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Case Report

Novel karyotypes of partial monosomy 21 and partial monosomy 1 and underlying etiology

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Abstract: We report two novel karyotypes in the siblings of a Chinese family, 45,XY,der(1)t(1;21)(q44;q21)mat,-21 and 45,XX,der(1)t(1;21)(q44;q21)mat,-21. These karyotypes are the result of unbalanced inheritance of a maternal balanced reciprocal translocation 46,XX,t(1;21)(q44;q21). Both patients share a phenotype of intellectual disability, facial malformation, and infertility. The infertility is manifest by: azoospermia in the brother and recurrent spontaneous abortions (RSA) in the sister. Database search revealed recurrent copy number losses associated with these translocated regions. Here we propose that the partial deletion of the gene SMYD3 is responsible for both the intellectual disability in both patients, as well as the azoospermia in the male patient. Altogether, SMYD3 may be an important candidate gene for future research on male fertility.

Keywords: Unbalanced translocation, azoospermia, miscarriage, CNV, FISH, SNP array, SMYD3

Introduction

Reciprocal translocations are exchanges of segments of DNA between non-homologous chromosomes. This is one of the most commonly observed chromosome abnormalities. The prevalence of balanced translocations is approximately 1 in 625 [1]. Usually the carriers of balanced translocations are nonsymptmatic, except in cases where the translocation disrupts certain. However, carriers can produce gametes with an unbalanced combination of the parental rearrangement, resulting in unbalanced translocations in their offspring [2-4]. The resulting chromosomal imbalance may cause a number of anomalies including infertility [4]. In this study, we report two familial cases of unbalanced translocations caused by a maternal balanced reciprocal translocation. These karyotypes have not been previously reported. Although both patients inherited a derivative chromosome, the unbalanced translocation was associated with azoospermia in the male, while the female sibling experienced multiple miscarriages.

Patients and methods

Clinical presentation

Case A: The proband was a 23-year-old male at the time of presentation with abnormal facial features, including a long narrow face, low set ears, long smooth philtrum, high and arched palate, macrognathia, elongated palpebral fissures, broad and large nasal bridge, and hypodontia (**Figure 1A**). Intellectual disability was found (IQ 57). His height was 184 cm and weight 50 kg with long and slender arms and legs. Physical exam revealed normal male external genitalia. Laboratory results demonstrated azoospermia. Endocrine and liver functions were normal. Both cardiac and abdominal ultrasound was normal.

Case B: This is one of the proband's sisters, who was 26-years-old at the time of presentation. She also had intellectual disability (IQ 61) and slightly abnormal facial features, including hypodontia, macrognathia, elongated palpebral fissures, and broad nasal bridge (**Figure 1B**).

