Decoding the Genetic Enigma: A Case Study on Congenital Anomalies with Developmental Delay and 9q Duplication Unveiled Via Comprehensive Whole Exome Sequencing and Cytogenetic Analysis

Reyhaneh Dehghanzad, PhD[1,2]; Mohsen Aghajanpour Mir, Msc[1,2]; Zahra Golchehre, PhD[2]; Mostafa Asadollahi, Msc[1,2]; Roghayeh Rahbar Parvaneh, Msc[2,3]; Behnoosh Tasharrofi, PhD[1,2]; Abbas Shakoori Farahani, MD, PhD[1]; Mohammad Keramatipour, MD, PhD[1,2]

[1]Department of Medical Genetics, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[2]Watson Genetic Laboratory, North Kargar Street, Tehran, Iran
[3]Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Received: 4 Jun 2024
Accepted: 23 Oct 2024
Published: 1 Jan 2026

Keywords:

Congenital anomalies
Intellectual disability
Developmental delay
Whole exome sequencing
Copy number variations

ABSTRACT

Objectives: Approximately 3% of newborns worldwide are affected by Congenital Anomalies (CAs) with or without Intellectual Disability (ID)/Developmental Delay (DD), often caused by genetic factors such as single-gene disorders or chromosome aberrations. Whole Exome Sequencing (WES) has become a highly effective first-tier test for identifying these genetic factors, detecting both Copy Number Variations (CNVs) and Single Nucleotide Polymorphisms (SNPs)/Insertion/Deletion Polymorphisms (INDELs), while conventional cytogenetic analysis can provide additional valuable information to confirm results where applicable.

Materials & Methods: The proband DNA was extracted and subjected to WES. Genetic variants were analyzed using the Genome Analysis Toolkit (GATK) following the American College of Medical Genetics and Genomics (ACMG) guidelines. Additionally, karyotyping of the child and her parents was conducted with high-resolution CTG banding after harvesting conventional cell cultures and performing Giemsa banding on metaphase spreads of cultured leukocytes.

Results: This study describes a patient with microcephaly, mild intellectual disability, and specific facial features, where initial WES did not identify any causative SNPs/INDELs. However, subsequent WES-based analysis for CNVs revealed the presence of dup (9) (q21.11q22.32). Further chromosomal analysis uncovered unique karyotypes for the patient [46, XX, t (5; 9) (p15.1; q22.1), add (14) (p11.1)] and her father [46, XY, t (5;9)(p15.1;q22.1)], and a normal karyotype for the mother.

Conclusion: The present study confirms the effectiveness of utilizing WES-based analysis of CNVs and SNPs/INDELs as the primary diagnostic test for identifying patients with CAs/ID/DD.

How to cite this article: Dehghanzad R, Aghajanpour Mir M, Golchehre Z, Asadollahi M, Rahbar Parvaneh R, Tasharrofi B, et al. Decoding the Genetic Enigma: A Case Study on Congenital Anomalies with Developmental Delay and 9q Duplication Unveiled Via Comprehensive Whole Exome Sequencing and Cytogenetic Analysis. Iran J Child Neurol. 2026;20(1): 79-84. https://doi.org/10.22037/ijcn.v20i1.45486.

Introduction

Approximately 3% of newborns are affected with Congenital Anomalies (CAs) with or without Intellectual Disability (ID)/Developmental Delay (DD) worldwide. In more than half of these patients, genetic factors are considered the cause. Single-gene disorders

Corresponding Author:
Mohammad Keramatipour. Department of Medical Genetics, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: keramatipour@sina.tums.ac.ir

and chromosome aberrations are the most common genetic causes of these conditions. However, due to the high genetic heterogeneity and complex etiological factors, the causes in about 50% of cases remain unsolved (1).

