Background: Recurrent pregnancy loss (RPL) is a common occurrence that affects up to 15% of couples in their reproductive years. In both males and females with RPL and infertility, chromosomal abnormalities play a significant impact. Aim: The study was designed to examine the involvement of chromosomal anomalies and the frequency of certain chromosomal variants persistent among couples experiencing RPL. Setting and Design: This case–control study was conducted on 1000 couples from January 2015 to September 2020 in the state of Odisha, India, strictly adhering to principles of Helsinki Declaration (1975). The study was performed at the School of Biotechnology, KIIT University in collaboration with inDNA Life Sciences Private Limited. Materials and Methods: A cohort of 1148 individuals with a history of RPL were selected for the study and they were screened with respect to fertile controls for the presence of any chromosomal anomaly using G-banding, nucleolar organizing region (NOR)-banding and fluorescence in situ hybridisation wherever necessary. Statistical Analysis: The connection between distinct polymorphic variations and the occurrence of RPL was assessed using Fisher’s exact test. Significant was defined as a \( P \leq 0.005 \). Results: One hundred and thirty-four individuals were found to harbor chromosomal anomalies. This study elucidates that along with balanced chromosomal translocations, the involvement of polymorphic variants also plays a significant role in cases of RPL. Conclusion: The cumulative occurrence of chromosomal anomalies and variants across our cohort of 1148 individuals indicates that the chromosomal assessment of all couples experiencing RPL must be performed by all the clinicians. This study aids us in identifying chromosomal polymorphisms as major players of RPL in addition to novel chromosomal translocations.

Keywords: Balanced translocation, G-banding, polymorphic variants, recurrent pregnancy loss

INTRODUCTION

The American Society for Reproductive Medicine, describes recurrent pregnancy failure as involuntary cessation of two or more clinically accepted pregnancies before 20 weeks of gestation period.[1] Recurrent pregnancy loss (RPL) is a universal problem and it affects up to 15% of couples in the reproductive age.[2–4] RPL is a multifactorial disease which encompasses endocrine

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dysfunction, various anatomical dysfunctions, autoimmune dysfunctions, parental age, chromosomal anomalies, infectious diseases and environmental toxins.\[5\]-\[8\] Chromosomal anomalies play a major role in both males and females affected with RPL and infertility.\[9\]-\[12\] Prior studies have revealed that the frequency of chromosomal aberrations is greater in couples with RPL compared to the general population.\[13\],\[14\] Generally, chromosomal anomalies in RPL couples may cause a genetic disproportion, which may in turn lead to various phenotypic defects in the foetus.\[15\],\[16\] Parental chromosomal anomalies establish a principal portion of the etiology of RPL.\[17\]-\[19\] Presence of parental chromosomal anomalies might result in uneven crossing-over in the progression of meiosis which may further lead to the development of defective gametes. Post fertilisation, clinical consequences of attaining pregnancy with chromosomal imbalances are usually lethal to the developing embryo resulting in fetal death.\[5\] In contrast to conventional chromosomal translocations, polymorphic variants may also play a crucial role in executing RPL.\[15\] The heterochromatin region of the chromosomes comprises highly repetitive sequences and several current studies suggest that polymorphism in these areas may have clinical effects such as RPL or infertility.\[20\],\[21\] It is well-known that heterochromatin of chromosomes benefits in the attachment of the spindles to chromosomes, movement of chromosomes along the spindles, meiotic pairing of the chromosomes and sister-chromatid cohesion. The short arm of acrocentric chromosomes also comprises of heterochromatin. Any variations in this region beyond the boundaries of acceptance may lead to RPL resulting in mal-segregation of the chromosomes during karyokinesis.\[15\] The occurrence of chromosomal modifications in couples with RPL ranges from 2% to 8%. Previous studies suggest that 80% of the couples sheltering chromosomal alterations may result in RPL.\[17\] Therefore, screening of parental chromosomes is a basic prerequisite for clinicians to understand the aetiology of RPL. The aim of the current study was to identify key chromosomal alterations and additionally, role of polymorphic variants and their respective frequencies present across our RPL cohort in the eastern region of India.

