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CASE REPORT

# Double deletion of a chromosome 21 inserted in a chromosome 22 in an azoospermic patient

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## Key Clinical Message

We report on a phenotypically normal 41-year-old azoospermic man with a 45 chromosomes karyotype including one normal chromosome 21, one normal chromosome 22, and a der(22)ins(22;21). Array CGH showed a 1.8 Mb terminal deletion of bands 21pter to 21q21.1 and a 341 kb terminal deletion on band 21q22.3.

## Keywords

Azoospermia, chromosome 21, chromosome 22, deletion, chromosome, insertion, karyotype, partial monosomy 21.

## Introduction

Several 21q partial monosomies have been reported in the literature, presenting phenotypes with highly variable severity and features, depending on size, position of the deletion, and associated chromosomal rearrangement. Some authors have delineated critical regions along the long arm of chromosome 21 according to the severity of the phenotype associated with the deletion. Many patients with 21q deletion and a very mild phenotype have been described, in particular paracentromeric and subtelomeric 21q deletions [3, 5, 18]. However, to our knowledge only one case of chromosome 21 deletion associated with a chromosome 21 insertion into chromosome 22 has been described to date, in a family whose proband had a partial trisomy 21 and other members were carriers of the recombinant chromosome in a 45 chromosome karyotype [2]. We report here a patient carrying two deletions on the long arm of a chromosome 21 inserted into a chromosome 22, and whose only phenotype was azoospermia.

## Materials and Methods

### Clinical description

Our patient was referred to the geneticist for infertility due to azoospermia. This 41-year-old man has normal intelligence; he is married and has a normal social and professional life. He does not show any particular sign or symptom besides infertility, and notably no sign of hypogonadism. He is the first of three children, born to non-related parents. His brother and sister are both healthy with normal karyotypes. His sister is the mother of two healthy boys. His father died of a stroke at the age of 39 and his mother, who was alcoholic, died at the age of 65. No genetic analysis is available for them. The patient also has a healthy first degree paternal cousin.

### Cytogenetic analysis

Peripheral blood from the patient was prepared according to the standard cytogenetic procedures and metaphases

were analyzed by R-banding and G-banding. A 550-band resolution karyotype was obtained.

### Array-CGH

Array-CGH was performed on a CytoChip ISCA 4x44K v1.0 (BlueGnome Ltd, Cambridge, U.K.) pangenomic oligonucleotide array, according to the standard procedures with a quatuor method (two "color swap" hybridizations with cyanine 3 and 5 staining). BlueFuse MULTI (BlueGnome Ltd) was used for informatic analysis of the results. Medical analysis was performed with Cartagenia (Leuven, Belgique) and results were compared with the data in DGV (Database of Genomic Variants) for benign CNVs (Copy Number Variants), to DECIPHER (Wellcome Trust Sanger Institute, Cambridge, U.K.) and ISCA (The International Standards for Cytogenomic Arrays Consortium) for deleterious CNVs. We used the human genome assembly hg19 (GRCh37) for all molecular analysis (Ensembl Genome Browser).

### Fluorescent in situ hybridization

Fluorescent in situ hybridization (FISH) was performed according to a standard protocol with an overnight hybridization followed by a SSC solution wash. To confirm the karyotype anomalies, D21S1446 and D22S105 subtelomeric probes of chromosomes 21 and 22, respectively (Kreatech, Amsterdam, the Netherlands) were used. Array-CGH anomalies were confirmed and structurally characterized using several probes: wcp (whole chromosome painting) of chromosomes 21 (Spectrum Red) and 22 (Spectrum Green) probes (Metasystems, Altlhusseim Germany), centromeric 13/21 and 14/22 probes (Kreatech Diagnostics), and Bacterial Artificial Chromosomes (BACs) probes mapped on chromosome 21: RP1-126N20 (21q11.2), RP11-482O14 (21q21.1), RP11-25F24 (21q21.2), RP11-71A7 (21q22.3), and RP11-323F14 (21q22.3) (BlueGnome, Illumina, Cambridge, U.K.).

