MOLECULAR CYTOGENETIC CHARACTERIZATION OF A RARE RECOMBINANT CHROMOSOME 22 CAUSED BY A MATERNAL INTRACHROMOSOMAL INSERTION

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The formation of recombinant chromosomes in the offspring of inversion and insertion carriers constitutes a significant challenge in clinical genetics due to the high risk of chromosomal abnormalities in children. Here, we present a clinical case. The aim of this study was to characterize the structure and origin of a chromosomal imbalance in a female patient presenting with delayed motor and speech development, craniofacial anomalies, and sensorineural hearing loss through molecular cytogenetic analysis of a recombinant chromosome 22. Chromosomal microarray analysis of the proband, who exhibited psychomotor delay and dysmorphic features, revealed three interstitial duplications: 22q11.21, 22q12.3–q13.1, and 22q13.2. Fluorescence in situ hybridization (FISH), using both commercial and homemade DNA probes, demonstrated that the mother carried a complex intrachromosomal rearrangement comprising an initial paracentric inversion of 22q11.21–q12.3, followed by an interstitial insertion of the 22q11.21 and 22q12.3–q13.1 segments into the nucleolar organizer region at 22p12. Accordingly, the recombinant chromosome identified in the proband resulted from meiotic segregation of the maternal complex intrachromosomal inversion and insertion. These findings highlight the diagnostic value of an integrated cytogenomic approach for the precise delineation of complex chromosomal rearrangements, determination of their origin, and assessment of genetic risk in clinical genetic counseling.

Keywords: intrachromosomal insertion, inversion, recombinant chromosome 22, FISH, homemade DNA probes, CNV, duplication

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Compliance with ethical standards: the study was approved by the Ethics Commitee of the Research Centre for Medical Genetics (protocol No. 4/2 dated 19 April 2021). The informed consent for participation in the research study was obtained from the patients.

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МОЛЕКУЛЯРНО-ЦИТОГЕНЕТИЧЕСКАЯ ХАРАКТЕРИСТИКА РЕДКОГО СЛУЧАЯ РЕКОМБИНАНТНОЙ ХРОМОСОМЫ 22 ВСЛЕДСТВИЕ МАТЕРИНСКОЙ ИНТРАХРОМОСОМНОЙ ИНСЕРЦИИ

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Формирование рекомбинантных хромосом в потомстве носителей инверсий и инсерций представляет собой серьезную проблему клинической генетики в связи с высоким риском рождения детей с хромосомной патологией. Представлен клинический случай. Целью исследования было в ходе молекулярно-цитогенетической диагностики рекомбинантной хромосомы 22 установить структуру и происхождение хромосомного дисбаланса у пациентки с задержкой моторного и психоречевого развития, черепно-лицевыми аномалиями и тугоухостью. При проведении хромосомного микроматричного анализа у пробанда с задержкой психомоторого развития и признаками дисморфогенеза были обнаружены три интерстициальные дупликации: 22q11.21, 22q12.3q13.1 и 22q13.2. FISH-анализ с использованием коммерческих и несерийных ДНК-зондов позволил установить, что у матери пациентки имеется сложная интрахромосомная перестройка: сочетание инициирующей парацентрической инверсии 22q11.21q12.3 и последующей межплечевой инсерции районов 22q11.21 и 22q12.3q13.1 в район ядрышкового организатора 22p12. Соответственно, выявленная у пробанда рекомбинантная хромосома является результатом сегрегации материнской сложной внутрихромосомной перестройки. Полученные результаты подчеркивают диагностическую ценность комплексного цитогеномного подхода для точной идентификации сложных хромосомных нарушений, определения их происхождения и оценки генетических рисков при медико-генетическом консультировании.

Ключевые слова: интрахромосомная инсерция, инверсия, рекомбинантная хромосома 22, FISH, несерийные ДНК-зонды, CNV, дупликация

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Recombinant chromosomes (rec) are detected in the offspring of carriers with balanced structural chromosomal rearrangements (CRs), such as inversions and insertions, and are associated with genomic imbalances — copy number variations (CNVs) in the form of deletions and duplications — linked to abnormal phenotypes [1, 2].