Copy-Number Variants (CNVs), which can alter the structure and function of the human genome, have been identified as the cause of about 25.7% of DD cases (2). Similarly, Single-Nucleotide Variants (SNVs) and Insertions and Deletions (INDELS) variants accounted for approximately 25% of DD cases (3). In contrast, karyotyping and Chromosomal Microarray Analysis (CMA) alone yield about 7.5% and 16% to 28% respectively, in the diagnosis of children with CAs with or without DD (other than suspected trisomies) (4).

The available clinical evidence strongly recommends using Whole Exome Sequencing (WES) /Whole Genome Sequencing (WGS) as a first-tier test in the routine diagnosis of patients with CAs/ID/DD (5). WES has been shown to be a more cost- and time-saving diagnostic approach than other cytogenomic techniques. In addition to routine, detailed analysis of pathogenic SNVs and indels in known genes, WES data analysis could detect large deletions or duplications of genomic content in patients (6).

Partial trisomy 9 is the fourth most common autosomal trisomy, often resulting from unbalanced segregation of a parental balanced translocation involving chromosome 9 and another autosome. The resulting phenotypes vary widely depending on the size and location of the duplicated region (7), which iskey to understanding the genotype-phenotype relationship. Accurate assessment of these CNVs through WES or WGS can help pinpoint the specific genes and genome locations involved, as well as their impact on the patient's phenotype.

This study investigated a patient suspected of having a genetic syndrome using WES and chromosomal analysis. The patient is a 13-year-old girl with intrauterine growth retardation (IUGR), microcephaly, poor feeding, delayed speech, mild intellectual disability, aggressive behavior, growth regression, micrognathia, moderate kyphosis, mild scoliosis, genu valgum, and diabetes. A combination of WES and karyotyping revealed significant duplication of chromosome 9 and its aberrant location on chromosome 14.

Case report

A 13-year-old girl from western Iran, presenting multiple syndromic features, was referred to Watson Genetics Laboratory, Tehran, Iran, for genetic analysis. She was born to non-consanguineous parents (a 32-year-old father and a 29-year-old primigravida mother) at full-term via cesarean section. Her physical exam at birth indicated intrauterine growth restriction (IUGR), with a birth weight of 2 kg, length of 44 cm, and head circumference of 31 cm. In infancy, she displayed microcephaly, restlessness, and poor feeding. Her motor development initially progressed normally; however, she experienced delayed speech acquisition and later demonstrated mild intellectual disability with an Intelligence quotient (IQ) of 75-80, alongside aggressive behaviors and growth regression.

Throughout childhood, additional physical and developmental abnormalities emerged, including micrognathia (corrected surgically at age 11), moderate kyphosis, mild scoliosis, genu valgum, and the onset of diabetes at age 12 years. Her brain MRI at nine months was unremarkable. Presently, she has mild ambulatory difficulty and poor speech, accompanied by distinctive facial features such as sparse eyebrows, a broad and prominent nasal bridge, downturned corners of the mouth, retrognathia, and a small pursed mouth with full cheeks.

In addition, she appears syndromic, with a head circumference remaining below age norms, consistent with her microcephaly. Facial findings include dysmorphic features: Sparse eyebrows, broad nasal bridge, downturned corners of the mouth, retrognathia, a small mouth with an expression resembling a "whistling face," and full cheeks. Examination of the hands reveals joint contractures of the fingers, with a tendency toward camptodactyly, ulnar deviation, and limited extension. Her feet show mild deformities with talipes equinovarus (clubfoot). Examining the spine reveals moderate kyphosis and mild scoliosis, while genu valgum affects her lower limb alignment and gait. Neurologically, she exhibits limited speech and communication ability, with mildly impaired gait. Endocrine assessment confirmed diabetes at age 12 years, which is under medical management.

The patient's complex syndromic presentation of craniofacial and skeletal anomalies, microcephaly, and developmental delay supports a probable genetic etiology for her condition.