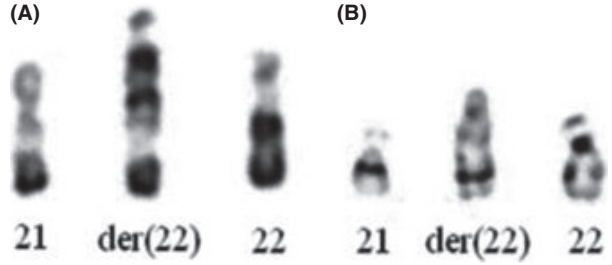


**Figure 1.** R-banding (A) and G-banding (B) partial karyotype of chromosomes 21, 22, and the derivative chromosome 22 due to the insertion of a segment of 21q.

## Results

Fifteen mitoses were analyzed, revealing a 45 chromosome karyotype with one normal chromosome 21, one normal chromosome 22, and a derivative chromosome 22 caused by insertion of a chromosome 21 into a chromosome 22 (Fig. 1).

Array-CGH showed a deletion on each end of chromosome 21 (Fig. 2). The proximal one, localized on 21q11.1q21.1 (15,499,877-17,224,547), is 1.7 Mb long and involves six OMIM genes including one OMIM morbid gene, LIP1. The distal one is a terminal deletion of 341 kb in 21q22.3 (47,797,296-48,090,288), encompassing four OMIM genes. Among them, the only OMIM morbid gene is PCNT. No other CNV on chromosome 22 was highlighted as previously seen on karyotype.

Fluorescent in situ hybridization analysis confirmed the deletions and complete inverted insertion of the remaining segment of the inserted chromosome 21. The other chromosome 21 and 22 are both normal (Fig. 3).

The final karyotype is:

45,XY,-21,der(22)ins(22;21).ish der(22)ins(22;21)(q13.2;q22.3q21.1)(wcp21+,RP11-25F24+,RP11-323F14+)del(21)(pterq21.1)(RP1-126N20-,RP11-482O14-)del(21)(q22.3)(RP11-71A7-)

## Discussion

A few years ago, monosomy 21 was thought to be the only viable autosomic monosomy. However, most of the reported cases turned out to be partial monosomies resulting from cryptic translocations or mosaics [4, 8, 10, 12, 15, 20, 22].

The case we report here shows an unusual rearrangement with a deletion of both ends of chromosome 21: the short arm, the centromere and a 1.7 Mb proximal segment of 21q are deleted, as well as a 341 kb subtelomeric portion of 21q, with only an acentric 21q segment left. This segment is preserved because of its insertion into a chromosome 22, leading to a 45 chromosomes karyotype with an apparent monosomy 21. To our knowledge, Aviv et al. described the only case of a familial complex rearrangement with a der(22)t(21;22) associated with a chromosome 21 pter to q21.2 deletion. The deleted regions on chromosomes 21 and possibly 22 were not precisely specified by array-CGH analysis. The carriers were diagnosed because this family included a child carrying a partial trisomy 21 due to the presence of two normal chromosomes 21 and the derivative chromosome 22. However, the subjects carrying the rearrangement had a more severe phenotype than our patient, including mild developmental delay, poor social adjustment, psychiatric and behavioral problems, without fertility issues. Nevertheless, these phenotypic features