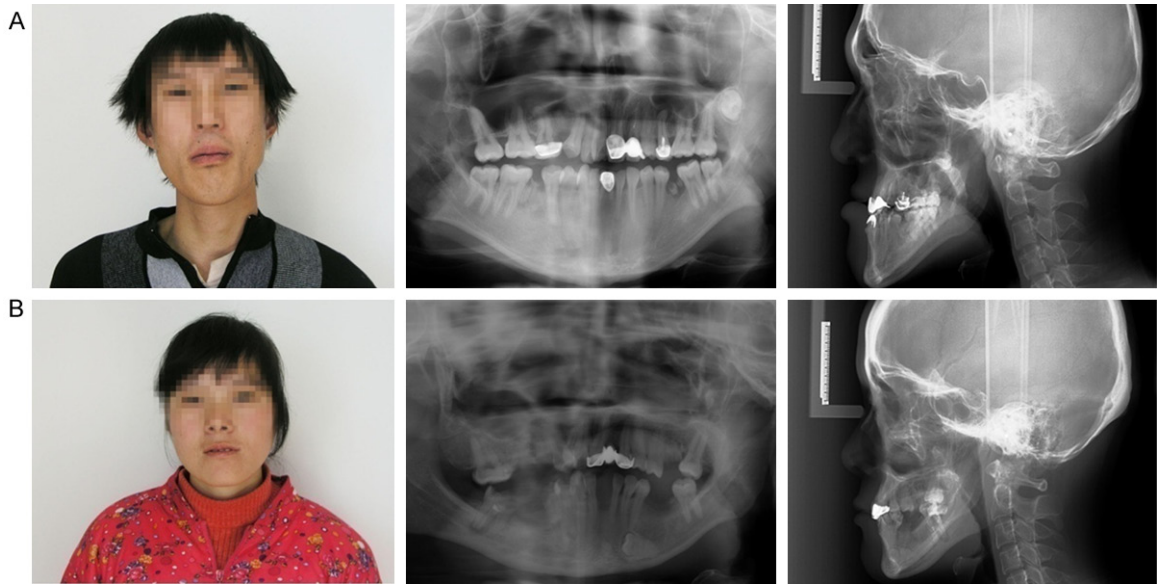

Figure 1. Facial features and dental X-rays of the proband and his affected sister. A. The facial features and dental X-rays of the proband, including the long narrow face, low set ears, long smooth philtrum, high and arched palate, macrognathia, elongated palpebral fissures, broad and large nasal bridge, and hypodontia. B. The facial features and dental X-rays of the proband's affected sister. Note that her abnormal facial appearance is less apparent than the proband's facial features. Hypodontia, macrognathia, elongated palpebral fissures, and a broad nasal bridge can be seen.

Her height was 162 cm and weight 48 kg. Her X-ray revealed scoliosis.

Cytogenetic study

Peripheral blood was collected from all family members, including the proband, his parents, two sisters, and his nephew. Cytogenetic analyses were performed on GTG-banded metaphase spreads prepared from phytohemagglutinine (PHA)-stimulated peripheral blood lymphocytes. Chromosomal analyses were done in 50 metaphases for each sample with a resolution of 320-400 bands. The karyotypes were described in accordance with the international system for human cytogenetic nomenclature (ISCN).

Fluorescent in situ hybridization (FISH) analysis

FISH analysis for chromosomes 1 and 21 were carried out on all of the family samples, using human genomic bacterial artificial chromosomes (BACs) clones spanning 1qter and 21qter. Preparation and labeling of probes and in-situ hybridization were performed according to kit protocol. Fluorescent-labeled probe BAC:RP11-152M6 (31887 bp, red) was used to identify chromosome 1q44 and BAC:RP11-687D14 (162531 bp, green) was used to label chromosome 21q21.1. The fluorescent signals were detected with a Leica fluorescence microscope. At least 10 metaphases per hybridization were analyzed.

Single nucleotide polymorphism (SNP) array analysis

A SNP array was performed to confirm the translocation and to identify the breaking points on chromosome 1 and 21 in these two patients. An Illumina human 610-Quad V1.0 genotyping Beadchip was used in this study, following the manufacturer's instructions. The Human 610-Quad Beadchip was imaged on the Illumina Bead Array Reader and the data was processed with both Illumina Genome Studio v2009.1 and KaryoStudio v.1.0.3 modules. Copy number variations and the size of the aberration were recorded through SNP data analysis.

Analysis of translocation regions with the UCSD genome browser and DECIPHER

To get further insights into understanding of the phenotypic outcome, we analyzed the translo-

Figure 2. Karyotypes of the proband, his affected sister, and their mother. A. The proband's karyotype demonstrates a derivative chromosome 1 and a single chromosome 21, described as 45,XY,der(1)(1q44;q21),-21. B. The sister has the same chromosomal abnormality as the proband, 45,XX,der(1)(1q44;q21),-21. C. Their mother carries a balanced translocation between chromosomes 1 and 21, described as 46,XX,t(1;21)(q44;q11).

