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DELINEATION OF PARTIAL CHROMOSOMAL ABNORMALITIES IN EARLY PREGNANCY LOSSES

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ABSTRACT

Pregnancy loss (PL), particularly early pregnancy loss (EPL), is a prevalent reproductive complication, with approximately 15% of confirmed pregnancies affected. Chromosomal abnormalities are implicated in more than half of EPLs, with trisomies being the most prevalent. Partial abnormalities, including segmental deletions, duplications, and unbalanced translocations, are detected in up to 10% of EPL cases. This study focuses on the precise characterization of partial chromosomal abnormalities, previously identified by Quantitative fluorescent polymerase chain reaction (QF-PCR) and multiplex ligation probe amplification (MLPA) analyses. By employing an array comparative genomic hybridization (aCGH), we analyzed 20 EPL samples, identifying 32 partial chromosomal abnormalities, including 18 deletions and 14 duplications, with an average size of 33.2 Mb. Notably, two abnormalities previously undetected by QF-PCR and MLPA were revealed (deletions in 7q36, and 1p36.32p36.31regions), emphasizing the necessity of high-resolution genomic tools. Chromosomes 1, 18, and 13 emerged as frequently involved, aligning with previous associations with recurrent pregnancy loss. Recurrent abnormalities were identified in six chromosomal regions, with chromosome 1p36.33-p36.32 exhibiting the highest frequency. Gene Ontology (GO) enrichment analysis of recurrent regions highlighted disruptions in critical biological processes, including molecular binding, enzymatic activity, and cellular development. Many genes in these regions are linked to multisystem syndromes, suggesting their involvement in early embryonic development and pregnancy viability.

Our findings underscore the complexity of EPL's genetic landscape, demonstrating that large CNVs, may disrupt multiple genes critical for development. Although, subtelomeric MLPA reliably detects telomeric partial chromosomal abnormalities in EPLs, aCGH is essential for detection and precise characterization of all CNVs, thus enhancing diagnostic and counseling strategies in affected couples.

Key words: Partial fetal chromosomal abnormalities, early pregnancy loss, EPLs, aCGH

INTRODUCTION

Pregnancy loss (PL), encompassing spontaneous abortion or miscarriage, refers to the premature termination of a pregnancy before fetal viability, typically before the 20th gestational week. Early pregnancy loss (EPL) denotes losses occurring within the first trimester (<12 weeks) [1]. Approximately 15% of couples with confirmed pregnancies experience EPL, with recurrent PL (RPL) affect around 2% of them [2]. The etiology of PL is complex, involving a confluence of maternal and fetal factors. Maternal factors include endocrine disturbances, uterine anomalies, implantation issues, and infections [3]. In the event of fetal cause of EPL, chromosomal abnormalities account for roughly half of EPL cases, whereas the underlying cause remains elusive in the other half. Chromosomal trisomies, are the most prevalent fetal chromosomal aberrations, constituting up to 56% of abnormal EPLs, with trisomy 16 being the most common [4, 5]. Triploidy and monosomy, each affecting about 15% of abnormal conceptuses, follow in frequency. Rarer chromosomal anomalies comprise a smaller proportion of these cases. Lately, with the development of the molecular

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genetic technologies, especially with the widespread application of the whole exome sequencing (WES), embryonic and fetal monogenic conditions, were also reported in EPLs. Genes linked to fetal death, such as *CPLANE1, CHD7, FBN1, FGFR3, NIPBL*, and *SOS1*, are often associated to multisystem disorders. Others are associated or related with specific conditions like cardiac anomalies (*CSRP3, GATA4, SCN5A*), skeletal dysplasia (*COL1A1, FGFR2*), kidney diseases (*GREB1L, NPHS1*), and CNS abnormalities (*PIK3R2*). This diversity suggests that EPL has varied etiologies [6-11].

Partial chromosomal aberrations, involving gain or loss of chromosomal content, represent a significant factor contributing to EPL. Our previous research identified these aberrations in 8% of abnormal EPLs, demonstrating heterogeneity across different chromosomes [4].

Quantitative fluorescent polymerase chain reaction (QF-PCR) and multiplex ligation probe amplification (MLPA) have been established as valuable screening tools for chromosomal abnormalities in the context of EPL. These techniques have enabled detailed analysis of chromosomal aberrations, including partial chromosome abnormalities, revealing their diverse nature and impact on pregnancy outcome [4, 12-14]. Consequently, while these techniques provide a valuable first step in the diagnostic process, they may not yield a definitive genetic diagnosis in all cases since they cannot detect all abnormalities, such as interstitial chromosomal abnormalities or determine the size and gene content of the detected partial chromosomal abnormalities. Understanding these abnormalities is crucial for improving diagnostic accuracy, genetic counseling, and potentially developing preventive strategies for recurrent pregnancy loss. Given the profound implications of partial chromosomal imbalances for fetal development and pregnancy outcome, a comprehensive and in-depth characterization of these aberrations is imperative.

In this study, we employed array comparative genomic hybridization (aCGH) to delineate the genomic architecture of previously detected partial chromosomal imbalances, including their size, location, and gene content. This granular level of analysis is expected to provide deeper insights into the pathogenic mechanisms associated with these aberrations and contribute to a more comprehensive understanding of the complex fetal etiology of early pregnancy loss.

MATERIALS AND METHODS

Study group and selection of samples

In a prior investigation, partial chromosomal abnormalities were identified in 16 out of 900 analyzed EPL samples [4]. More recently, an additional four samples exhibiting partial chromosomal abnormalities were detected and incorporated into the current analysis, bringing the total number of EPL samples included in this study to 20. These samples consisted of single chromosomal abnormalities in half, while the other half exhibited double chromosomal abnormalities.