Materials and Methods

Subjects and karyotype analysis

This case-control study was conducted on 1000 couples from January 2015 to September 2020 in the state of Odisha, India, strictly adhering to principles of Helsinki Declaration (1975). The study was performed at the School of biotechnology, KIIT University in collaboration with inDNA Life Sciences Private Limited. A detailed consent form was duly signed by all the participants before including them in this study. A total of 2000 individuals (1272 individuals with RPL and 728 fertile controls) were included in this study. Karyotyping was carried out on heparinised peripheral blood of couples experiencing RPL. Peripheral blood lymphocytes were stimulated and cultured for 72 h. After harvesting and fixing the lymphocytes, metaphase spreads were prepared using prechilled slides. Giemsa trypsin Giemsa-banding technique was used to stain the aged slides. A minimum of 25 metaphase spreads were scored for each case, and 5 best metaphases were karyotyped using Olympus BX61 upright light microscope and Cytovision software version 7.2. Four hundred–five hundred and fifty was the average banding resolution achieved. NOR banding was performed to support our karyotype findings. The study design is summarised in [Figure 1].

Along with structural and numerical chromosomal aberrations, individual cases were also scored for the presence of heteromorphisms according to the International System for Chromosome Nomenclature 2016. Any visual polymorphic alteration in the size of centromeric heterochromatin in the long arms of chromosome 1, 9 and 16 and the distal heterochromatic region of chromosome Y were documented. Distinct polymorphic variants of the size of the satellite and the length of the stalks of all the acrocentric chromosomes were also scored. To be classified as variants, polymorphisms needed to be at least twice the size of the corresponding region of the other homologous chromosome. For detection of the presence of heteromorphic variants in the chromosomes, the karyotype results were scored by three independent individuals to avoid bias and variable results.

The study was approved by an independent ethical committee (EC no. RPL/IND/15-AIC03). Detailed informed consent was obtained from all the participants before inclusion in the study.

One hundred and twenty-four individuals were eliminated from the study for reasons including: Age above 38 years, female partner with ovulation dysfunction (polycystic ovarian syndrome), male or female partner with anatomic defects, male or female partner with a history of infections in the reproductive tract, anatomic defects in the reproductive tracts, endocrinological dysfunctions, male partner with azoospermia or extended sperm DNA fragmentation.

The remaining 1148 individuals were segregated in three groups namely, Group I: separates the couples into two categories based on the sex of the individual that is male and female, Group II divides the couples into five categories based on their age that is <20, 20–25, 26–30, 31–35 and 36 and above, Group III segregates the couples into four categories based on the total...
number of miscarriages experienced that is, 2, 3, 4 and above. Individuals diagnosed with polymorphic variants were again retrospectively followed up after 5 years for receiving a response of their recent reproductive outcome(s). RPLs should be studied in a larger cohort for a better correlation of the frequency of chromosomal anomalies or variants across RPL individuals. A sample size estimation has not been performed.

**Statistical analysis**

All the statistical analysis was performed using Graph Pad Prism 8.0.2 software. Fisher’s exact test was performed to evaluate the association between different polymorphic variants and occurrence of RPL. \( P \leq 0.005 \) was considered to be significant.

