparable cost, similar turnaround-time, and using a standardized and reproducible protocol as presented herein (Table S8), it is clear that low-pass GS can enable greater detection and provide higher precision than a combination of chromosomal analysis and CMA.

Recurrent miscarriage represents a management challenge because of the uncertainty of the outcome of subsequent pregnancies. Described general interventions such as the use of aspirin or heparin[38] and progesterone[39] failed to improve live births in clinical trials. Identifying RM-affected couples with a chromosomal structural rearrangement, particularly for the rearrangements cryptic to karyotype analysis, has direct clinical relevance for genetic counseling and individualized treatment interventions. IVF and PGT have made possible a significant reduction of miscarriage or birth with untoward outcomes for such RM-affected couples.

IVF with PGT-A is a controversial intervention offered to RM-affected couples around the world, and a survey of 386 clinics in 70 countries found that RM is the indication in 31% of PGT cycles.[40] However, the largest study of PGT to date, analyzing 46,439 day-3 and day-5 embryos,[28] found no increased number of aneuploidies when the testing indication was only RM. This supports the rationale that RM couples with normal karyotypes will not receive any benefit from PGT-A unless in the setting of advanced maternal age.[41] In addition, the study also demonstrated that carrier couples of translocations, apart from segregation of unbalanced gametes,[42,43] have an increased risk of having an embryo with non-mosaic aneuploidy due to meiotic (OR = 2.2, 95 CI, 1.8 to 3.4 in day 3 and OR = 1.4, 95 CI, 1.1 to 2.4 in day 5) but not mitotic errors.[28] However, studies show that for embryos with mosaic aneuploidies, a self-correction mechanism such as trisomy rescue might exist, resulting in a livebirth.[44,45] Such a mechanism is not applicable to embryos with non-mosaic aneuploidy[25,46] or with unbalanced translocations, which can lead to impaired trophoblastic differentiation causing implantation failure or miscarriage.[47] Therefore, detection of parental chromosomal abnormalities is an invaluable tool of diagnostic importance in the management of RM-affected couples.

It has been suggested that IVF and PGT-A/SR would improve the pregnancy outcome for the carrier couples by significantly reducing the miscarriage rate.[42,43] This possibility is further confirmed by our secondary preliminary analysis: 26 couples with diagnosed chromosomal abnormalities who pursued pregnancy by PGT achieved a low miscarriage rate (5.0%) with an absolute reduction of 27.2% by PGT compared to 68 couples with normal GS and PGT-A. Although increased maternal age in the non-carrier group was observed, we noted the reduction in RM after intervention with IVF and PGT was greater in "carrier" couples compared to "non-carrier" couples. The effect of increased maternal age in increased rate of aneuploidies was minimized by excluding embryos with aneuploidies through IVF and PGT-A. Because the sample size in this aspect of our study is limited, a larger follow-up sample size in our future investigations is warranted to substantiate the observation. Furthermore, future clinical trials should assess the primary goal of decreasing miscarriage rate and comparing the usage of PGT-A/SR in these couples with chromosomal rearrangements as defined by GS.

Overall, our study supports that GS has an increased resolution and detection rate. Comparing karyotype as the gold standard versus GS, GS has a sensitivity of 90.57% (95% CI, 83.33–95.38), specificity of 100% (95% CI, 99.82–100.00), with positive predictive value (PPV) of 100%, negative predictive value (NPV) of 99.52% and 99.54% accuracy. The increased diagnostic yield of GS with demonstrably improved management in RM-affected couples challenges the current concerns raised by various medical societies (Table 1) and provides the basis of a

Table 3. Eight Translocations Only Reported by Low-Pass GS

Sample ID[a]	Karyotype	Low-Pass GS	Length of Translocated Segments		Suspected Reasons of Missed Detection by Chromosome Analysis
			ChrA (Mb)	ChrB (Mb)	
SD-RSA_10835[Y]	46,XX	46,XX,t(12;21)(p11.21;q21.1)	31.6	30.2	similar banding patterns
SD-RSA_10949	46,XX	46,XX,t(2;17)(q37.3;p11.2)	3.2	16.8	involving G light band
SD-RSA_10970[N]	46,XX	46,XX,t(7;17)(p22.2;p13.1)	4.3	8.5	cryptic translocation
SD-RSA_11189[Y]	46,XX	46,XX,t(9;19)(q34.3;p13.3)	2.4	3.1	cryptic translocation
SD-RSA_11359[Y]	46,XX	46,XX,t(5;14)(p13.2;q24.2)	38.1	35.7	similar banding patterns
SD-RSA_20504	46,XY	46,XY,t(20;21)(p12.1;q22.11)	13.5	13.7	similar banding patterns
SD-RSA_21394	46,XY	46,XY,t(2;21),der(2) t(2;21)(p24.3;q21.3),der(21) t(2;21)(p24.3;q21.3), inv(21) (q21.1q21.3)	13.2	20.9	involving G light band
SD-RSA_22134[N]	46,XY	46,XY,t(1;19)(p36.22;q13.2)	16.1	12.6	similar banding patterns

[a]Y (Yes) or N (No) refer to couple with miscarriage(s) or fetal structural abnormalities identified in subsequent pregnancy following cytogenetic testing with normal reports

rational recommendation for couples and clinicians. Given the increased diagnostic yield, enhanced precision and comprehensive identification of chromosomal abnormalities, our results call for the reconsideration of the relevance of chromosomal rearrangements in the pathogenesis of RM and support a paradigm shift for applying low-pass GS in routine clinical use to expand the scope of genetic diagnosis for chromosomal rearrangements in RM-affected couples.

Supplemental Data

Supplemental Data can be found online at https://doi.org/10.1016/j.ajhg.2019.10.003.

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Declaration of Interests

F.X., J. Yuan, L.Y., J.X., L.C., Q.L., X.Z., J.L., A.C., W.Z., T.M., W.-J.W., Y.J., K.K., H.Y., and H.J. are current employees of BGI-Shenzhen. Y.J. is also a current employee of Complete Genomics and has stock in this company. The other authors declare no conflict of interest.

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Web Resources

Database of Genomic Variants, http://dgv.tcag.ca/dgv/app/home
Human Phenotype Ontology, http://human-phenotype-ontology.github.io/
Online Mendelian Inheritance in Man (OMIM), https://www.omim.org

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