All EPL samples referred to the Research Center for Genetic Engineering and Biotechnology "Georgi D. Efremov", at the Macedonian Academy of Sciences and Arts, Skopje for analysis of chromosomal aneuploidies were selected by a collaborative team of gynecologists and pathologists. Standard histopathological analysis was performed on all EPLs, as described previously [14, 15]. To ensure ethical compliance, written informed consent was obtained from all study participants, and the research protocol received approval from the institutional ethics committee (Macedonian Academy of Sciences and Arts, 09-1047/6 from 04.05.2016).

All EPL samples were processed using standard phenol-chloroform or automated extraction methods to obtain genomic DNA for further analysis. To initially exclude maternal contamination and to screen for common aneuploidies, we employed quantitative fluorescent polymerase chain reaction (QF-PCR) with a panel of short tandem repeat (STR) markers targeting chromosomes 13, 18, 21, and the sex chromosomes [16]. This method allowed for rapid detection of trisomies and monosomies involving chromosomes 13, 18, 21 and X, as well as triploidies. To complement QF-PCR, multiplex ligation-dependent probe amplification (MLPA), using P036-Subtelomere Mix 1 and P070-Subtelomere Mix 2B probemixes, was implemented to identify chromosomal imbalances on all chromosomes.

Array comparative genomic hybridization (aCGH)

To further elucidate the genomic architecture of the observed partial chromosomal abnormalities, array comparative genomic hybridization (aCGH) analysis was conducted following Agilent Technologies' SureTag Complete DNA Labeling Kit (Agilent Technologies, Santa Clara, CA, USA). Genomic DNA extracted from the analyzed samples, along with sex-matched control DNA, was enzymatically fragmented using the restriction enzymes AluI and RsaI to ensure uniform DNA fragment sizes. Subsequently, the fragmented DNA samples were fluorescently labeled: the experimental DNA was labeled with Cyanine 5 (Cy5), while the control DNA was labeled with Cyanine 3 (Cy3). After purification, the labeled experimental and control samples were combined and co-hybridized onto Agilent Technologies' CGH Constitutional G3 4x180k microarrays. Following hybridization, data acquisition was performed using Agilent Technologies' DNA microarray scanner with surescan technology, ensuring accurate cap-

ture of fluorescence signals. The resulting data were subsequently processed and analyzed using Agilent's Genomic Workbench software platform. This analysis provided a comprehensive visualization of CNVs, with detailed genomic annotations based on the UCSC hg19 genome assembly, facilitating precise mapping and interpretation of the chromosomal imbalances observed in the study.

Identifying Recurrent Chromosomal Regions and Gene Ontology (GO) enrichment analysis

To gain insights into the genetic factors underlying early pregnancy loss, we conducted a comprehensive analysis of a total of 32 chromosomal abnormalities detected by aCGH in 20 EPLs with partial chromosomal abnormalities. We have established a criterion for identifying recurrent chromosomal regions: a chromosomal region, included in deletion or duplication, had to be observed in more than two EPLs. We determined the smallest overlapping region (SOR) for each recurrent chromosomal region. This approach enabled us to pinpoint the specific genes and pathways that are likely involved in the pathogenesis of early pregnancy loss.

To gain a deeper understanding of the biological functions and processes associated with the identified genes, we conducted a Gene Ontology (GO) enrichment analysis utilizing the Panther software [12]. This analysis compares the frequency of specific GO terms within a group of genes involved in SORs. Significantly enriched GO terms were visualized using a bar plot, thus providing a clear and intuitive way to interpret the results of the enrichment analysis and identify the biological functions and processes and that are most likely to be involved in EPL.

RESULTS

General findings

To investigate the genomic landscape of the previously detected partial chromosomal abnormalities in EPLs with QF-PCR and subtelomeric MLPA methods, we have performed array CGH analysis on a total of 20 samples.

All 30 previously detected chromosomal abnormalities among the 20 studied EPLs were confirmed and 2 additional chromosomal abnormalities previously not detected with QF-PCR and subtelomeric MLPA methods were revealed. These additional abnormalities were out of the ligation sites of the used subtelomeric MLPA probes, therefore they were not detected previously.

Among the 20 investigated samples, the total number of detected partial chromosomal abnormalities was 32, of which 18 (56% of all detected abnormalities) were deletions and 14 were duplications (44%). Nine samples had single partial chromosomal abnormality, 10 samples carried double abnormalities, while one sample carried three partial abnormalities. All the characterized abnormalities were on distal chromosomal sites, except one sample which had an additional aberration located proximally (7q22.1q34) of the expected single chromosomal abnormality on chromosome 7q36, previously not detected with the QF-PCR and subtelomeric MLPA methods. Also, another sample with deletion on 1p region and duplication on 1q region detected previously by MLPA, was found to carry an additional duplication involving the 1p36.32p36.31 chromosomal region. Schematic presentation of the detected chromosomal abnormalities among the affected chromosomes is shown on Figure 1. The sizes of