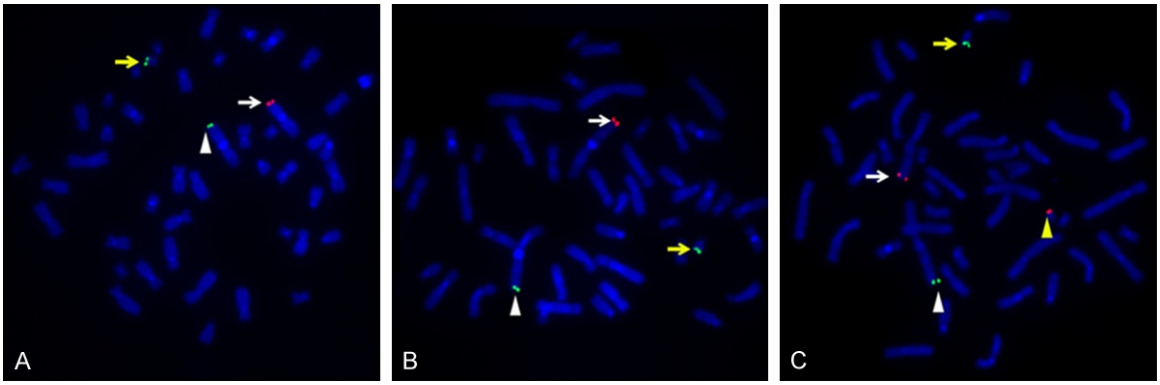

Figure 3. FISH analysis using subtelomeric DNA probes. The termini of the long arms of chromosomes were probed with BAC:RP11-152M6 (Chromosome 1, red) and BAC:RP11-687D14 (Chromosome 21, green). A. The proband's result showed a pair of chromosome 1 that stained green (indicated by the white triangle) and red (white arrow). Only one homologous chromosome 21 was labeled green (yellow arrow). B. The sister has the same abnormal result. C. Their mother carries a balanced translocation: a normal chromosome 1 labeled red (white arrow), a derivative chromosome 1 in green (white triangle), a normal chromosome 21 in green (yellow arrow), and a derivative chromosome 21 in red (yellow triangle).

cation regions using UCSC Genome Browser (https://genome.ucsc.edu/). We chose the human Dec. 2013 (GRCh38/hg38) assembly and retrieved the data of ClinVar variants, OMIM genes and OMIM phenotype loci. To analyze the translocation region on chromosome 1, we used "chr1 245919238 248956422" as the custom track and "chr21 1 20691654" was used as the custom track for the translocation region on chromosome 21. We also searched CNVs on DECIPHER (https://decipher.sanger.ac.uk/). We searched "1:245919238-248956422" and "21:1-20691654" to find the CNVs within the translocation regions on chromosome 1 and 21.

Results

Cytogenetic study

GTG-banded chromosome analysis of 50 cells at 320-400 band resolution revealed abnormal karyotypes in both the proband (**Figure 2A**) and his affected sister (**Figure 2B**). They each have a derivative chromosome 1 and lack a normal chromosome 21, resulting in partial monosomy 1 and partial monosomy 21. The karyotypes were described as 45,XY,der(1)t(1;21)(q44;q21),-21 and 45,XX,der(1)t(1;21)(q44;q21),-21. However, their mother and another sister were both found to carry a balanced reciprocal translocation. The karyotypes of the mother and unaffected sister were described as 46,XX,t(1;21)(q44;q21) (**Figure 2C**). The karyotypes of the father and the nephew did not reveal any abnormalities.