The majority of pathogenic microdeletions and microduplications represent the recurrent CNVs, which arise due to specific features of the genomic architecture and lead to sporadic cases of chromosomal imbalance [3]. In contrast, non-recurrent CNVs may result from other mechanisms of formation, for example, from the recombination events occurring during in meiosis I in phenotypically normal heterozygous carriers of inversion, as well as from intra- and interchromosomal insertions. A between-arm intrachromosomal insertion represents a segment of chromatin from one arm inserted into a breakpoint site on the opposite arm [4]. Thus, after bivalent formation between the chromosome carrying a between-arm insertion and its normal homologue, the inserted segment becomes reoriented to ensure the maximum degree of synapsis within the bivalent. A single (or any odd number of) crossovers within the centromeric segment (the chromosome region containing the centromere) results in the formation of recombinant chromosomes — one with a duplication of the inserted segment and the other with a deletion. Gametes (and subsequently zygotes) carrying recombinant chromosomes give rise to interstitial CNVs in the offspring (Fig. 1).

The contribution of balanced intrachromosomal insertions to the spectrum of chromosomal abnormalities is significant, as the genetic risk is expected to be high and, in theory, could approach 50%, which is of crucial importance for medical genetic counseling [4, 5]. In this context, chromosome 22 — characterized by a high concentration of low-copy repeats (LCRs) — is of particular interest. This genomic architecture predisposes the region to a broad spectrum of balanced and unbalanced chromosomal rearrangements (CRs) mediated by non-allelic homologous recombination (NAHR) [6]. The best-studied examples are the 22q11.2 reciprocal deletion and duplication syndromes, also known as genomic sister disorders [7, 8]. However, non-recurrent copy number variations (CNVs) in chromosome 22 are also of significant value. Such cases, which reveal complex and diverse mechanisms of genomic structural organization — including replication-mediated and multi-step genomic events — may present with multiple regions of chromosomal imbalance [9, 10]. These observations not only expand our understanding of the structural variability of chromosome 22 but are also of practical importance for medical genetic counseling, particularly in selecting strategies for prenatal and preimplantation genetic diagnosis in families with an affected child [11].

In this paper, we present a unique case of a recombinant chromosome 22 in a patient with an abnormal phenotype. The study aimed to characterize, using molecular cytogenetic methods, a rare rec(22) with three interstitial duplications resulting from meiotic crossing over during gametogenesis in the mother, who was a carrier of a complex intrachromosomal rearrangement.

METHODS

Clinical data

The patient was a 7-month-old girl referred to the Research Centre for Medical Genetics because of psychomotor developmental delay and craniofacial dysmorphism. The family history revealed an older child, a 5-year-old girl, who was healthy and had no apparent clinical abnormalities (as reported by the parents). Both parents were healthy and non-consanguineous. The mother's obstetric history included two missed miscarriages (Fig. 2A).

The patient was born at 36 weeks of gestation. The Apgar score was 7/7. Birth weight was 2260 g, body length 45 cm, and head circumference 32 cm. After birth, she was admitted to the neonatal pathology unit with a diagnosis of grade I–II cerebral ischemia and motor dysfunction syndrome. From birth, delayed motor development and bilateral sensorineural hearing loss were noted. At the first examination (age 7 months), the following phenotypic features were observed: orbital hypertelorism, epicanthus inversus, short palpebral fissures, bulbous nasal tip, trapezoid-shaped upper lip, long philtrum, high-arched palate, and long fingers. Anthropometric parameters: body length 68 cm (between the 50th and 75th centiles), body weight 6000 g (below the 3rd centile). Gross motor development was markedly delayed: the patient could hold her head only in the prone position and was unable to roll over. At follow-up at the age of 2 years, she continued to show a pronounced delay in motor development: she could sit unsupported at 1.5 years and began standing and stepping with support at 2 years. Self-care skills were absent, and speech development was mildly delayed — she could pronounce a few simple words.

Molecular cytogenetic studies

To detect CNV, the chromosomal microarray analysis of the DNA sample from the patient's peripheral blood was conducted using the CytoScan HD Array oligonucleotide microarrays (Affymetrix, Santa Clara, USA) in accordance with the manufacturer's protocols. The analysis of CNVs was performed using the Chromosome Analysis Suite (ChAS) ver.