Genetic analysis in the proband was performed following DNA extraction from EDTA whole blood using the Blood SV-mini kit (GeneAll Biotechnology Co., LTD, South Korea). WES was conducted with the Twist Human Core Exome Plus kit (Twist Bioscience, USA) and run on an Illumina NovaSeq platform (Illumina Inc., CA, USA). Data were analyzed using the Genome Analysis Toolkit (GATK-v4.4.0), and variants were annotated and filtered, and final interpretation followed the American College of Medical Genetics and Genomics (ACMG) guidelines.

WES of the proband revealed no pathogenic or likely pathogenic SNVs or Indels in the known genes

related to the patient's phenotype. Analysis of copy number variations in WES data was performed using the GATK gCNV caller. CNV analysis suggested duplication of a large fragment of chromosome 9 with an approximate size of 27 Mb. The duplicated fragment roughly spanned positions Chr9:71,627,900 to 99,017,200 (Figure 1). According to the UCSC genome browser, this region encompasses 276 genes, including 109 protein-codinggenes, 52 non-coding RNA genes, and 115 pseudogenes. Among them, 36 genes have Online Mendelian Inheritance in Man (OMIM) phenotypes (Supplementary Table 1). According to the ACMG guidelines, this variant can be classified as pathogenic. Although WES identified the duplicated region, the fragment's current chromosomal location could not be defined by WES data. In other word, this study were unable to determine whether the duplicated segment is on chromosome 9 or has moved to another chromosome. To validate the WES findings and define the chromosomal nature of this duplication, karyotype analysis was performed for the patient and her parent. Therefore, peripheral venous blood samples were obtained in sodium heparin tubes. Conventional cell culture, harvesting, and Giemsa (GTG)-banding were performed on metaphase spreads of cultured leukocytes, using standard techniques. Karyotype was determined for the patient and her parents at a resolution of 500-550 bands using the high-resolution GTG-banding technique. Twenty metaphases were analyzed for each individual.

According to the International System for Human Molecular Cytogenomic Nomenclature (ISCN, 2020), the karyotype of the proband was 46, XX, t (5; 9) (p15.1; q22.1), add (14) (p11.1) (Figure 2A), the karyotype of her father was 46, XY, t (5; 9) (p15.1; q22.1) (Figure 2B) and her mother had a normal female karyotype (46, XX) (Figure 2C). Based on cytogenetic analysis, the father has only a balanced translocation only. No imbalance, gain, or loss of chromosomal segment is seen in the father. The proband has an apparently balanced translocation involving chromosomes 5 and 9, inherited from his father. In both the proband and his father, breakage and reunion occurred at bands 5p15.1 and 9q22.1, with an exchange of the segments distal to these bands. However, only the proband has an additional duplication of the segment from chromosome 9 (specifically 9q21.11 to 9q22.32), as indicated by her WES data. Detailed analysis confirmed that the duplicated segment was inserted at band 14p11.1 on chromosome 14 in the proband. Thus, this aberrantly added fragment on chromosome 14 originated from chromosome 9 during paternal meiosis, resulting in a unique duplication in the proband.