Fluorescent in situ hybridization (FISH) analysis

Subsequent targeted FISH analysis of the proband and his affected sister confirmed the findings of karyotype analysis. They had two

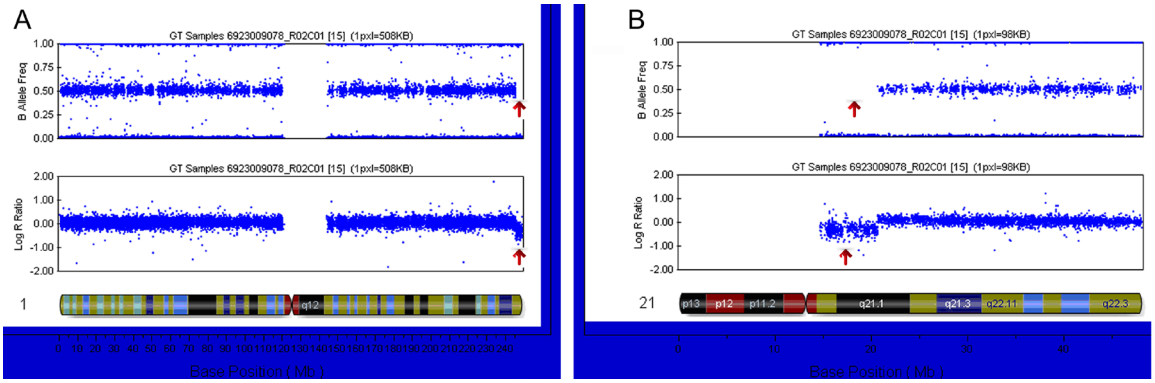

Figure 4. SNP array analysis confirms the derivative chromosomes. A. SNP array results of chromosome 1 showed one copy number loss of 1q44 (bp245,919,238-249,202,755). The lost region is indicated by the red arrows, where B Allele Frequency (BAF) is 0 and the LogR Ratio is decreased. Although the values in the middle of chromosome 1 were missing, it did not interfere with our analysis. B. SNP array analysis of chromosome 21 showed the breakpoint is within the band q21.1 and between 17,531,989-20,691,654 bp. A copy number loss on chromosome 21 was also confirmed by BAF of 0 and decreased LogR Ratio.

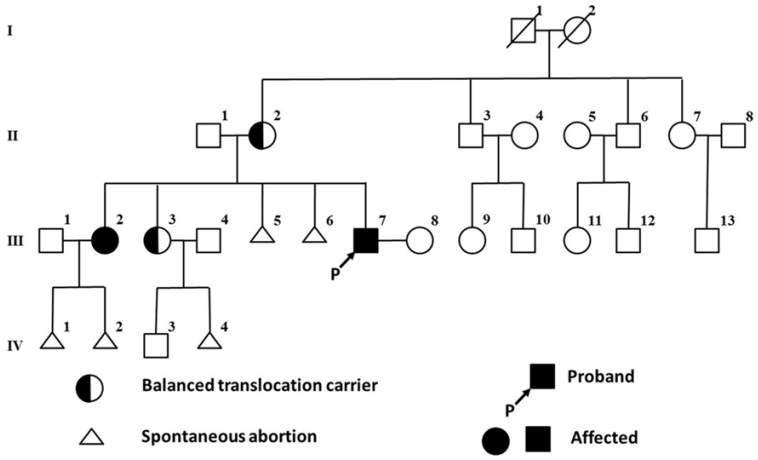

Figure 5. Pedigree of the family. The proband (III7) and his affected sister (III2) are affected. In addition, both of them are infertile. The proband has azoospermia while his sister has experienced recurrent miscarriages. The proband's unaffected sister (III3) and their mother (II2) carry the balanced translocation. Both III3 and II2 are phenotypically normal but have a history of miscarriage. All the other members of the family are normal.

chromosome 1 labels in either green or red, but only one chromosome 21 labeled in green, demonstrating monosomy of chromosome 21 and the presence of a derivative chromosome 1 (**Figure 3A** and **3B**). The FISH analysis of their mother and another sister showed a balanced reciprocal translocation. Two copies of chromosome 1 and 21 were observed, but each pair was labeled by different probes, consistent with balanced reciprocal translocation.