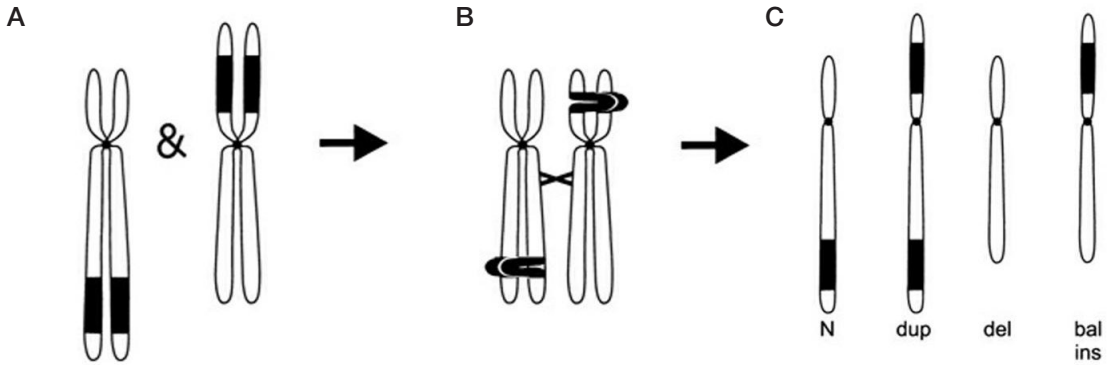

Fig. 1. Gamete production following recombination between the sites of rearrangement in a between-arm intrachromosomal insertion. **A**. Normal chromosome (left) and chromosome with insertion (right); **B**. Crossover event; **C.** Types of gametes. (Adapted from Gardner R. J., Amor D. J., 2018.)

Table. Nucleotide sequences of the primers used in the study

Name	DNA primer sequences, 5'-3'	PCR product length (bp)	Genomic coordinates (hg19)
dup22q12.3_1	F:GTGGGGTGGAAATAGAGGAGGAAAAGTG	9118	chr22:36,911,962–36,938,387
	R: ACACAATAACGCAGAGAGTGAAATGGGT		
dup22q12.3_2	F: ACCCATTTCACTCTCTGCGTTATTGTGT	9818	
	R: CCAGCTTCATCTCATTCCTCTCTTGTCC		
dup22q12.3_3	F: CACTCTTGCTGCTCTAGGGTTTCTTCTC	9964	
	R: ATGGGAATCTATTTTGTCTCCTGTCGCC		
22q13_1	F: GTCTCCCCCTCAAAAATGCTGGTGATAA	9874	chr22:41,525,966–41,555,314
	R: CAAGTAGCCTTCAGAGTTCCATCTGCTC		
22q13_2	F: AAGACAGACGGATGGAAAACCTAGTTGC	9002	
	R: GACATACTTGAGACACTGGAGCTTGACC		
22q13_3	F: AACTCTCCTATAATGCCTCCAGGGTCTC	9520	
	R: GCAACACACAAGTTCAGCAAAAACCAAC		
22q-invG-1	F: GTTCAAAGCCCCCACAGTCTTCCCAATG	9890	chr22:24,426,053–24,452,928
	R: AGGAGGAGGTCACAAGTCCCATACCACT		
22q-invG-2	F: AGTGGTATGGGACTTGTGACCTCCTCCT	9643	
	R: ACATGCTGGCGGGGAAAGAGACAGTTTA		
22q-invG-3	F: GAGTAGGGAGGGATGCTGCTGGGTTAAG	9182	
	R:GAGGAGACCAGAGAAGAGGGTGGCAATG		
22q-invR-1	F: GTGGAGAGGAGAGTGTGAATAGGGAAGT	9068	chr22:34,120,020–34,162,291
	R: GGTTGTTGCGAGATGAATGAAGCCAAAT		
22q-invR-2	F: CACCCCACATTCCTGAAGATGACACTAC	9095	
	R: TGAGTGAGTGATCGCCTCCTTTATGAGA		
22q-invR-3	F: AAACCTCTACCTCCAAAACCTATCCCCA	9259	
	R: TCCCACATTCTCTCCATCCTCTTCTTGT		

4.0 software (Thermo Fisher Scientific Inc.; USA), and the results were interpreted in accordance with the International System for Human Cytogenomic Nomenclature (ISCN, 2020). The CNV identified was matched to the data published in the scientific literature and the information from the publicly available databases: Database of Genomic Variants (DGV) (http://dgv.tcag.ca/dgv/app/home), DECIPHER (http://decipher.sanger.ac.uk/), and OMIM (http://www.ncbi.nlm.nih.gov/omim). Genomic coordinates are provided in accordance with the Human Genome February 2009 assembly (GRCh37/hg19). Clinical significance of variants was assessed in accordance with the standards of the American College of Medical Genetics and Genomics (ACMG) [12].