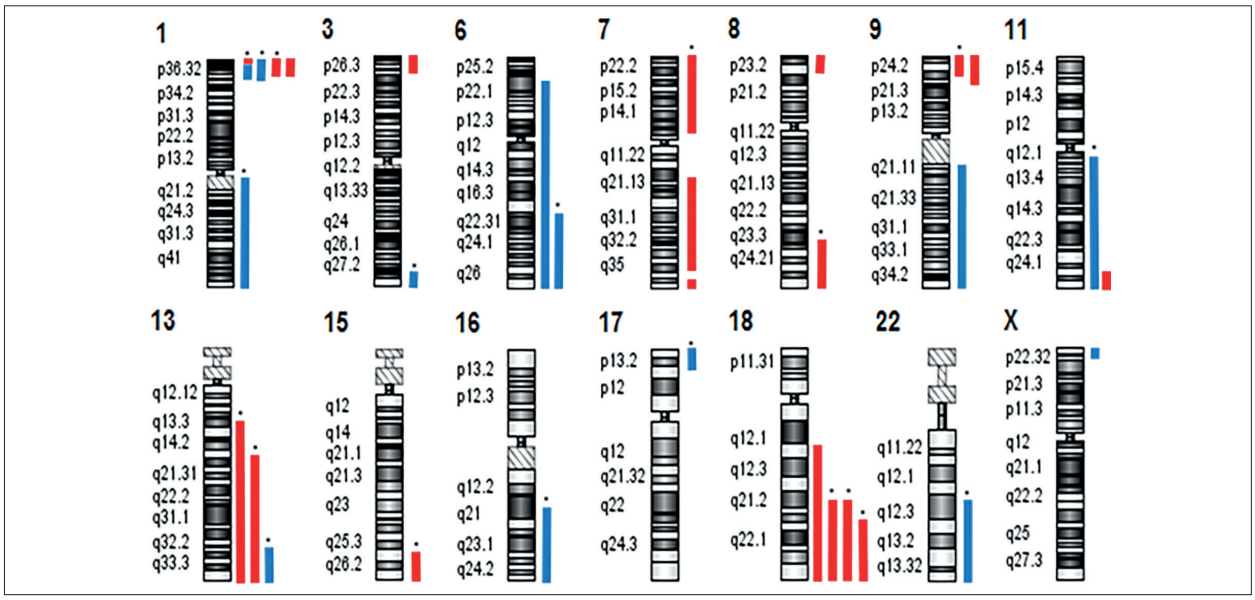

Figure 1. Figure 1. Schematic presentation of the detected chromosomal abnormalities among the affected chromosomes. (Red bars-deletions; blue bars-duplications), *chromosomal abnormalities detected together with other abnormality, see Table 1.

Table 1. Detailed genomic locations of the detected partial chromosomal abnormalities

	Sample	Chromosome band	Start-stop positions	Deletion/Duplication	Length (Mbp)
1	Abp-24	3p26.3-p26.1	73914-7761159	Deletion	7.68
2	Abp-118	6p22.3-q27	20695423-170911240	Duplication	150.21
3	Abp-141	17p13.3-p13.2	59739-4424713	Duplication	4.36
		18q21.2-q23	49010709-78010032	Deletion	28.99
4	Abp-154	8p23.3-p23.1	176814-12686483	Deletion	12.51
5	Abp-264	Xp22.33	169064-2344976	Duplication	2.17
6	Abp-359	11q24.2q25	127546842-134927114	Deletion	7.38
7	Abp-498	9p24.3-p23	204193_9092170	Deletion	8.88
8	Abp-565	8q24.13-q24.3	123993466-146294098	Duplication	22.3
		13q14.3-q34	54627609-115089535	Deletion	60.46
9	Abp-612	3q25.33-q29	160109099-197845254	Duplication	37.73
		9p24.3-p22.3	204193-14453418	Deletion	14.25
10	Abp-627	7q22.1-q34	101658846-140064713	Deletion	38.4
		7q36.3	158074913-159118566	Deletion	1.04
11	Abp-760	18q12.1-q23	25698937-78010032	Deletion	52.31
12	Abp-805	1p36.33-p36.32	564424-2352253	Deletion	1.78
		1p36.32p36.31	2394396-5710339	Duplication	3.31
		1q21.1q44	142617943-249212668	Duplication	106.59
13	Abp-815	1p36.33-p36.13	564424-17886109	Deletion	17.32
14	Abp-840	13q13.3-q34	39536567-115093155	Deletion	75.55
		16q21-q24.3	64826408-90148400	Duplication	25.32
15	Abp-882	1p36.33-p36.11	564424-24570889	Deletion	24.01
		11q11-q25	55361638-134904063	Duplication	79.54
16	Abp-914	13q32.3-q34	101077865-115105297	Duplication	14.02
		18q21.31-q23	55906486-78010032	Deletion	22.1
17	Abp-941	9q21.11-q34.3	70984337-141087916	Duplication	70.1
18	Abp-942	6q22.31-q27	122649716-170911237	Duplication	48.26
		7p22.3-p13	45064-45032669	Deletion	44.98
19	Abp-990	1p36.33-p35.2	746608-31284865	Duplication	30.53
		18q21.2-q23	49862572-78010032	Deletion	28.14
20	Abp-998	15q26.1-q26.3	93296596-102388476	Deletion	9.09
		22q13.1-q13.33	37984158-51219009	Duplication	13.23

the abnormalities ranged from 1.04 Mb to 150.21 Mb, with average size of 33.2 Mb. Detailed genomic locations of the detected partial chromosomal abnormalities is presented in Table 1 and the gene content of the detected abnormalities is described in Supplementary Table 1.

Chromosome 1 exhibited the highest frequency of abnormalities, with six of the detected alterations mapped to this chromosome. Chromosome 18 was the second most affected, harboring four abnormalities, followed by chromosome 13 with three abnormalities.

Recurrent chromosomal regions and GO enrichment analysis

To identify significant CNV regions and to determine the molecular functions and biological processes of the genes contained in these regions who are associated with early pregnancy loss (EPL), we first focused on recurrent regions (>2) among the 32 detected partial chromosomal alterations determining the SOR regions.