**RESULTS**

Thousand one hundred and forty eight out of 1272 individuals were included in the study with known history of RPL. The results of the RPL couples were compared with age- and sex-matched 728 fertile individuals. Constitutional chromosomal anomalies were found in 38 individuals (3.31%) whereas polymorphic variants were observed in 104 individuals (9.06%). Among these 142 individuals screened with chromosomal anomalies and variants, 77 (54.225%) were female and 65 (45.774%) were male. The mean age was 27.3 years for females and 31.4 years for males. 1148 RPL individuals comprised 574 males and 574 females whereas, 728 fertile individuals comprised 364 males and females respectively. Chromosomal anomalies and variants were present in 142 (12.37%) of RPL individuals [Figure 2]. Prominent chromosomal translocations were found in 15 (1.306%) RPL individuals, which comprised of 8 translocations in male RPL group (0.696%) and 7 translocations in the female RPL group (0.609%). The involvement of different chromosomes in translocation has been summarised in Supplementary Table 1. The major translocations found in this study are listed in Table 1. On the contrary, no chromosomal translocation was observed in the fertile couples. Notable polymorphic variants were also scored in RPL group and compared with the fertile group. All polymorphic variants found in our cohort have been represented in [Figure 2], with the help of partial karyotypes. Prominent polymorphisms were found in 104 individuals (9.06%) out of 1148 RPL individuals. We further segregated the polymorphisms observed in our cohort into stalks (short, average, long) and satellites (short and prominent) [Figure 3] and 5-year follow-up was performed to know their current reproductive status [Table 2]. It was observed that the individuals with longer stalks and larger satellites have a higher frequency of reproductive failures as compared to less prominent stalks and satellites. Correlation

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**Table 1: List of chromosomal translocations found in 574 couples screened in this study**

| Karyotype | Male | n (%) |
|-----------|------|-------|
| 46,XYt (10:15)(p12;q21),9qh+ | 1 | (0.174) |
| 46,XY,t (7;14)(q31;p12) | 1 | (0.174) |
| 45,XY,rob (13;14)(q10;q10) | 2 | (0.348) |
| 46,XY,t (4;18)(q26;q22) | 1 | (0.174) |
| 46,XY,t (10;17)(q11.2;p11.2) | 1 | (0.174) |
| 46,XY,t (14;22)(q10;q10) | 1 | (0.174) |
| 46,XY,t (19;22)(q13.4;q11.1),13ps+ | 1 | (0.174) |

| Karyotype | Female | n (%) |
|-----------|--------|-------|
| 46,XXt (2;9)(q32.2;p24.3) | 1 | (0.174) |
| 45,inv (11)(p12q13), rob (13;14)(q10;q10) | 1 | (0.174) |
| 46,XX,t (10;15)(p15;q23) | 1 | (0.174) |
| 45,XX,t (14;21)(q10;q10) | 2 | (0.348) |
| 46,XX,t (8;18)(p23;q12) | 1 | (0.174) |
| 46,XX,t (2;13)(p11.2;p11.2) | 1 | (0.174) |

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**Figure 1:** Pictorial representation of the design of this study

**Figure 2:** Pictorial representation of the design of this study

**Figure 3:** Pictorial representation of the design of this study

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**Table 2:** List of chromosomal anomalies among RPLs in eastern India

| Karyotype | n (%) |
|-----------|-------|
| 46,XYt (10:15)(p12;q21),9qh+ | 1 (0.174) |
| 46,XY,t (7;14)(q31;p12) | 1 (0.174) |
| 45,XY,rob (13;14)(q10;q10) | 2 (0.348) |
| 46,XY,t (4;18)(q26;q22) | 1 (0.174) |
| 46,XY,t (10;17)(q11.2;p11.2) | 1 (0.174) |
| 46,XY,t (14;22)(q10;q10) | 1 (0.174) |
| 46,XY,t (19;22)(q13.4;q11.1),13ps+ | 1 (0.174) |

**Table 1:** List of chromosomal translocations found in 574 couples screened in this study
between polymorphic variants and occurrence of RPL was calculated using Fisher’s exact test. It was observed that all the polymorphic variants in our study possess significant association with the risk of RPL [Table 3]. On the other hand, polymorphisms were also observed in 20 (2.747%) fertile individuals out of 728 fertile individuals [Figure 4]. The polymorphisms were mainly found in 9, 13, 14, 15, 16, 21, 22, and Y chromosomes. Among the previously mentioned chromosomes, chromosomes 9, 14, 15 and 21 were involved in most of the cases [Figure 4b]. Out of the 574 male RPL individuals studied, inversions were present in 2 cases comprising of 1 case of inv (4) (0.174%), 2 cases of inv (9) (p11q13) (0.348%) and 1 case of inv (11) (p12;q13) (0.174%). Whereas, polymorphic variants were found in 61 cases (10.627%). These 61 cases mainly comprised of 7 cases of 1qh+ (1.219%), 23 cases of 9qh+ (4.006%), 2 cases of 16qh+ (0.348%), 20 cases of single acrocentric chromosome polymorphism (3.484%) and 9 cases of multiple acrocentric chromosome polymorphisms (1.567%). The frequency of polymorphic variants in our cohort is summarised in [Table 4].