Fluorescence *in situ* hybridization (FISH) on chromosomal preparations from cultured peripheral blood lymphocytes was performed using commercially available locus-specific (22q11.2 LSI *TBX1*/22q13 *SHANK3*) and sub-telomere (22qter) DNA probes (Kreatech, Netherlands), as well as a DNA probe specific to the short arms of acrocentric chromosomes (Acro-p) (MetaSystems, Germany), in accordance with the manufacturers' protocols. Denaturation and hybridization were performed using the ThermoBrite hybridization system (StatSpin, USA), and assessment was conducted using the AxioImager M.1 epifluorescence microscope (Carl Zeiss, Germany) and the Isis softwate tool for digital image processing (MetaSystems, Germany).

The development of homemade DNA probes to refine the structure of the patient's recombinant chromosome 22 and assess the parental chromosomes parents represented an important stage of the study. Primers were selected using the Primer-BLAST NCBI software tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and the UCSC Genome Browser database (http://genome.ucsc.edu). The OligoAnalyzer™ Tool (https://eu.idtdna.com/pages/tools/oligoanalyzer/) was used to verify specificity of the selected primers. Primers were synthesized by Evrogen (Russia). The nucleotide sequences of the primers selected are provided in Table.

Sequences of the selected DNA primers were used to conduct LR-PCR using the BioMaster LR HS-PCR (2x) (BiolabMix, Russia) in the GeneAmp PCR System 9700 (Applied Biosystems, USA) in accordance with the manufacturer's protocol. The resulting amplicons were purified on the columns using the diaGene DNA purification kit for DNA isolation from reaction mixtures (Dia-M, Russia) in accordance with the manufacturer's instructions, with subsequent combining of purified PCR products in one test tube aimed at obtaining a DNA probe approximately 30 kb in length. A nick-translation method was used to introduce a fluorescent label into a DNA probe. FISH with homemade DNA probes involved separate denaturation of DNA in the chromosomal preparation and the DNA probe [13–15].

Chromosomes were counterstained with DAPI I (Abbott Molecular, USA) in Vectashield mounting medium (Vector Laboratories, USA) at a 1:20 ratio. Metaphase chromosome images were analyzed using the ISIS digital imaging system (MetaSystems, Germany) integrated with an Axio Imager M1 epifluorescence microscope (Carl Zeiss, Germany).

RESULTS

In the first stage of molecular cytogenetic testing of the patient with motor and speech developmental delay, craniofacial anomalies, and hearing loss, chromosomal microarray analysis (CMA) was performed. The analysis revealed three interstitial micrtoduplications located in the long arm of chromosome 22: arr[hg19] 22q11.21(18037572_21915207)