Single nucleotide polymorphism (SNP) array analysis

SNP array analysis showed an approximately 3.28 Mb distal deletion on chromosome 1 in both the proband and his affected sister (**Figure 4A**). The breakpoint on the long arm of chromosome 1 is between 245,919,238-249,202,755 bp. A deletion on chromosome 21 in the pter-21.1 region was also observed (**Figure 4B**). Although the SNP array analysis of the chromosome 21 short arm was uninformative, we were still able to confirm the breakpoint on the long arm of chromosome 21 was between 17,531,989-20,691,654 bp. These findings were consistent with copy number losses in the proband and his affected sister: loss of one copy 1q44(bp245,919,238-249,202,755) →qter and one copy of 21pter→q21.1(17531-989-20691654). These results were consistent with karyotype and FISH analyses.

Analysis of translocation regions with the UCSD Genome Browser and DECIPHER

Both of the fragments are large and gene-rich regions, as shown by the UCSD Browser.

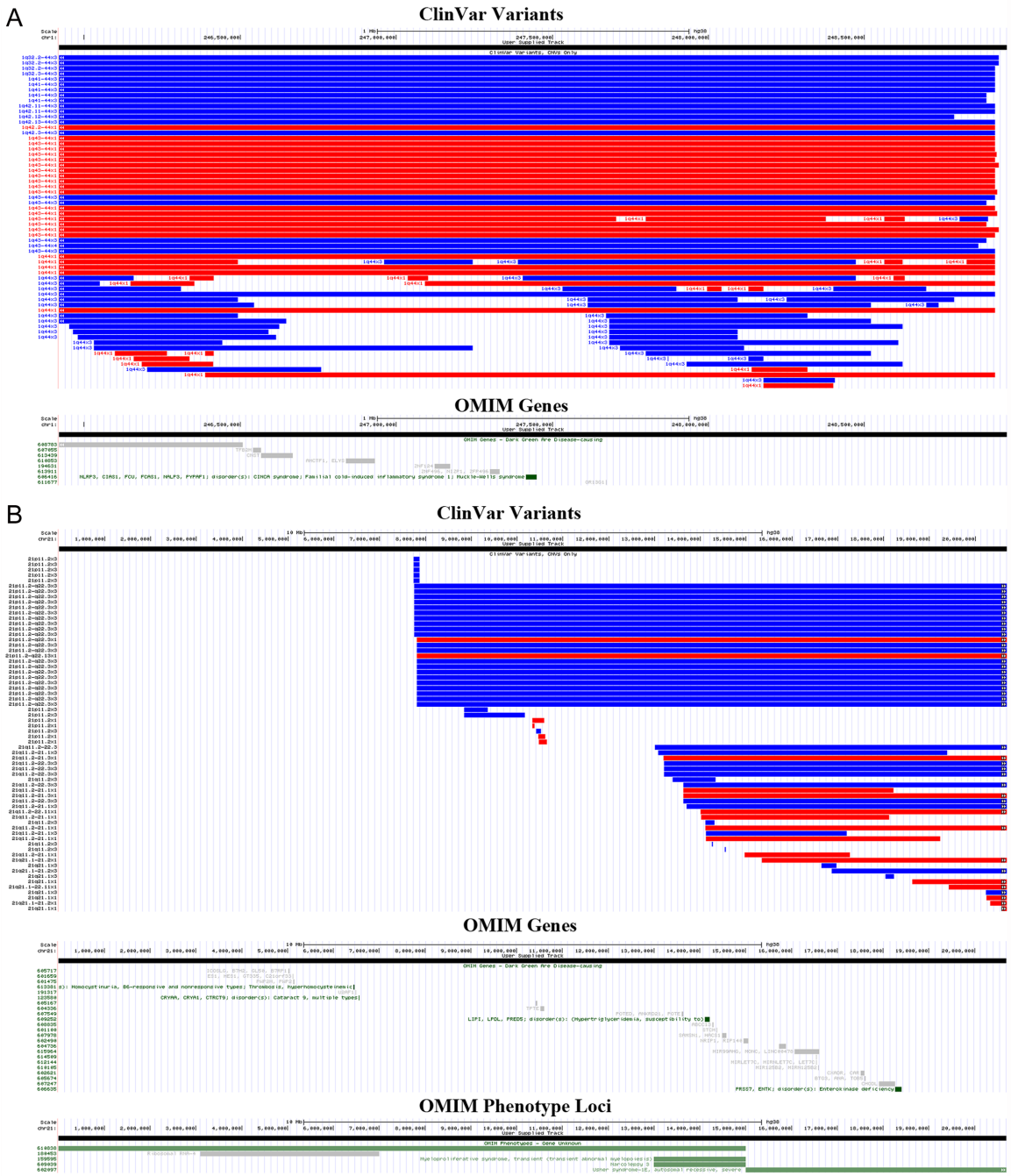

Figure 6. Copy Number Losses Shown by the UCSD Genome Browser. A. Copy number losses and associated OMIM genes within the translocation region on Chromosome 1. B. Copy number losses and associated OMIM genes and OMIM phenotype loci within the translocation region on Chromosome 21.

According to the ClinVar variants track, two pathogenic and three DNA copy number losses of uncertain significance (VOUS) have been reported in 1q44→qter while four pathogenic copy number losses within 21pter→q21.1 have been reported (**Figure 6**; **Table 1**). According to DECIPHER, three VOUSs, three likely pathogenic copy number losses, and one pathogenic copy number loss have been reported within these two regions, after ruling out patients with more than one CNV. The associated phenotype, intellectual disability, is consistent with the clinical presentation of our patients (**Table 2**). OMIM also showed eight disorders associated with these regions (**Figure 6**; **Table 3**).