With this approach, we identified a total of six recurrent events. The most common recurrent event was on the terminal part of the chromosome 1p, with SOR of 1.78 Mb in the 1p36.33-p36.32 regions, shared by five deletions/duplications Common recurrent events were found on the terminal part of chromosomes 18q (SOR of 22.1 Mb in the 18q21.31-q23 region) and chromosome 13q (SOR of 14.02 in the 13q32.3-q34 region). With two recurrent events were chromosomes 6q, 9p and 11q, with SOR of 48.26 Mb in the 6q22.31-q27 region, SOR of 8.88 Mb in the 9p24.3-p23 region and SOR of 7.38 Mb in the 11q24.2q25 region.

Schematic presentation of the SOR regions is presented on Figure 2. The OMIM genes in every recurrent abnormality were identified and the respective diseases caused by those genes were paired. The most common disorders caused by the identified genes were multisystem syndromes, which may explain their pathogenicity in early human development. The OMIM genes and the associated disorders are shown in Supplementary Table 2.

Gene Ontology (GO) enrichment analysis was conducted using Panther, and significantly enriched GO terms were visualized through bar plots (Figures 3 and 4). The analysis revealed that the genes are predominantly associated with molecular binding activities, enzymatic functions as catalytic molecules, or ATPase activity.

In terms of biological processes, the recurrent genes identified in this study primarily encode proteins involved in essential functions such as biological regulation, cellular processes, and metabolic processes (Figure 4). Furthermore, several genes within the affected regions were linked to developmental processes, indicating that their dysfunction could contribute to early developmental abnormalities, potentially leading to pregnancy loss.

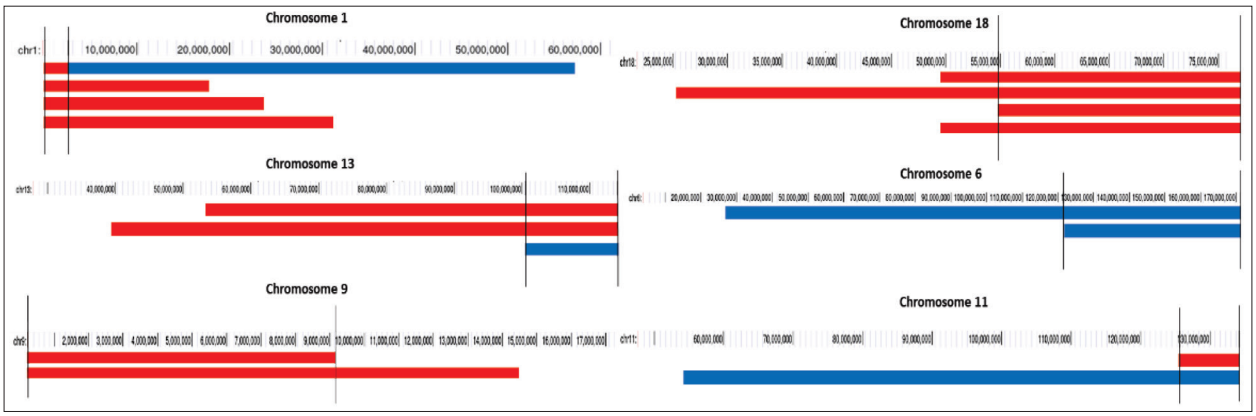

Figure 2. The detected smallest overlapping regions (SORs), (Red bars-deletions; blue bars-duplications).