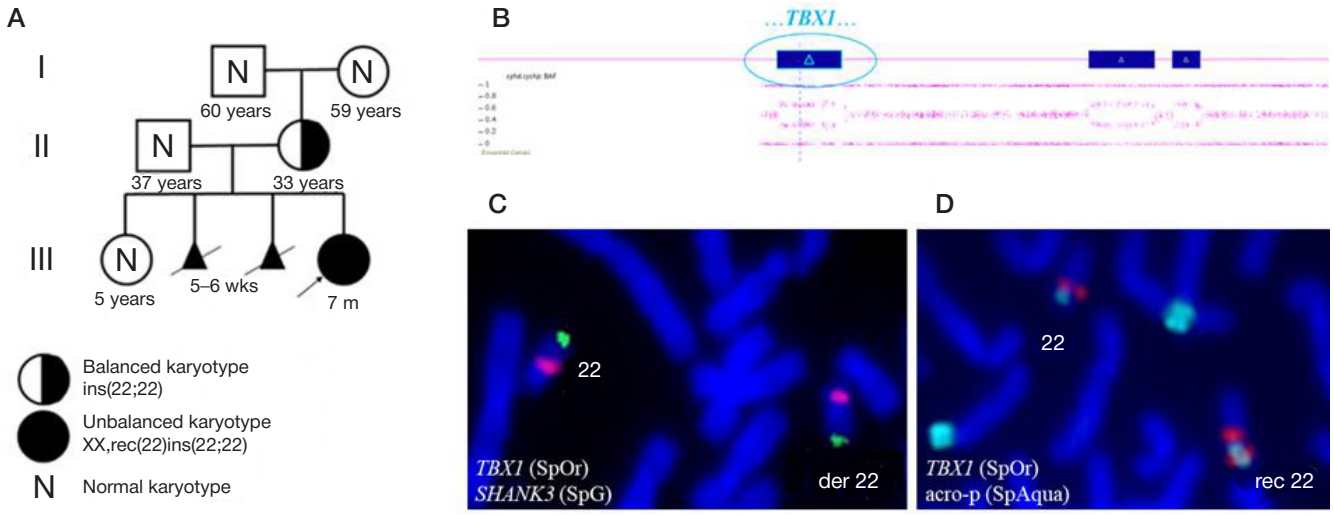

Fig. 2. Familial case of intrachromosomal ins(22;22). **A**. Three-generation pedigree of the family with the *de novo* heterozygous carrier of the intrachromosomal insertion on chromosome 22. **B**. CMA hybridization profile of chromosome 22 demonstrating three duplications in the patient. **C**. FISH results for the patient's mother showing hybridization with the DNA probe for the 22q11.2 region (*TBX1*, SpOrange) and the control probe for SHANK3 (SpGreen), which is not involved in the rearrangement. **D**. FISH results for the patient showing the recombinant chromosome 22, in which the duplicated fragment containing *TBX1* (SpOrange) is inserted into the nucleolar organizer region (p12) (acro-p, SpAqua).

x3,22q12.3q13.1(36793141_40756125)x3,22q13.2 (41818449_43449759)x3 (Fig. 2B) sized 3.9 Mb, 4 Mb, and 1.6 Mb, respectively.

To verify the data obtained, FISH analysis of the chromosomal preparations of the patient, her sister and parents was conducted that involved the use of the commercially available DNA probe for the 22q11.21 chromosomal locus (*TBX1* gene). It was found that the patient's mother was a carrier of an intrachromosomal between-arm insertion involving the 22q11.2 region inserted into the nucleolar organizer region (p12) (Fig. 2C). Thus, the duplication including the *TBX1* gene resulted from the maternal meiotic crossing over event within the centromeric segment of chromosome 22, which led to the formation of the recombinant chromosome 22 observed in the patient (Fig. 2D). Examination of the maternal grandparents revealed no insertion, indicating that the mother's chromosomal rearrangement occurred *de novo*.

To verify the second and third microduplications, it was hypothesized that there was a "single" duplication in the 22q12.3q13.2 region. For this purpose, homemade locus-specific DNA probes were designed for two regions of chromosome 22: (hg19):36,911,962–36,938,387 and (hg19):41,525,966–41,555,314. The primer design is provided in Table. The FISH analysis showed that the patient's mother carried an intrachromosomal insertion involving the 22q12.3q13.1 region into the nucleolar organizer region (p12) of chromosome 22 (Fig. 3B). Thus, the patient's recombinant chromosome 22 contains both the duplication including the *TBX1* gene (22q11.2) and the 22q12.3q13.1 duplication of maternal origin (Fig. 3C).

Based on the data obtained, we proposed a hypothesis regarding the complex mechanism underlying the chromosomal rearrangement in the patient's mother. A long disomic region of 14.9 Mb was identified between the proband's first (22q11.21) and second (22q12.3) duplications (Fig. 3A). This suggested that the 22q11.21 and 22q12.3q13.1 regions on the maternal chromosome 22 were likely close together due to a paracentric inversion (chr22(hg19):18,037,572–36,793,141). Therefore, both of these regions were inserted together into the nucleolar organizer region (p12) of chromosome 22 as a result of the initial *de novo* rearrangement that occurred during meiosis in one of her parents, whose karyotypes were assessed and turned out to be normal. To verify this hypothesis, two additional homemade locus-specific DNA probes for the 22q11.23 and 22q12.3 regions were designed (chr22(hg19):24,426,053–24,452,928 and chr22(hg19):34,120,020–34,162,291 (Table). The schematic representation of the complex chromosomal rearrangement is shown in Fig. 4.