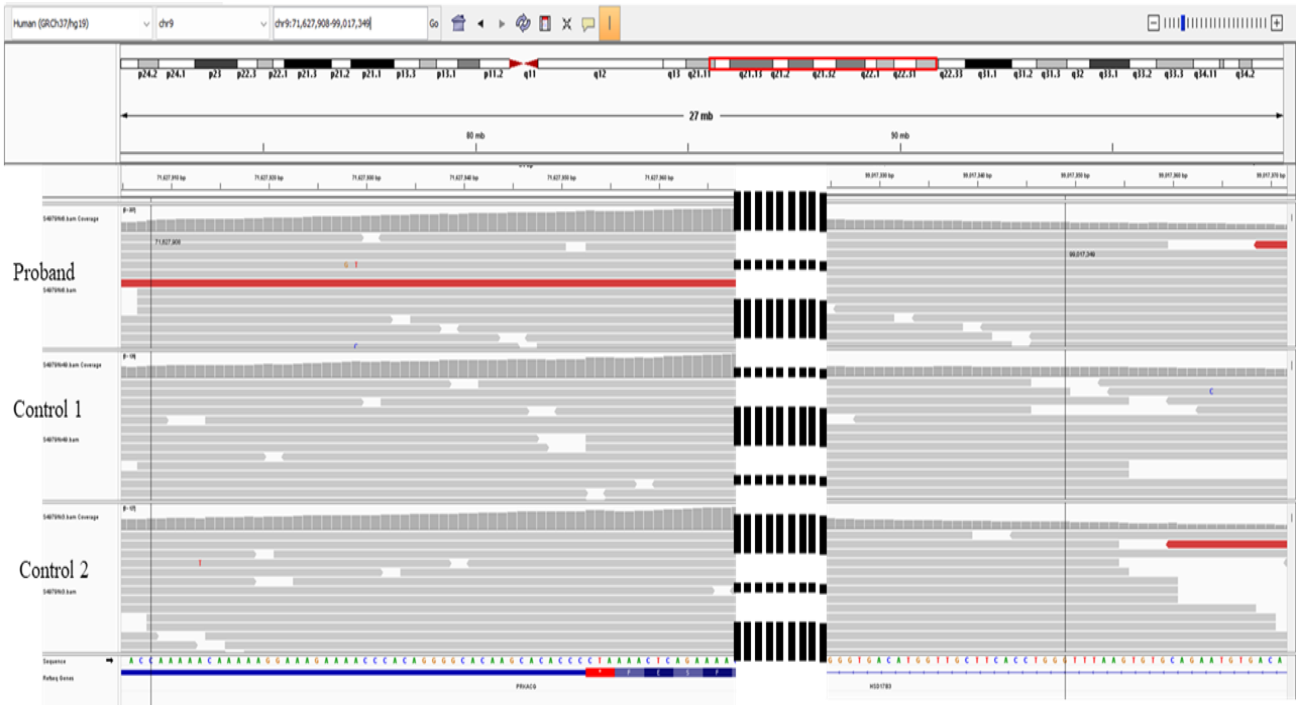

Figure 1. The visualization of the 27Mb duplicated fragment in the proband using IGV (Integrative Genomics Viewer) contrasts with the absence of such duplication in two healthy controls.