Table 1. Copy number losses reported by ClinVar

ClinVar ID	Position	Band	Size (Mp)	Clinical significance
RCV000141991	chr1:246147446-246352642	1q44	0.21	VOUS
RCV000141609	chr1:246097511-246265839	1q44	0.17	VOUS
RCV000143307	chr1:246184097-246413863	1q44	0.23	VOUS
RCV000054069	chr1:247092432-248918469	1q44	1.83	Pathogenic
RCV000054068	chr1:246386899-248918469	1q44	2.53	Pathogenic
RCV000141944	chr21:13634136-18211199	21q11.2-21q21.1	4.58	Pathogenic
RCV000052800	chr21:14019847-18125051	21q11.2-21q21.1	4.11	Pathogenic
RCV000052801	chr21:14127526-19238720	21q11.2-21q21.1	5.11	Pathogenic
RCV000052802	chr21:14971617-17270231	21q11.2-21q21.1	2.3	Pathogenic

Note: Benign and likely benign CNVs were excluded.

Table 2. Copy number losses reported by DECIPHER

DECIPHER ID	Position	Band	Size (Mp)	Clinical significance	Phenotypes
282528*	chr1:246428941-246550260	1q44	0.12	VOUS	Intellectual disability, moderate, Postnatal growth retardation
282529*	chr1:246261013-246518362	1q44	0.26	VOUS	Intellectual disability, moderate
284767*	chr1:246027111-246201500	1q44	0.17	Likely pathogenic	Intellectual disability, moderate
299859*	chr1:246261013-246518362	1q44	0.26	Likely pathogenic	Behavioral/Psychiatric Abnormality, Cognitive impairment
277597	chr21:18894835-20311763	21q11.2-21q21.1	1.42	Pathogenic	Delayed speech and language development, Mild conductive hearing impairment, Receptive language delay
289281	chr21:18339940-18882441	21q11.2-21q21.1	0.54	Likely pathogenic	Global developmental delay
260943	chr21:15412471-20329123	21q11.2-21q21.1	4.92	VOUS	

*Copy number losses within SMYD3 gene. Note: Benign and likely benign CNVs were excluded. Patients with more than one CNV were also excluded.