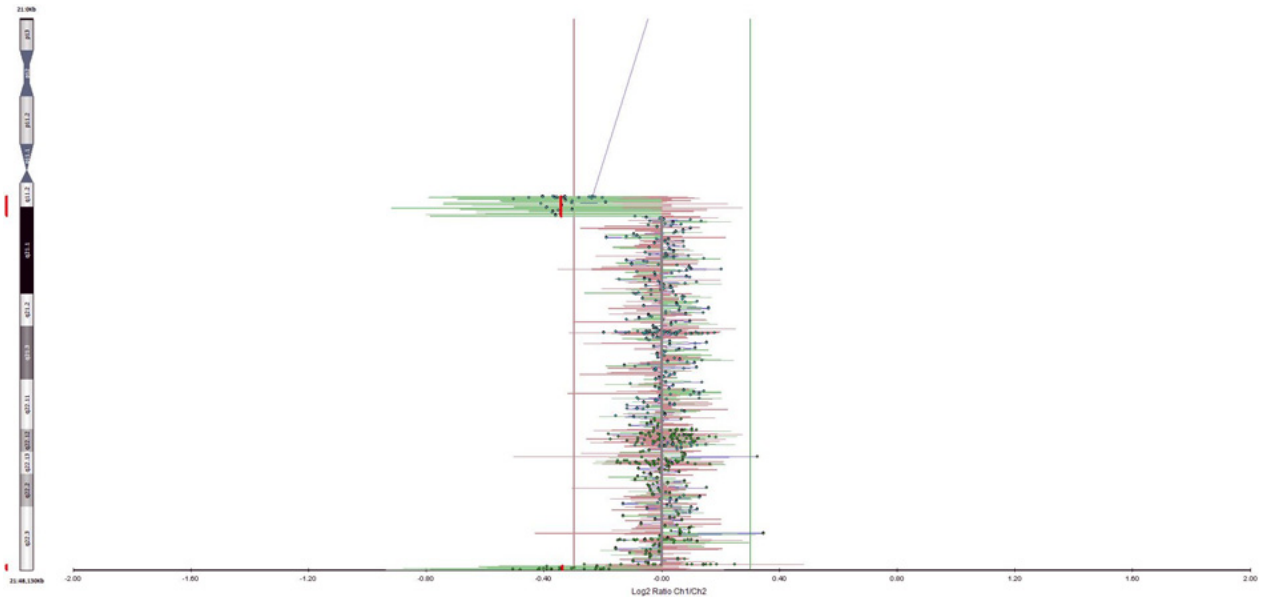


**Figure 2.** Array-CGH showing deletions of the proximal and distal ends of chromosome 21, respectively, 1,7 Mb and 341 kb.

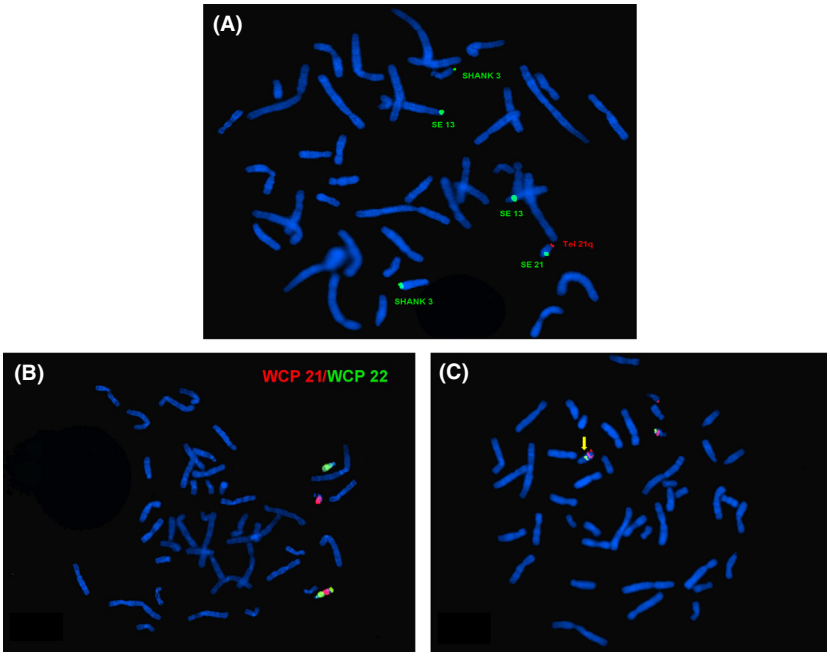


**Figure 3.** (A) D21S1446 (21q telomere), SE13/21 (centromeres of chromosomes 13 and 21), and SHANK3 (22q telomere) FISH probes showing the deletion of a 21q subtelomeric region and the integrity of both 22q subtelomeric regions. (B) Painting wcp 21 (chromosome 21) and wcp 22 (chromosome 22) FISH probes showing the insertion of chromosome 21 into chromosome 22. (c) FISH using a red RP11-25F24 (21q21.2), a green RP11-323F14 (21q22.3) BAC probes, and a red D22S105 probe (22q telomere) showing the inversion of the inserted chromosome 21 segment. Yellow arrow: chromosome 21 inserted in chromosome 22.

may partly be explained by the psychosocial environment.