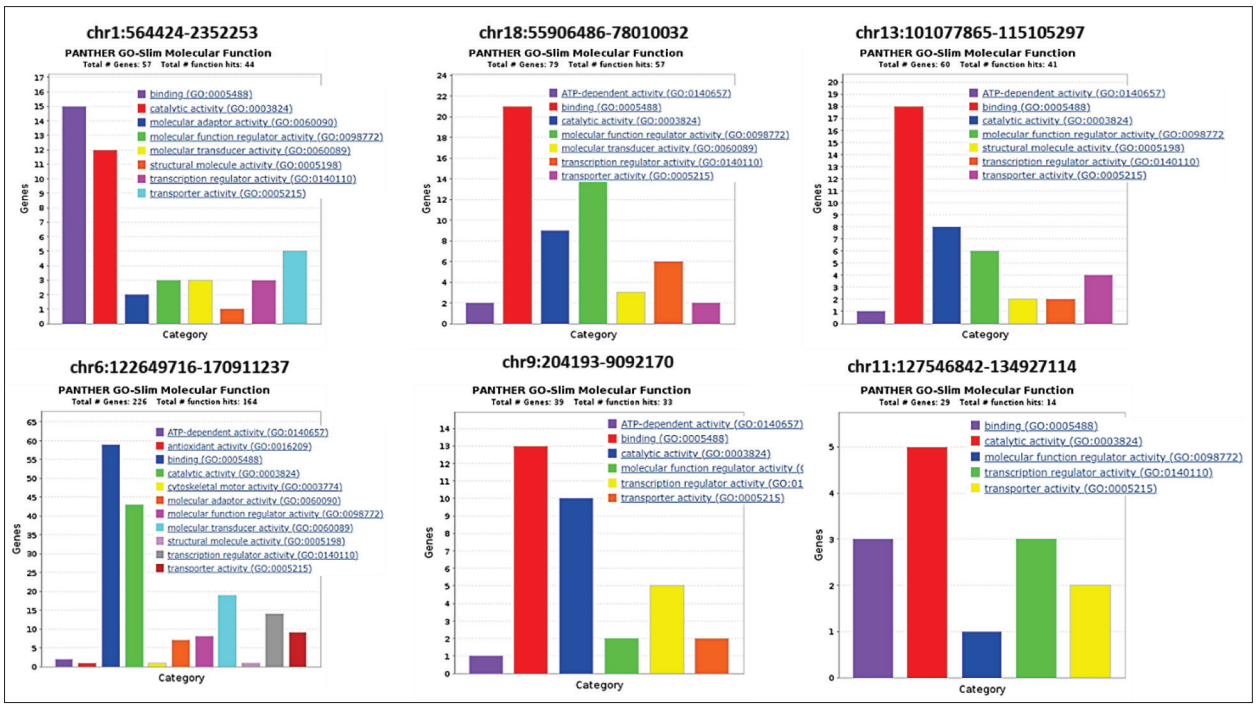

Figure 3. Molecular function profiling of recurrent genes in the studied regions.

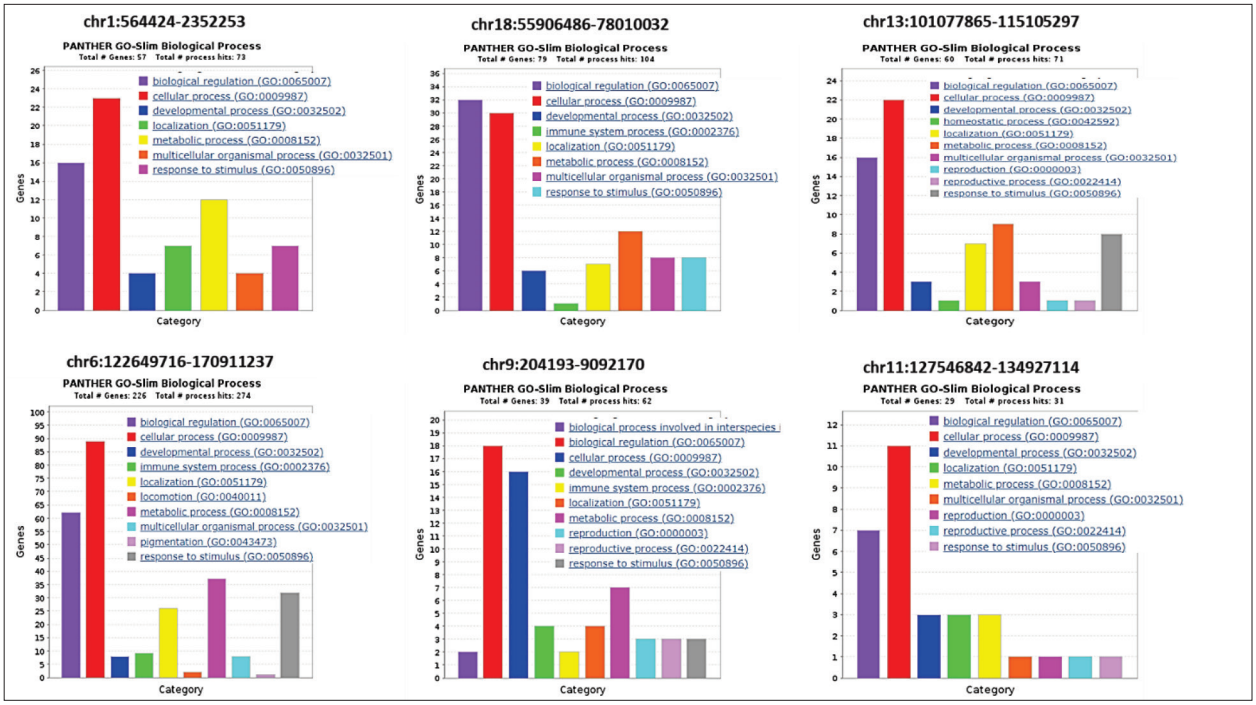

Figure 4. An analysis of the biological processes associated with frequently occurring genes within the specified recurring regions.

DISCUSSION

This study aimed to refine the characterization of previously identified partial chromosomal abnormalities in EPLs from early pregnancy losses (EPLs) by employing aCGH. Our findings significantly expand upon the initial QF-PCR and subtelomeric MLPA data, providing a detailed understanding of the genomic landscape in these cases. The identification of multiple abnormalities within individual samples, unidentified by the previous analyses underscores the complex genetic etiology of EPLs. For instance, in one sample we identified an additional abnormality located proximally to the expected single chromosomal abnormality on chromosome 7q36, which had not been previously detected using the QF-PCR and subtelomeric MLPA. In another sample, we discovered an additional duplication on the 1p36.32p36.31 chromosomal regions, despite the initial distal deletion and duplication on 1p and 1q regions respectively. Interstitial chromosomal abnormalities account for approximately 5–10% of chromosomal anomalies in early pregnancy losses (EPLs) [18, 19]. These abnormalities cannot be detected using methods such as the previously used QF-PCR and subtelomeric MLPA, underscoring the limitations of these techniques. This highlights the critical need for high-resolution approaches, such as aCGH in EPLs with no chromosomal abnormality detected by subtelomeric MLPA.