Apart from conventional structural chromosomal anomalies, numerical chromosomal anomalies were observed in the RPL group mainly in the sex chromosomes as compared with the fertile group. Among 574 RPL males, 9 males were found to have Klinefelters syndrome (1.567%), and 1 male showed sex reversal (0.174%). On the other hand, out of 574 RPL females, 1 case of del (Xq) (0.174%), 1 case of inv (X) (p11.4;q22) (0.174%), 2 cases of i (Xq) (0.348%), 1 case of 47, XXX (0.174%) and 2 cases of sex reversal (0.348%) were observed. From the cohort of 1148 RPL individuals and 728 fertile individuals, subjects were further divided into five categories based on their age, namely <20, 20–25, 26–30, 30–35 and 36 and above. It was observed that females in the age group of 20–30 harboured most of the chromosomal anomalies, followed by the next age group of 30–35 in which both males and females were equally affected with chromosomal anomalies [Figure 5a]. Furthermore, our cohort was categorised based on the total number of pregnancy loss experienced by the couples, it was observed that females with chromosomal anomalies were involved more in the group of where the couples experienced two RPLs [Figure 5b]. Males and females were involved equally in the group where the couples...
Chakraborty, et al.: The frequency and spectrum of chromosomal anomalies among RPLs in eastern India

Table 2: Correlation between various polymorphisms and the risk of recurrent pregnancy loss

| Type of polymorphisms | Number of patients | Occurrence of recurrent pregnancy loss | Recurrent pregnancy loss |
|-----------------------|--------------------|---------------------------------------|-------------------------|
|                       |                    | Yes                                   | No                      |
| 1qh+                  |                    | 08                                    | 08                      | 00                      |
| Yes                   | 08                 | 08                                    | 126                     | 1742                    |
| No                    | 1868               | <0.0001 (significant)                 |                         |                         |
| 9qh+                  |                    | 31                                    | 26                      | 05                      |
| Yes                   | 31                 | 26                                    | 108                     | 1845                    |
| No                    | 1845               | <0.0001 (significant)                 |                         |                         |
| 16qh+                 |                    | 04                                    | 04                      | 00                      |
| Yes                   | 04                 | 04                                    | 130                     | 1742                    |
| No                    | 1872               | <0.0001 (significant)                 |                         |                         |
| Yqh+ (Only in males)  |                    | 07                                    | 07                      | 00                      |
| Yes                   | 07                 | 07                                    | 78                      | 853                     |
| No                    | 931                | <0.0001 (significant)                 |                         |                         |
| 13pstk+ and 13ps+     |                    | 16                                    | 16                      | 02                      |
| Yes                   | 16                 | 16                                    | 118                     | 1858                    |
| No                    | 1860               | <0.0001 (significant)                 |                         |                         |
| 14pstk+ and 14ps+     |                    | 23                                    | 22                      | 01                      |
| Yes                   | 23                 | 22                                    | 112                     | 1741                    |
| No                    | 1853               | <0.0001 (significant)                 |                         |                         |
| 15pstk+ and 15ps+     |                    | 38                                    | 34                      | 04                      |
| Yes                   | 38                 | 34                                    | 100                     | 1738                    |
| No                    | 1838               | <0.0001 (significant)                 |                         |                         |
| 21pstk+ and 21ps+     |                    | 29                                    | 27                      | 02                      |
| Yes                   | 29                 | 27                                    | 107                     | 1847                    |
| No                    | 1847               | <0.0001 (significant)                 |                         |                         |
| 22pstk+ and 22ps+     |                    | 27                                    | 26                      | 01                      |
| Yes                   | 27                 | 26                                    | 108                     | 1741                    |
| No                    | 1850               | <0.0001 (significant)                 |                         |                         |

**DISCUSSION**

Recurrent miscarriage can have adverse emotional effects on a woman and her partner. The miscarriage rate among clinically documented pregnancies is 15%, with maximum losses in the first trimester.[22] Chromosomal polymorphism variation or chromosomal heteromorphism, was considered normal for a longer period. In recent years more number of studies have shown an increased frequency of chromosomal heteromorphism in RPL as well as infertile couples.[23] Greater escalation in the frequency of chromosomal variants in RPL males (7.491%) and RPL females (10.627%) compared with control males (2.472%) and females (3.021%) in our study suggests that variants could be associated with RPL.