Chettouh et al. [3] defined five critical regions on the long arm of chromosome 21, responsible for the main clinical signs associated with 21q monosomies. Subsequently, Lyle et al. [13] suggested another model dividing chromosome 21 in 3 critical regions. Deletions from the centromere to 32.3 Mb (to the beginning of band q22.11)

 

are associated with a severe phenotype and deletions from 32.3 to 37.1 Mb (q22.11 to beginning of q22.12) with an even more severe phenotype, whereas deletions from approximately 37.1-38.6 Mb (q22.12) to the telomere are associated with a mild phenotype. This classification remains imprecise especially for the first region, because they considered three patients with large deletions spanning from the centromere to bands 21q21.3 or 21q22.11. But other authors have described patients with a proximal deletion upstream of 21q22 without major phenotypic effect. The familial rearrangement with a 21pter→21q21.2 deletion previously described was revealed only after the birth of a partial 21 trisomy carrier [2]. In the same way, subject GMU [3] showed a 45,XX,-21,der(9)t(9;21)(pter;q21) karyotype with a partial monosomy 21 (pter→q21), without any notable phenotypic impact. Finally, patient 3 from Lindstrand et al. [11] had a 14 Mb interstitial deletion at 21q11.1q21.3 and only showed balance and gross motor difficulties at age 5. In fact, the severity of the phenotype described by Lyle et al. in this proximal centromere-32.3 Mb region is probably caused by the deletion of band 21q22 in two out of three patients, corresponding to the description of "monosomy 21q syndrome" [19]. The latest described patient carrying a 21q22 deletion is a 19-month-old child with a global developmental delay, dysmorphic features, and cardiac and neurological malformations [6]. Deletion of the more distal segment of 21q is the most common 21q deletion and is not responsible for severe phenotypes and intellectual deficiency according to the division of chromosome 21 by Lyle et al. [13], in agreement with other described cases. Two patients were reported with a large 21q22.2q22.3 deletion, respectively, approximately 7.9 and 4.7-7.3 Mb, associated with a phenotype including mild intellectual deficit, a few dysmorphic features, epilepsy, marfanoid habitus and, for the latter, isthmic stenosis of the aorta [5]. Four further cases of 21q terminal deletion also had a minimum size of 4.86 Mb, associated with a mild phenotype including mild intellectual deficit and dysmorphic features without any visceral malformation [17].

Infertile men with a chromosome 21 deletion have been previously described. Alkhalaf et al. [1] reported a 24-year-old man with a 46,XY,del(21)(pter→q11.2) who had hypogonadism and azoospermia. Gekas et al. [7] identified a patient with a 46,XY,r(21) karyotype among a cohort of 2196 infertile men, but the ring 21 was not precisely described. We hypothesize that the same mechanism led to the formation of this ring 21 and of the der (22)ins(22;21) of our patient, with a loss of both telomeric extremities of the 21 chromosome followed by a rearrangement in order to stabilize the deleted chromosome. Our patient's azoospermia could be the consequence of the haploinsufficiency, although none of the deleted genes have been involved in spermatogenesis to date. However, the chromosome rearrangement itself is more likely to be responsible for a chromosome missegregation during meiosis, leading to spermatogenesis failure because of the impossibility of producing balanced gametes. According to McKinlay Gardner and Sutherland [14], a chromosome rearrangement, especially when involving an acrocentric chromosome, might disrupt the integrity of the X-Y bivalent and impair the synapsis of homologous segments in the normal and the rearranged chromosomes, leading to disruption of spermatogenesis.

In spite of this particular chromosomal rearrangement with two deletions on chromosome 21, our patient has a normal phenotype, apart from the azoospermia. As previously discussed, the location of the deletions can account for the normal phenotype. The low number of genes involved is another argument. Only 10 OMIM genes are deleted, and among them only two OMIM morbid genes, *LIP1 (609252)* and *PCNT(605925)*, for which haploinsufficiency is not known to be associated with pathology [16], [21].

In conclusion, we report the first case of a patient carrying a deletion of both extremities of a chromosome 21 and an insertion of this chromosome into a chromosome 22, with no phenotypic consequence other than a secretory azoospermia.

## Conflict of Interest

None declared.

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