The comprehensive molecular cytogenetic analysis involving the use of both commercially available and homemade DNA probes confirmed the hypothesis about the complex mechanism underlying the maternal chromosomal rearrangement. It was shown that the patient's recombinant chromosome 22 with two microduplications resulted from two consecutive events that occurred during the maternal meiosis: an initial paracentric inversion in 22q11.21q12.3 and the subsequent between-arm insertion of the regions 22q11.21 and 22q12.3q13.1 into the nucleolar organizer region 22p12. In this study, we did not assess the third duplication; further analysis is planned to clarify the complete mechanism underlying the formation of the complex CR in the patient's mother.

Thus, combining CMA and FISH with both the commercially available and homemade DNA probes made it possible to visualize the mechanism underlying the development of chromosomal imbalance. According to the family's three-generation pedigree, the estimated empirical risk of giving birth to a child with a chromosomal/genomic imbalance or spontaneous abortion is 75%, which is considered a high genetic risk (Fig. 2A).

DISCUSSION

The present case demonstrates a rare instance of recombinant chromosome 22 formation resulting from the meiotic segregation of a complex maternal intrachromosomal rearrangement, specifically a combination of inversion and insertion. Similar mechanisms have been previously described in studies on rec(22) derived from maternal intrachromosomal rearrangements. For example, several rec(22) cases have been reported in which such maternal rearrangements led to duplications of distal 22q regions in the offspring [16, 17]. One report describes a female patient with a 7 Mb duplication in 22q13.1q13.2, which was subsequently identified as a meiotic segregation product of a maternal insertion [18].