Table 3. Disorders on OMIM associated with the translocated regions

OMIM ID	Disorders	Inherited pattern	Genes	Locus
607115	CINCA syndrome	Autosomal dominant	NLRP3	1q44
120100	Familial Cold Autoinflammatory Syndrome 1	Autosomal dominant	NLRP3	1q44
191900	Muckle-Wells syndrome	Autosomal dominant	NLRP3	1q44
226200	Enterokinase deficiency	Autosomal recessive	PRSS7	21q21.1
610838	Susceptibilityto Autism	Autosomal dominant	AUTS12	21p13-q11
159595	Myeloproliferative syndrome, transient		MST	21q11.2
609039	Narcolepsy 3		NRCLP3	21q11.2
612347	Jervell and Lange-Nielsen Syndrome	Autosomal dominant	KCNE1	21q22

Discussion

It is commonly known that balanced reciprocal translocations lead to a higher incidence of abortion and malformed children [5]. In this report, the mother of the proband is a carrier of a balanced translocation t(1;21)(q44;q21) and, according to the family pedigree (**Figure 5**), has experienced two miscarriages. The unaffected sister, who also carries the same balanced translocation, has a history of spontaneous abortion as well. Both the mother and unaffected daughter are otherwise phenotypically normal. However, the proband and his affected sister inherited an unbalanced translocation, in which they each inherited the derivative chromosome 1 from their mother and lost the maternal chromosome 21, resulting in partial monosomy 1 and partial monosomy 21. The underlying cause was likely 3:1 segregation of chromosomes during maternal meiosis I [6]. The two pairs of homologous chromosomes