Our study's findings are particularly noteworthy given the large size of the CNVs detected (averaging 33.2 Mb), suggesting that these genetic alterations may disrupt multiple genes and are essential for normal embryonic development. The frequent involvement of chromosomes 1, 18, and 13 in these abnormalities is in line with their established association with pregnancy loss [20, 21]. These chromosomes have previously been implicated in recurrent pregnancy loss (RPL), particularly in studies of aneuploidy and large chromosomal rearrangements [22]. Chromosomal copy number variations (CNVs) in key regions such as 1p36.33-p36.32, 9p24.3-p23, 11q24.2-q25, 13q32.3-q34, and 18q21.31-q23 have been strongly implicated in early pregnancy loss (EPL). These regions host genes critical for apoptosis, placental development, and cellular signaling. For example, 1p36.33-p36.32 harbors genes essential for apoptosis, while abnormalities in 11q24.2-q25 involve genes like *ETS1*, crucial for connective tissue integrity. Disruptions in 18q21.31-q23 affect placental function, and 13q abnormalities interfere with vascular development. Advances in next-generation sequencing have enhanced the identification of these CNVs in EPL cases, confirming their significance in embryonic viability [23-26]. In live-born individuals, similar abnormalities may result in congenital disorders, developmental delays, and physical malformations, but often allow for survival past the prenatal period. The key difference lies in the severity of the

genetic disruption and the associated clinical outcomes, with EPL cases often involving more pronounced, lethal alterations. By contrast, live-born individuals may exhibit milder phenotypes due to mosaicism or less severe genetic disruptions [27].

Around 1/3 of all genes in the recurrent regions are associated with multisystem syndromes, as shown by the OMIM genes enrichment analysis (Supplementary Table 2). Some of the genes are strongly associated with EPLs due to their critical roles in embryonic development and placental function. For instance, *PEX10* and *PEX3*, linked to peroxisomal biogenesis disorders, often result in embryonic lethality due to metabolic dysfunction [28]. *COL4A1* and *COL4A2*, involved in vascular integrity, are associated with placental abnormalities and pregnancy complications [29]. Additionally, *CITED2* plays a vital role in placental development, and its deficiency is linked to embryonic lethality [30]. The results of our Gene Ontology (GO) enrichment analysis provide additional insights into the biological processes potentially disrupted by these chromosomal abnormalities. Our data suggest that the recurrent genes identified are primarily involved in essential biological processes such as molecular binding, enzymatic activity, ATPase activity, growth, metabolism, reproduction, and developmental processes. Dysregulation of these genes could plausibly lead to early embryonic developmental abnormalities, contributing to pregnancy loss. This finding is consistent with previous studies highlighting the role of genetic dysregulation in critical pathways such as apoptosis, cell cycle control, and placental development in EPL [31-36].

It is essential to acknowledge the limitations of this study, including the retrospective design and relatively small sample size. These factors may restrict the generalizability of our findings. The majority of the detected chromosomal abnormalities could have arisen from parental balanced chromosomal translocations [37]. Unfortunately, this cannot be confirmed at the time, since we were unable to perform parental karyotypes. The probabilities of different reproductive outcomes for carrier individuals of reciprocal balanced translocations are commonly based on an estimate of the likelihood of a fetus to survive with chromosomal imbalances resulting from the adjacent-1 segregation, while conceptions with other unbalanced segregations may not be viable [38]. This can lead to a more precise risk assessment and proper genetic counselling for the next pregnancies of the couples.

The focus on CNVs also does not exclude the possibility of other genetic factors contributing to EPLs, such as single nucleotide variants (SNVs) or epigenetic changes that were not assessed in this study. Furthermore, while aCGH offers higher resolution than many traditional methods, it does not capture all types of genomic variation [39]. Future studies should aim to integrate CNV data with whole-genome sequencing and epigenetic profiling to develop a more comprehensive understanding of the genetic landscape of EPLs. Functional validation studies using in vitro and in vivo models are critical to elucidating how these genetic changes affect cellular and developmental processes [40-42]. This comprehensive research approach will be essential for understanding the underlying mechanisms of EPLs.

CONCLUSION

In conclusion, our study provides valuable insights into the partial chromosomal abnormalities associated with EPLs. The identification of recurrent CNV regions and their associated genes represents a significant step towards understanding the complex genetic etiology of this condition. Although, subtelomeric MLPA reliably detects telomeric partial chromosomal abnormalities in EPLs, aCGH is essential for detection and precise characterization of all CNVs, thus enhancing diagnostic and counseling strategies in affected couples.

Acknowledgments

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Ethics Committee Approval

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Macedonian Academy of Science and Arts (09-1047/6 from 04.05.2016). Written informed consent was obtained from all study participants.

Author Contributions

Concept-Gj.B.; D.P.K; Design-D.P.K; Supervision-D.P.K; Materials-K.K.S.; Data Collection-Gj.B, M.T, K.K.S.; Analysis and Interpretation-Gj.B, M.T.; Literature Review- Gj.B, D.P.K.; Writing-Gj.B, D.P.K.; Critical Review-Gj.B, M.T., K.K.S., D.P.K.

Conflict of Interest

The authors declared no conflict of interest.