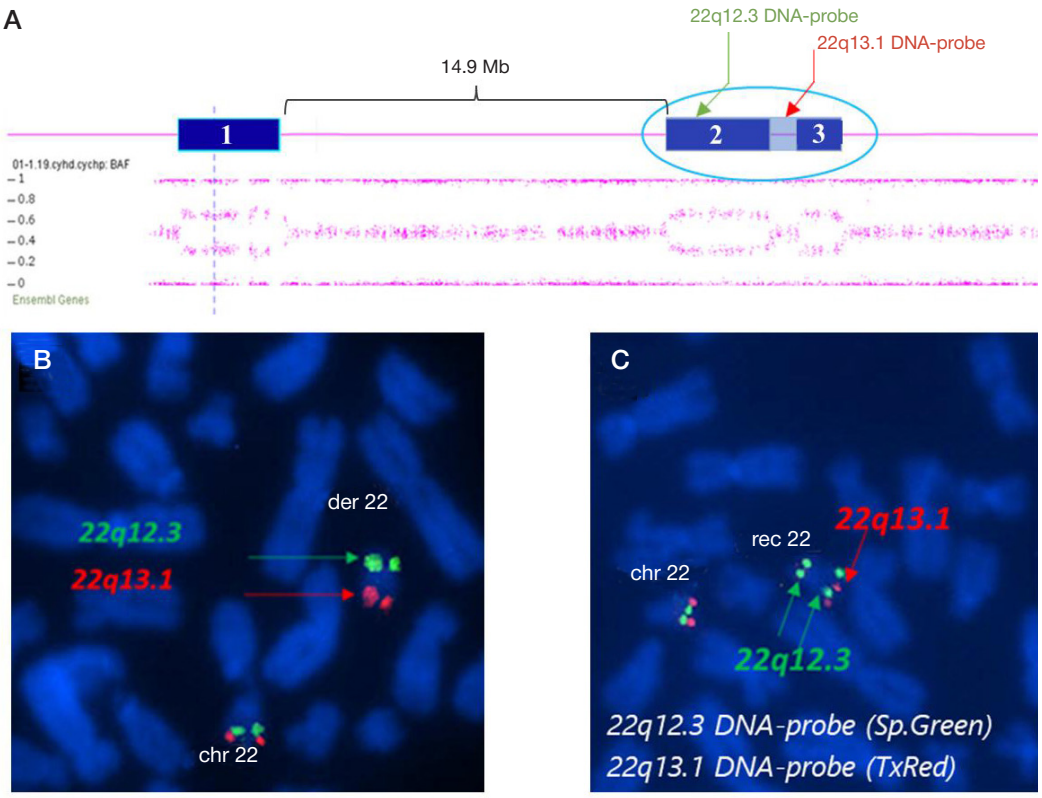

Fig. 3. FISH analysis with homemade DNA probes disproving the hypothesis of a "single" duplication. **A**. CMA hybridization profile of chromosome 22 in the patient and schematic representation of the genomic localization of the homemade DNA probes designed to test the "single duplication" hypothesis. **B**. FISH results with homemade DNA probes on maternal metaphase chromosomes, showing that the derivative chromosome 22 resulted from insertion of the 22q12.3 fragment into the nucleolar organizer region (p12). The 22q13.1 region is not involved in the rearrangement. **C**. FISH results with homemade DNA probes on the patient's metaphase chromosomes, showing that the recombinant chromosome 22 contains an insertion of the duplicated 22q12.3 fragment into the nucleolar organizer region (p12). The 22q13.1 region is not duplicated and is represented by a single copy on each homologue

Our case extends these observations by demonstrating that a recombinant chromosome 22 containing multiple CNVs can arise from the inheritance of a complex maternal intrachromosomal between-arm insertion combined with an inversion.

The acrocentric chromosome 22 is one of the shortest human chromosomes and contains the highest density of low-copy repeats (LCRs) within its long arm. LCRs are DNA blocks ranging in size from 10 to 400 kb that share a high degree (95–97%) of nucleotide sequence identity. This homology facilitates non-allelic homologous recombination (NAHR), the mechanism underlying typical 22q11.2 deletions and duplications [6, 19]. Optical genome mapping has confirmed a high degree of LCR22 variability in the population, which increases the likelihood of non-recurrent and complex chromosomal rearrangements [20]. At the same time, more complex, multi-stage events like the one we report here are thought to be explained by other replication-mediated

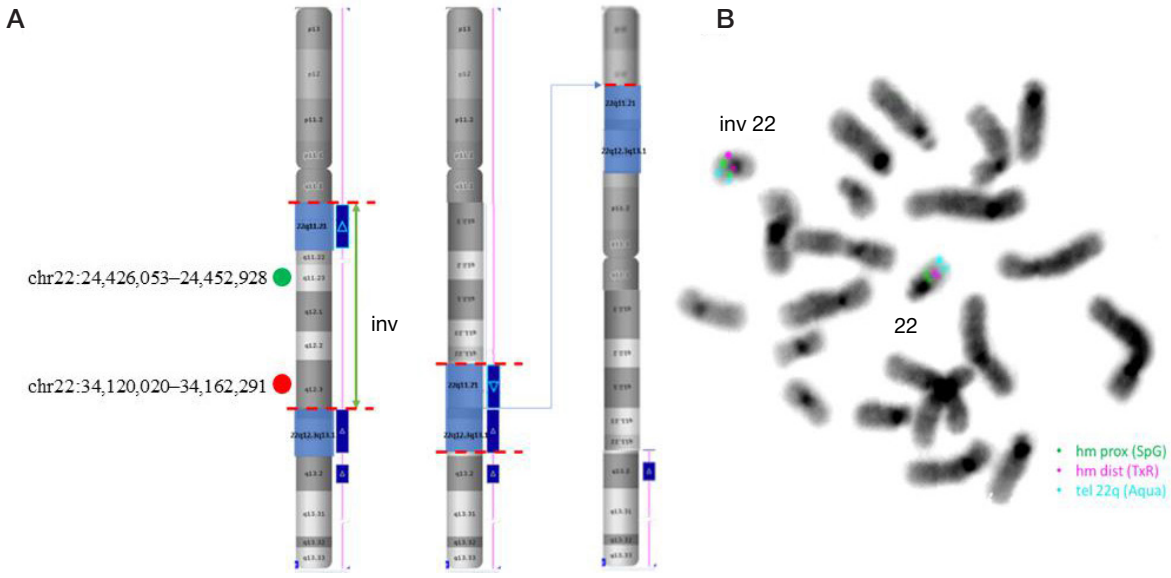

Fig. 4. A. Schematic representation of the mechanism underlying formation of the complex maternal *de novo* CR. **B**. Results of FISH analysis using the distal homemade DNA probe (TxRed), the proximal DNA probe (SpGreen) for the inversion region (chr22(hg19):18,037,572–36,793,141) and the commercially available DNA probe for the subtelomeric region of the long arm of chromosome 22 (SpAqua)

mechanisms, specifically Fork Stalling and Template Switching (FoSTeS) and Microhomology-Mediated Break-Induced Replication (MMBIR) [3, 9, 10, 21].