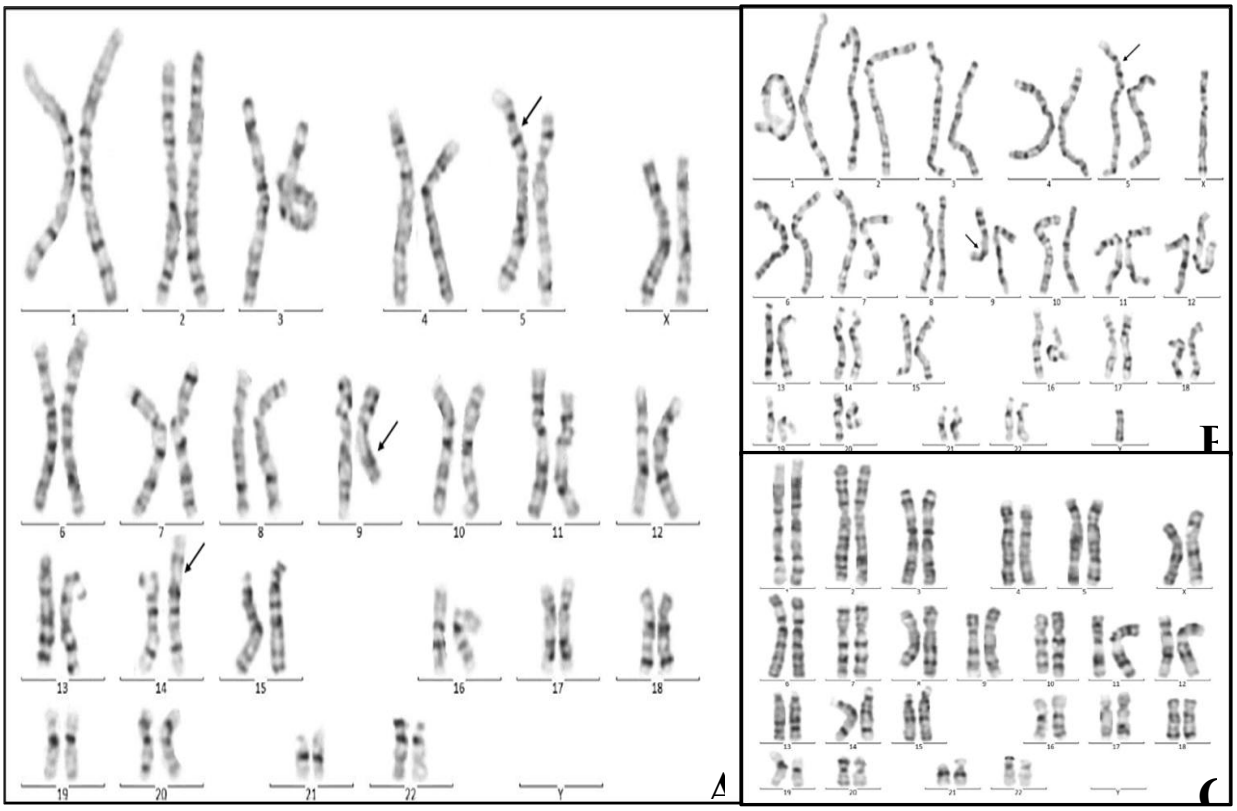

Figure 2. Karyotype results of the proband (A), her father (B) and her mother (C). The black arrows appoint the breakage locations on chromosomes 5 and 9, and the added fragment to chromosome 14 in the proband.

Discussion

In genetic analysis, WES has become the first-tier test for patients with CAs/ID/DD. This advanced technique has been shown to be highly effective in detecting both CNVs and SNPs/INDELs that can contribute to these conditions. However, conventional cytogenetic analysis should be considered to confirm WES results when applicable, as it can provide valuable complementary information that improves diagnostic yield (8) and the interpretation of genetic findings.

The present investigation describes a case study involving a female patient exhibiting mild intellectual disability, developmental delay, and distinctive facial features, where a diagnosis of 46, XX, t (5;9)(p15.1;q22.1), ins (14;9) (p11.1;q21.11q22.32) was established through the synergistic integration of WES and karyotyping techniques. However, the present case is unique as she has a parental translocation between 5p and 9q, along with an excess of a partial 9q segment on the third chromosome (14p). Thus, its interpretation is not consistent with the expected meiotic segregation pattern in reciprocal translocation carriers, and her identified cytogenetic abnormality has not been previously reported.

To the best of our knowledge, chromosomes involved in reciprocal translocations form quadrivalents during meiosis. These complex structures segregate through various modes, such as alternate, adjacent-1, adjacent-2, 3: 1, or 4: 0. As a result, gametes with distinct balanced or unbalanced chromosome combinations are formed (9). The present findings indicate that the meiotic processes can be more complex than anticipated, particularly for specific chromosome rearrangements. These intricacies necessitate a comprehensive understanding through the utilization of models and artificial intelligence (AI) tools.

On the flip side, our understanding of the complete functionality of the human genome remains incomplete. In patients with genomic rearrangements of a somewhat similar nature, examining gene dosage alterations and rearrangements can offer significant insights into the functions of associated genes and the impact of variations on human health and disease (10). Based on the literature review, some inherited balanced reciprocal translocations involving 5p15.1 or 9q22.1, including 46, XX, t (9; 15) (q22; q11.2) and 46, XY, t (5; 11) (p15.1; q14.2) in normal individuals have been reported previously (11). In addition, Dvorah Abeliovich et al. reported a mother with a balanced translocation, 46, XX, t (5, 14) (p15; q13) who had a 1 year old child with peculiar head, apparently low-set ears, hypotelorism, depressed nasal bridge, down-