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REFERENCES

1. Macklon NS, Geraedts JPM, Fauser BCJM. Conception to ongoing pregnancy: the "black box" of early pregnancy loss. Hum Reprod Update. 2002;8:333–343.
2. Ford HB, Schust DJ. Recurrent pregnancy loss: etiology, diagnosis, and therapy. Reviews in obstetrics and gynecology. 2009;2(2):76.
3. Turesheva A, Aimagambetova G, Ukybassova T, Marat A, Kanabekova P, Kaldygulova L, Amanzholkyzy A, Ryzhkova S, Nogay A, Khamidullina Z, Ilmaliyeva A. Recurrent pregnancy loss etiology, risk factors, diagnosis, and management. Fresh look into a full box. Journal of clinical medicine. 2023 Jun 15;12(12):4074.
4. Bozhinovski G, Terzikj M, Kubelka-Sabit K, Jasar D, Lazarevski S, Livrinova V, Plaseska-Karanfilska D. Chromosomal Abnormalities in Early Pregnancy Losses: A Study of 900 Samples. Balkan Journal of Medical Genetics. 2023 Dec 1;26(2):11-20.
5. Kubelka-Sabit K, Bozinovski G, Jasar D, Filipovski V, Lazarevski S, Ivanovski M, Plaseska-Karanfilska D. Detection of placental chromosomal aberrations in early spontaneous abortions in correlation with the histologic findings. Macedonian Medical Review. 2017;71(1):64-71.
6. Bozhinovski G, Terzikj M, Kubelka-Sabit K, Plaseska-Karanfilska D. High Incidence of CPLANE1-Related Joubert Syndrome in the Products of Conceptions from Early Pregnancy Losses. Balkan Medical Journal. 2024 Mar;41(2):97.
7. Rajcan-Separovic E. Next generation sequencing in recurrent pregnancy loss-approaches and outcomes. Eur J Med Genet. 2020;63:103644.
8. Zhao C, Chai H, Zhou Q, et al. Exome sequencing analysis on products of conception: a cohort study to evaluate clinical utility and genetic etiology for pregnancy loss. Genet Med. 2021;23:435-442.
9. Al Qahtani NH, AbdulAzeez S, Almandil NB, et al. Whole-genome sequencing reveals exonic variation of ASIC5 gene results in recurrent pregnancy loss. Front Med (Lausanne). 2021;8:699672.
10. Buonaiuto S, Biase ID, Aleotti V, et al. Prioritization of putatively detrimental variants in euploid miscarriages. Sci Rep. 2022;12:1997.
11. Kline J, Vardarajan B, Abhyankar A, et al. Embryonic lethal genetic variants and chromosomally normal pregnancy loss. Fertil Steril. 2021;116:1351-1358.
12. Donaghue C, Mann K, Docherty Z, Mazzaschi R, Fear C, Ogilvie C. Combined QF-PCR and MLPA molecular analysis of miscarriage products: an efficient and robust alternative to karyotype analysis. Prenatal Diagnosis: Published in Affiliation With the International Society for Prenatal Diagnosis. 2010 Feb;30(2):133-7.
13. Saxena D, Agarwal M, Gupta D, Agrawal S, Das V, Phadke SR. Utility and limitations of multiplex ligation-dependent probe amplification technique in the detection of cytogenetic abnormalities in products of conception. Journal of postgraduate medicine. 2016 Oct;62(4):239.
14. Kubelka-Sabit K, Jasar D, Filipovski V, Bozinovski G, Plaseska-Karanfilska D. Molecular and histological characteristics of early triploid and partial molar pregnancies. Polish Journal of Pathology. 2017 Sep 1;68(2):138-43.
15. Kubelka-Sabit KB, Prodanova I, Jasar D, Bozinovski G, Filipovski V, Drakulevski S, Plaseska-Karanfilska D. Molecular and immunohistochemical characteristics of complete hydatidiform moles. Balkan Journal of Medical Genetics. 2017 Jun 30;20(1):27-34.
16. Noveski P, Terzic M, Vujovic M, Kuzmanovska M, Sukarova Stefanovska E, Plaseska-Karanfilska D. Multilevel regression modeling for aneuploidy classification and physical separation of maternal cell contamination facilitates the QF-PCR based analysis of common fetal aneuploidies. Plos one. 2019 Aug 20;14(8):e0221227.
17. Thomas PD, Ebert D, Muruganujan A, Mushayahama T, Albou LP, Mi H. PANTHER: Making genome-scale phylogenetics accessible to all. Protein Science. 2022 Jan;31(1):8-22.
18. Liao N, Zhang Z, Liu X, Wang J, Hu R, Xiao L, Yang Y, Lai Y, Zhu H, Li L, Liu S. A chromosomal microarray analysis-based laboratory algorithm for the detection of genetic etiology of early pregnancy loss. Frontiers in Genetics. 2023 Jun 29;14:1203891.
19. Xiang J, Ding Y, Tang H, Zhang W, Mao J, He Q, Zhang Q, Wang T. Genetic analysis of pregnancy loss and fetal structural anomalies by whole exome sequencing. Orphanet Journal of Rare Diseases. 2024 Sep 9;19(1):330.
20. Essers R, Lebedev IN, Kurg A, Fonova EA, Stevens SJ, Koeck RM, von Rango U, Brandts L, Deligiannis SP, Nikitina TV, Sazhenova EA. Prevalence of chromosomal alterations in first-trimester spontaneous pregnancy loss. Nature Medicine. 2023 Dec;29(12):3233-42.