De novo chromosomal variants could be clinically more significant compared with those that are inherited from previous generations.[24] Pericentromeric regions and nucleolar organizing regions on human chromosomes have been ignored owing to their negligible euchromatin content though they comprise a substantial amount of heterochromatin, which is rich in DNA satellite repeats.[24] The possible association of chromosomal polymorphic variations with higher-order organisation of genomic DNA around fundamental histone proteins and its part in epigenetic mechanisms of gene regulation and control should not be overlooked.[24] Chromosomal variants have been previously reported to be associated with spermatogenesis.[25] Higher occurrence rate of polymorphic variants has been reported to be involved with poor spermatogenesis.[25] Heterochromatin has been previously reported to have a major role in spindle anchorage, chromosome movement, meiotic pairing and sister chromatid exchange.[25,26] Pericentromeric heterochromatin brings a silencing effect on euchromatic genes when they are brought into close proximity in a subgroup of cells where these genes would be normally expressed otherwise.[27] The result of the chromosomal polymorphisms, primarily the size of the heterochromatin blocks, could also, in turn, lead to restricting or inhibiting the binding of certain transcription factors.[28] The metaphase nucleolar organiser regions (NOR) comprises of ribosomal genes which encodes proteins such as upstream binding factor and RNA polymerase I, these genes are localised on the short arm stalks of human acrocentric chromosomes, and following polymorphic alterations in these regions, may result into constraints in transcriptions.[15] In addition, carriers of balanced chromosomal translocations have a major risk of having chromosomally unbalanced progeny with the likelihood for developmental defects as well as multiple congenital anomalies. Recognising

experienced three RPLs, and males with chromosomal anomalies were involved more in the group where the couples experienced more than 4 RPLs. In addition, we evaluated the implication of detecting chromosomal anomaly/variant in both the male and female with the pregnancy outcome as compared to one partner showing any chromosomal anomaly/variant. Our data suggest that frequency of pregnancy loss is higher if both the RPL partners have been detected with chromosomal anomaly/variant as compared to one partner detected with any chromosomal anomaly/variant [Figure 5c].
such a carrier status, therefore, helps in guiding the couple about the future threats associated with the pregnancy, and the various reproductive options available.\textsuperscript{[22]}

In this study, we were able to identify a wide repertoire of chromosomal anomalies across both male and female RPL groups. As high as 12.36% of the couples were identified with chromosomal anomalies in our cohort. The chromosomal anomalies included balanced chromosomal translocation, inversion, deletion, duplication, polymorphisms, extended stalks and satellites, numerical anomalies and sex reversal. The frequency of detection of polymorphic variants in RPL couples was highest among other chromosomal anomalies. In addition, the likelihood of detection of