turned angles of the mouth, and coloboma of the iris and abnormal cytogenetic result, 47, XY, + der (14) (14pter -> 14q13::5p15 -> 5pter) mat (12). Moreover, 46, XX, -5, +der (5) t (5; 11) (5qter -> 5p15::11p12 -> 11pter) pat has been reported in a newborn female infant with hypotonia, joint hyperextensibility, cardiac murmur, macroglossia, and hepatosplenomegaly (13). A female patient who died on the third day after birth, with severe retrognathia, low-set and dysmorphic ears with a preauricular appendix on the left side, prominent nasal bridge, hypotonia of muscles, cutix laxa, truncus arteriosus communis persistent and a persistent foramen ovale, an additional lobe of the right part of the liver, spina bifida occulta, right kidney showed multiple cysts of the tubuli and only rudiments of glomerula had 46, XX, t (5;10) (5qter -> 5p15::10p11 -> 10pter;10qter -> 10p11::5p15 -> 5pter) in the result of her mother balanced reciproca1 translocation, 46, XX, t (5;10) (p15;p11) (14).

Partial trisomy 9 ranks as the fourth most prevalent autosomal trisomy, preceded by trisomies 21, 18, and 13. Features that often occur in people with chromosome 9q duplication include developmental delay, intellectual disability, behavioral problems, craniofacial abnormalities, and distinctive facial features (7). Partial trisomy of 9q typically results from imbalanced meiotic segregation of a parental balanced translocation between chromosome 9 and another autosomal chromosome (15). Moreover, Keith Tiong et al. reported a 23-year-old female with de novo duplication of 9q22.1 to q32. She exhibited dysmorphic features, developing slowly from six months, learning difficulties and growing poorly (16).

Partial trisomy 9 can result in widely varying phenotypes based on the size and location of the duplicated region. Notably, researchers are still actively working to understand the relationship between genotype and phenotype fully (17). Kosuke Izumi et al. reported de novo duplication of 9q22.3 in three family members who had growth retardation, mild intellectual disability, and mild facial dysmorphism. In their study, the PTCH1 was identified as a potential candidate gene associated with their phenotype, alongside 14 other genes within the region (18). In the current case, PTCH1 is part of the duplication region, including 276 genes, and it is plausible that this finding could offer insights into the phenotype observed.

In Conclusion

The present study reaffirms that WES-based analysis of CNVs and SNPs/INDELs can serve as the most efficacious first-tier test in genetic clinics for diagnosing patients presenting with CAs/ID/DD. In addition, in the postgenomic era marked by advancing genome analysis techniques, fine-tuning case studies related to genome variations can significantly enhance our understanding of genome function.

Acknowledgment

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of Watson Genetic Laboratory, Tehran, Iran and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study.

Authors' Contribution

Reyhaneh Dehghanzad conceptualized and designed the study, performed WES data analysis, and drafted the manuscript. Mohsen Aghajanpour Mir, Zahra Golchehre, Mostafa Asadollahi, and Behnoosh Tasharrofi collected the data, reviewed the literature, and contributed to drafting the manuscript. Roghayeh Rahbar Parvaneh performed the molecular experiments. Abbas Shakoori Farahani reviewed and revised the manuscript. Mohammad Keramatipour supervised the project, interpreted the data, reviewed and revised the manuscript, and approved the final version for submission

Conflict of Interest

The authors declared no conflict of interest.