21. Soler A, Morales C, Mademont-Soler I, Margarit E, Borrell A, Borobio V, Muñoz M, Sánchez A. Overview of chromosome abnormalities in first trimester miscarriages: a series of 1,011 consecutive chorionic villi sample karyotypes. Cytogenetic and genome research. 2017 Feb 1;152(2):81-9.
22. Wang Y, Li Y, Chen Y, Zhou R, Sang Z, Meng L, Tan J, Qiao F, Bao Q, Luo D, Peng C. Systematic analysis of copy-number variations associated with early pregnancy loss. Ultrasound in Obstetrics & Gynecology. 2020 Jan;55(1):96-104.
23. Fan L, Wu J, Wu Y, Shi X, Xin X, Li S, Zeng W, Deng D, Feng L, Chen S, Xiao J. Analysis of chromosomal copy number in first-trimester pregnancy loss using next-generation sequencing. Frontiers in Genetics. 2020 Oct 20;11:545856.
24. Shen Y, Zhang W, Hua P, Qian F. Genetic Analysis of 1p36 Deletions for Six Aborted Fetuses. Alternative Therapies in Health & Medicine. 2024 Oct 1;30(10).
25. Wang Y, Li Y, Chen Y, Zhou R, Sang Z, Meng L, Tan J, Qiao F, Bao Q, Luo D, Peng C. Systematic analysis of copy-number variations associated with early pregnancy loss. Ultrasound in Obstetrics & Gynecology. 2020 Jan;55(1):96-104.
26. Wu X, Su L, Xie X, He D, Chen X, Wang M, Wang L, Zheng L, Xu L. Comprehensive analysis of early pregnancy loss based on cytogenetic findings from a tertiary referral center. Molecular Cytogenetics. 2021 Dec;14:1-8.
27. Verdoni A, Hu J, Surti U, Babcock M, Sheehan E, Clemens M, Drewes S, Walsh L, Clark R, Katari S, Sanfilippo J. Reproductive outcomes in individuals with chromosomal reciprocal translocations. Genetics in Medicine. 2021 Sep;23(9):1753-60.
28. Mast FD, Li J, Virk MK, Hughes SC, Simmonds AJ, Rachubinski RA. A Drosophila model for the Zellweger spectrum of peroxisome biogenesis disorders. Disease Models & Mechanisms. 2011 Sep 1;4(5):659-72.
29. Coste T, Vincent-Delorme C, Stichelbout M, Devisme L, Gelot A, Deryabin I, Pelluard F, Aloui C, Leutenegger AL, Jouannic JM, Héron D. COL4A1/COL4A2 and inherited platelet disorder gene variants in fetuses showing intracranial hemorrhage. Prenatal diagnosis. 2022 May;42(5):601-10.
30. Kuna M, Soares MJ. Cited2 is a key regulator of placental development and plasticity. BioEssays. 2024 Aug;46(8):2300118.
31. Davalieva K, Bozhinovski G, Kiprijanovska S, Kubelka-Sabit K, Plaseska-Karanfilska D. Proteomics analysis of human chorionic villi reveals dysregulated pathways that contribute to recurrent pregnancy loss. PROTEOMICS–Clinical Applications. 2024 Nov;18(6):e202400020.
32. Ni X, Li X, Guo Y, Zhou T, Guo X, Zhao C, Lin M, Zhou Z, Shen R, Guo X, Ling X. Quantitative proteomics analysis of altered protein expression in the placental villous tissue of early pregnancy loss using isobaric tandem mass tags. BioMed research international. 2014;2014(1):647143.
33. Xin L, Xu B, Ma L, Hou Q, Ye M, Meng S, Ding X, Ge W. Proteomics study reveals that the dysregulation of focal adhesion and ribosome contribute to early pregnancy loss. PROTEOMICS–Clinical Applications. 2016 May;10(5):554-63.
34. Li J, Wang L, Ding J, Cheng Y, Diao L, Li L, Zhang Y, Yin T. Multiomics studies investigating recurrent pregnancy loss: an effective tool for mechanism exploration. Frontiers in immunology. 2022 Apr 27;13:826198.
35. Davalieva K, Terzikj M, Bozhinovski G, Kiprijanovska S, Kubelka-Sabit K, Plaseska-Karanfilska D. Comparative proteomics analysis of decidua reveals altered RNA processing and impaired ribosome function in recurrent pregnancy loss. Placenta. 2024 Jun 8;154:28-37.
36. Davalieva K, Kocarev D, Plaseska-Karanfilska D. Decoding recurrent pregnancy loss: insights from comparative proteomics studies. Biology of Reproduction. 2025 Jan;112(1):1-7.
37. Priya PK, Mishra VV, Roy P, Patel H. A study on balanced chromosomal translocations in couples with recurrent pregnancy loss. Journal of human reproductive sciences. 2018 Oct 1;11(4):337-42.
38. Verdoni A, Hu J, Surti U, Babcock M, Sheehan E, Clemens M, Drewes S, Walsh L, Clark R, Katari S, Sanfilippo J. Reproductive outcomes in individuals with chromosomal reciprocal translocations. Genetics in Medicine. 2021 Sep;23(9):1753-60.
39. Boormans EM, Birnie E, Wildschut HI, Schuring-Blom HG, Oepkes D, van Oppen CA, Nijhuis JG, Macville MV, Kooper AJ, Huijsdens K, Hoffer MV. Multiplex ligation-dependent probe amplification versus karyotyping in prenatal diagnosis: the MAKE study. BMC Pregnancy and Childbirth. 2008 Dec;8:1-5.

40. Gourhant L, Bocher O, De Saint Martin L, Ludwig TE, Boland A, Deleuze JF, Merviel P, Dupré PF, Lemarié CA, Couturaud F, Le Maréchal C. Whole exome sequencing, a hypothesis-free approach to investigate recurrent early miscarriage. Reproductive BioMedicine Online. 2021 Apr 1;42(4):789-98.
41. Fu M, Mu S, Wen C, Jiang S, Li L, Meng Y, Peng H. Whole exome sequencing analysis of products of conception identifies novel mutations associated with missed abortion. Molecular medicine reports. 2018 Aug 1;18(2):2027-32.
42. Byrne AB, Arts P, Ha TT, Kassahn KS, Pais LS, O'Donnell-Luria A, Babic M, Frank MS, Feng J, Wang P. Genomic autopsy to identify underlying causes of pregnancy loss and perinatal death. Nature Medicine. 2023 Jan;29(1):180-9.