| Presence of stalks (number of individuals) | Individuals with successful pregnancy (5 years follow-up) | Presence of satellite (Number of individuals) | Individuals with successful pregnancy after (5 years follow-up) |
|------------------------------------------|----------------------------------------------------------|-----------------------------------------------|---------------------------------------------------------------|
| 13pstk+                                  |                                                          | 13ps+                                         |
| Short stalks (2)                         | 2                                                        | Short satellites (3)                          | 3                                                             |
| Average stalks (2)                       | 2                                                        | Prominent satellites (2)                     | 2                                                             |
| Long stalks (1)                          | 0                                                        |                                               |                                                                |
| 14pstk+                                  |                                                          | 14ps+                                         |
| Short stalks (3)                         | 3                                                        | Short satellites (3)                          | 3                                                             |
| Average stalks (2)                       | 2                                                        | Prominent satellites (1)                     | 0                                                             |
| Long stalks (3)                          | 1                                                        |                                               |                                                                |
| 15pstk+                                  |                                                          | 15ps+                                         |
| Short stalks (1)                         | 1                                                        | Short satellites (8)                          | 6                                                             |
| Average stalks (1)                       | 1                                                        | Prominent satellites (7)                     | 3                                                             |
| Long stalks (4)                          | 3                                                        |                                               |                                                                |
| 21pstk+                                  |                                                          | 21ps+                                         |
| Short stalks (1)                         | 1                                                        | Short satellites (6)                          | 5                                                             |
| Average stalks (2)                       | 2                                                        | Prominent satellites (2)                     | 2                                                             |
| Long stalks (2)                          | 1                                                        |                                               |                                                                |
| 22pstk+                                  |                                                          | 22ps+                                         |
| Short stalks (2)                         | 2                                                        | Short satellites (1)                          | 1                                                             |
| Average stalks (1)                       | 1                                                        | Prominent satellites (4)                     | 3                                                             |
| Long stalks (4)                          | 3                                                        |                                               |                                                                |

Figure 4: (a) Frequency distribution of both structural and numerical chromosomal anomalies of recurrent pregnancy loss couples as compared to control fertile couples. (b) Heat map showing the involvement of different chromosomes in recurrent pregnancy loss individuals among male and female individuals.
increased length of stalks and size of satellites over acrocentric chromosomes was significantly higher in RPL couples as compared with fertile individuals. Notably, few chromosomes were the most affected in our study, namely chromosome 15 (2.961%) was the most involved chromosome, followed by chromosome 9 (2.7%), chromosome 21 (2.351%) and chromosome 22 (2.264%), also chromosome 14 was involved in (1.916%) of the cases. Further analysing gender-wise distribution of these chromosomes, it was observed that chromosomes were involved equally across both genders except chromosome 9. The frequency of involvement of chromosome 9 in RPL females was found to be higher (4.355%) as compared to the RPL males (1.045%). In concordance with previously published literature,[17,29-34] a significant association of polymorphic variants with risk of RPL was observed in our cohort, which suggests that couples harboring polymorphic variants might be predisposed to a greater risk for RPL. Among other chromosomes involved in RPL, the sex-chromosomes were also found to have involvement in both RPL males and RPL females. A higher frequency of sex-chromosomal anomalies was documented in the RPL group as compared to the fertile controls.

In the age-wise distribution of the RPL individuals with chromosomal anomalies, the highest frequency of chromosomal anomalies was harbored by the RPL females belonging to the age group of 20–25 and 26–30, whereas, in the age group of 36 and above, the RPL males were the major contributor of chromosomal anomalies. Furthermore, the total cohort was further sub-categorised

### Table 4: Frequency of polymorphic variants in heterochromatin, stalks and satellite in 574 couples with recurrent pregnancy loss in eastern India

| Polymorphisms                      | Men, n (%) | Women, n (%) | Total, n (%) |
|------------------------------------|------------|--------------|--------------|
| Heterochromatin                    |            |              |              |
| 1qh+                               | 1 (0.174)  | 7 (1.219)    | 8 (0.696)    |
| 9qh+                               | 5 (0.871)  | 23 (4.006)   | 28 (2.364)   |
| 16qh+                              | 1 (0.174)  | 2 (0.348)    | 3 (0.261)    |
| Yqh+                               | 7 (1.219)  | NA           | 7 (0.609)    |
| Increased stalks and satellite region |          |              |              |
| 13pstk+ and 13ps+                  | 11 (1.916) | 5 (0.871)    | 16 (1.393)   |
| 14pstk+ and 14ps+                  | 14 (2.439) | 8 (1.393)    | 22 (1.916)   |
| 15pstk+ and 15ps+                  | 18 (3.135) | 16 (2.787)   | 34 (2.961)   |
| 21pstk+ and 21ps+                  | 13 (2.264) | 14 (2.439)   | 27 (2.351)   |
| 22pstk+ and 22ps+                  | 13 (2.264) | 13 (2.264)   | 26 (2.264)   |