References

1. Hsu R, Lee C, Chien Y, Lin S, Hung M, Chen N, et al. Utility of whole-exome sequencing for patients with multiple congenital anomalies with or without intellectual disability/developmental delay in East Asia population. Mol Genet Genomic Med. 2023;e2160.
2. Yuan H, Shangguan S, Li Z, Luo J, Su J, Yao R, et al. CNV profiles of Chinese pediatric patients with developmental disorders. Genetics in Medicine. 2021;23(4):669–78.
3. Chérot E, Keren B, Dubourg C, Carré W, Fradin M, Lavillaureix A, et al. Using medical exome sequencing to identify the causes of neurodevelopmental disorders: experience of 2 clinical units and 216 patients. Clin Genet. 2018;93(3):567–76.
4. Leite AJ da C, Pinto IP, Leijsten N, Ruiterkamp-Versteeg M, Pfundt R, de Leeuw N, et al. Diagnostic yield of patients with undiagnosed intellectual disability, global developmental delay and multiples congenital anomalies using karyotype, microarray analysis, whole exome sequencing from Central Brazil. PLoS One. 2022;17(4):e0266493.
5. Lindstrand A, Ek M, Kvarnung M, Anderlid BM, Björck E, Carlsten J, et al. Genome sequencing is a sensitive first-line test

to diagnose individuals with intellectual disability. Genetics in Medicine. 2022;24(11):2296–307.

6. Zanardo ÉA, Monteiro FP, Chehimi SN, Oliveira YG, Dias AT, Costa LA, et al. Application of whole-exome sequencing in detecting copy number variants in patients with developmental delay and/or multiple congenital malformations. The Journal of Molecular Diagnostics. 2020;22(8):1041–9.

7. Vahedi S, Vahedi M, Mirzaei F, Soltani N, Pazhand Birjandi H. A rare case of partial trisomy 9q with dysmorphic clinical features and Hirschsprung's disease. Int J Pediatr. 2022;10(12):17187–91.

8. Wiener EK, Buchanan J, Krause A, Lombard Z. Retrospective file review shows limited genetic services fails most patients–an argument for the implementation of exome sequencing as a first-tier test in resource-constraint settings. Orphanet J Rare Dis. 2023;18(1):1–9.

9. Ogilvie CM, Scriven PN. Meiotic outcomes in reciprocal translocation carriers ascertained in 3-day human embryos. European Journal of Human Genetics. 2002;10(12):801–6.

10. Zhou W, Kanai M, Wu KHH, Rasheed H, Tsuo K, Hirbo JB, et al. Global Biobank Meta-analysis Initiative: Powering genetic discovery across human disease. Cell Genomics. 2022;2(10).

11. Chen CP, Wu PC, Lin CJ, Su YN, Chern SR, Tsai FJ, et al. Balanced reciprocal translocations detected at amniocentesis. Taiwan J Obstet Gynecol. 2010;49(4):455–67.

12. Abeliovich D, Yagupsky P, Bashan N, Opitz JM. 3: 1 meiotic disjunction in a mother with a balanced translocation, 46, XX, t (5, 14)(p15; q13) resulting in tertiary trisomy and tertiary monosomy offspring. Am J Med Genet. 1982;12(1):83–9.

13. Aleck K, Williams J, Mongkolsmai C, Knight S, Taysi K. Partial trisomy 11p with interatrial septal aneurysm. Case report and literature review. In: Annales de Genetique. 1985. p. 102–6.

14. Back E, Vogel W, Hertel C, Schuchmann L. Trisomy 10p due to t (5; 10)(p15; p11) segregating in a large sibship. Hum Genet. 1978;41(1):11–7.

15. Aftimos SF, Hoo JJ, Parslow MI. Partial trisomy 9q due to maternal 9/17 translocation. American Journal of Diseases of Children. 1980;134(9):848–50.

16. Tiong K, Cotterill A, Falhammar H. Adult case of partial trisomy 9q. BMC Med Genet. 2010;11(1):1–5.

17. Zhou Y, Zhang C, Zhai J, Li T, Wu Q, Li W, et al. A patient with unusual features and a 69.5 Mb duplication from a de novo extra der (9): a case report. Mol Med Rep. 2015;12(1):155–8.

18. Izumi K, Hahn A, Christ L, Curtis C, Neilson DE. Familial 9q22. 3 microduplication spanning PTCH1 causes short stature syndrome with mild intellectual disability and dysmorphic features. Am J Med Genet A. 2011;155(6):1384–9.