In this case, we identified a complex intrachromosomal rearrangement in the mother, consisting of a paracentric inversion in 22q11.21q12.3 (chr22(hg19):18,037,572–36,793,141), followed by a between-arm intrachromosomal insertion of the 22q11.21 and 22q12.3q13.1 regions into the nucleolar organizer region at 22p12. The proximal inversion breakpoint is located within LCR22 A–B (~18–21 Mb), a well-known genomic instability "hot spot" that results in NAHR-mediated chromosomal rearrangements. According to the literature, inversions spanning the LCR22A–B or LCR22B–D/C blocks can initiate non-recurrent chromosomal rearrangements [6, 22]. The distal inversion breakpoint on the long arm (~36.8 Mb) lies outside the classic LCR22 boundaries in a region of unique sequences at 22q12.3, making the involvement of replication-mediated mechanisms (FoSTeS/MMBIR) more likely [21–23]. The insertion of a large 22q segment into the short arm (22p) is also consistent with known genomic architecture. The p-arms of acrocentric chromosomes, which contain the nucleolar organizer regions (NORs) and multiple repetitive blocks, represent highly dynamic genomic regions [24]. Therefore, the combination of an LCR22-associated inversion and an insertion into the 22p NOR creates a unique structural background predisposing to multiple duplications during meiotic segregation.

Our case expands the spectrum of previously reported rec(22) cases and demonstrates that the presence of a complex maternal CR can lead to multiple interstitial duplications in the offspring. This mechanism integrates two key factors: the variable genome architecture resulting from the abundance of low-copy repeats on chromosome 22, and the dynamic nature of the ribosomal repeat arrays within the nucleolar organizer region on its short arm.

The clinical manifestations in our patient, who carries multiple CNVs on chromosome 22, include motor and speech developmental delay, craniofacial dysmorphism, hypotonia, and hearing loss. These clinical features align with the phenotypic spectrum of 22q11.2 duplication syndrome, which is characterized by developmental delay, behavioral disorders, hearing loss, craniofacial anomalies, and variable degrees of cognitive impairment. The phenotypic abnormalities associated with 22q11.2 duplication are highly heterogeneous. This variability is thought to be associated with dose-dependent effects of key genes such as *TBX1*, as well as the influence of additional duplicated segments in the 22q12–q13 region, which may contribute to neurodevelopment and hearing function [8, 25, 26].

In terms of methods, this case emphasizes the need for a comprehensive cytogenomic approach. Standard karyotyping cannot detect CNVs smaller than 10 Mb; therefore, CMA and FISH were pivotal in this study. CMA revealed three interstitial microduplications but did not clarify the mechanism underlying their formation. Only FISH, using both commercially available and homemade DNA probes, enabled us to determine the topology of the chromosomal rearrangement and demonstrate that two of the three duplications resulted from the abnormal meiotic segregation of a complex maternal intrachromosomal rearrangement. It is important to emphasize that the use of homemade locus-specific DNA probes, obtained by long-range PCR, was critical: this allowed us to distinguish between the hypothesis of a "single" duplication and the presence of two distinct duplications, and to confirm a paracentric inversion in the long arm of chromosome 22 as the key starting point for the formation of the complex chromosomal rearrangement in the mother.

Thus, the applied cytogenomic strategy—integrating CMA and FISH with both commercial and homemade DNA probes—enabled us to determine the origin and mechanism of formation, and to reconstruct the complex intrachromosomal rearrangement in a patient presenting with motor and speech developmental delay and dysmorphic features. These findings also indicate a high genetic risk for the family to have another child with a chromosomal abnormality.

CONCLUSIONS

This study describes a rare case of multiple interstitial duplications on chromosome 22 resulting from meiotic segregation of a complex intrachromosomal rearrangement that arose *de novo* in the proband's mother. Our findings show that a combination of a paracentric inversion and subsequent insertion events can generate recombinant chromosomes harboring multiple CNVs, leading to a clinically significant chromosomal imbalance.

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