NA=Not available

**Figure 5:** (a) Comparison of percentage of chromosomal anomalies between different age groups of recurrent pregnancy loss and control group. (b) Comparison of percentage of chromosomal anomalies across different recurrent pregnancy loss groups segregated according to the total number of pregnancy losses. (c) Comparison of pregnancy outcome of recurrent pregnancy loss couples detected with chromosomal anomaly/variants in single partner and both the partners.
into three groups based on the total number of pregnancy losses. It was found that, in the category of 3–4 pregnancy losses, the RPL males were found to be the major contributors, whereas, in the category of 2 pregnancy losses, the females were found to be the major contributors towards chromosomal anomalies.

In this study, we described the role of polymorphic chromosomal variants in RPL other than conventional chromosomal anomalies such as balanced translocations, duplications, insertions and inversions. Polymorphic variants were previously not given proper importance to be considered an important parameter whilst screening chromosomal anomalies in RPL cases.\(^\text{[22]}\) In this study, major involvement of polymorphic variants (9.059%) in our cohort of RPL couples was observed. However, the occurrence rate of polymorphic variants in the control group was found to be very low (2.060%) as compared to the RPL group. A significant co-relation was observed between the occurrence of all the polymorphic variants in the karyotype and the event of RPL. Moreover, the severity of RPL was observed in individuals with longer length of stalks and satellites. Additionally, increased frequency of RPL was observed in a group where both the partners harbored chromosomal anomaly/variant as compared to single partner. Our results are similar to previous studies\(^\text{[5,14,15,26]}\) and the alterations in the occurrence rate may be due to the dissimilarity in environmental factors and population ethnicity. From the above study, we can suggest that RPL experienced by the couples may arise due to the presence of polymorphic variants in their karyotype results and couples harboured with polymorphic variants may develop a higher likelihood of RPLs.

Our study is the first study from the eastern part of India evaluating the frequency of chromosomal anomalies or variations involving 2000 individuals including control subjects. Our results suggest that chromosomal anomalies along with polymorphisms play a pivotal role in RPLs. Higher frequency of polymorphic variants in RPL group with respect to control individuals in our cohort emphasizes the need to evaluate their role in RPLs. Furthermore, this study involves karyotyping with a band resolution of 400–500 band resolution which may not have detected every possible polymorphic variant found in the RPL couples in our cohort. In addition, molecular studies involving chromosomal variants were not accessed in this study. Therefore, we suggest that the overall high frequency of polymorphic variants in RPL males and females needs to be confirmed with further molecular investigations with a larger sample size for better understanding of the involvement of polymorphic variants in RPLs.

**Data availability statement**

The authors are willing to share the data of this study upon a reasonable request.

**Acknowledgement**

We would like to acknowledge all the participants in this study for providing their consent.

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**Conflicts of interest**

There are no conflicts of interest.

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### Supplementary Table 1: Distribution of the different types of translocations in the study

| CHR | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | X  | Y  | Total |
|-----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|
| 1   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 2   | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 2  |
| 3   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 4   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  |
| 5   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 6   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 7   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  |
| 8   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  |
| 9   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 10  | 0  | 0  | 0  | 0  | 0  | 2  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 3  |
| 11  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 12  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 13  | 0  | 3  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 3  |
| 14  | 0  | 0  | 0  | 0  | 0  | 0  | 2  | 0  | 1  | 0  | 0  | 3  |
| 15  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 16  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 17  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 18  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 19  | 0  | 0  | 0  | 1  | 0  | 0  | 1  |
| 20  | 0  | 0  | 0  | 0  | 0  | 0  |
| 21  | 0  | 0  | 0  | 0  |
| 22  | 0  | 0  | 0  |
| X   | 0  | 0  | 0  |
| Y   | 0  | 0  | 0  |
| Total | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 1  | 5  | 2  | 0  | 1  | 1  | 0  | 0  | 2  | 2  | 0  | 0  | 15  |

CHR=Chromosomes