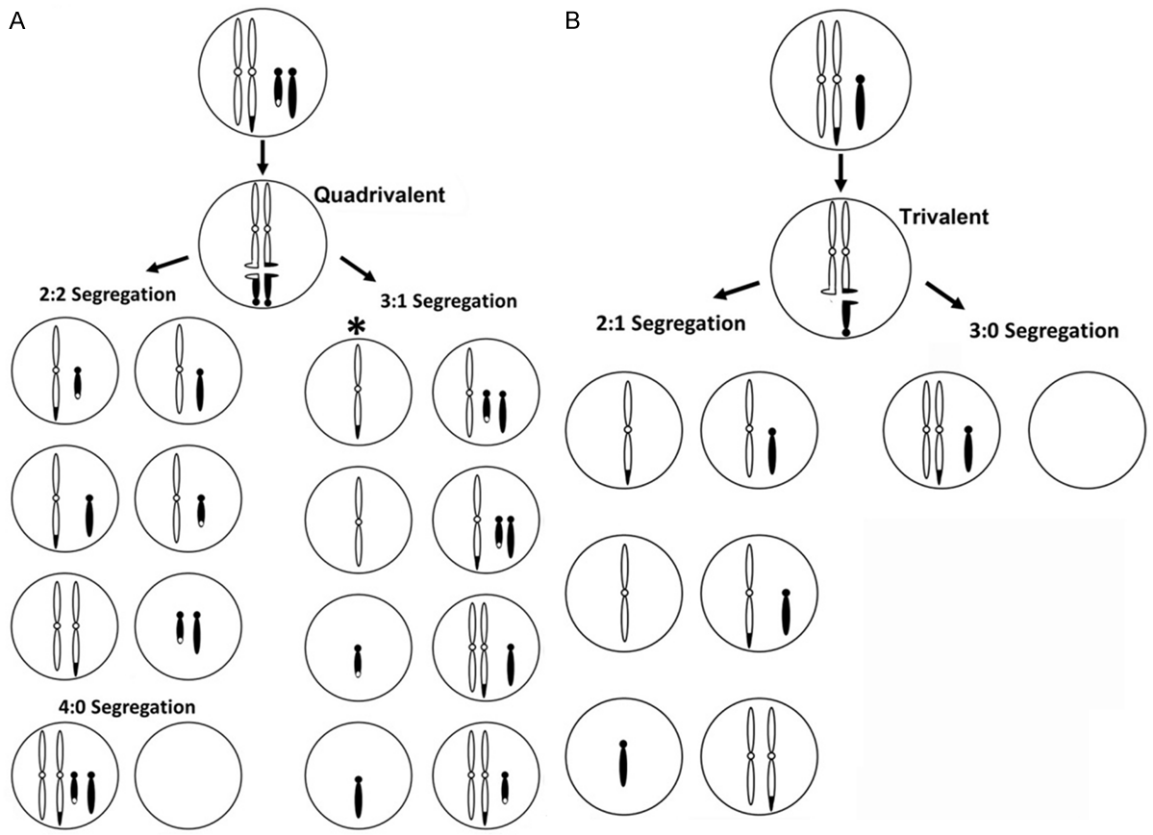

Figure 7. Schematic diagram of the reproductive consequences of the mother and the affected sister. A. With a balanced translocation, a quadrivalent chromosome group is formed during maternal meiosis. The haploidy inherited by the proband and his affected sister is marked with an asterisk (*). B. A trivalent chromosome group is formed during the meiosis of the affected sister, theoretically resulting in 1 out of every 8 oocytes containing a typical distribution of genetic information.

involved in the balanced translocation form a quadrivalent in a reciprocal translocation synapse during meiosis I (**Figure 7A**) [7]. From this point, there are three broad modes of chromosome segregation from a quadrivalent: 2:2, 3:1, and 4:0 [7]. In our case, the mother had a 3:1 segregation, which yielded a gamete containing the derivative chromosome 1 but no chromosome 21, which led to aneuploidy in the son and the daughter after fertilization.

To further understand the etiology in this case, we searched the DECIPHER GRCh37 database [8] and other annotation databases using the UCSC genome browser for human Dec. 2013 (GRCh38/hg38) assembly [9]. The proband has a 3.28 Mb deletion in the region 1q44→qter (245919238-248956422 bp) and a 20.7 Mb deletion in the region 21pter→q21.1 (1753-1989-20691654 bp). Both of the fragments are large and gene-rich regions. According to the ClinVar database, two pathogenic and three DNA copy number losses of VOUS were reported in 1q44→qter while four pathogenic copy number losses within 21pter→q21.1 were found. According to DECIPHER, three VOUSs, three likely pathogenic copy number losses, and one pathogenic copy number losses were reported within these two regions after ruling out patients with more than one CNV. The associated phenotype, intellectual disability, is consistent with our patients' clinical presentations. Recurrent pathogenic CNVs suggest the copy number losses in these patients are also pathogenic. Although OMIM also indicated eight disorders associated with these regions, none of them is consistent with our patients' phenotypes. Interestingly, the likely pathogenic copy number loss (DECIPHER ID: 284767), which is associated with moderate intellectual disability and contains only one gene (SMYD3; SET and MYND domain-containing protein 3), is the best

fit in our patients' presentation. SMYD3 was mapped to chromosome 1: 245,912,642-246,670,614 (SHGC-76633), partially overlapping the fragment 1q44→qter (245919238-248956422 bp). As a result, the translocation in our patients disrupted this gene. SMYD3 is a histone methyltransferase that regulates cell proliferation, immune response, and embryonic development [10-12]. SMYD3 also plays an important role in carcinogenesis and metastasis through multiple signaling pathways, including androgen receptor transcription and the MAPK pathway [13, 14]. Therefore, we searched for SMYD3 variants using the UCSD Browser. While there were no pathogenic variants retrieved from ClinVar and OMIM (data not shown), the DECIPHER database contained four cases of copy number losses within SMYD3. All of them demonstrated a phenotype of moderate intellectual disability (**Table 2**). This result suggests that SMYD3 is likely, at least partially, responsible for our patients' intellectual phenotype. SMYD3 has also been shown to be involved in oocyte maturation and spermatogenesis [11, 15, 16]. However, in our patients, the partial monosomy 1 and 21 led to azoospermia and recurrent spontaneous abortions, suggesting that this chromosomal abnormality had different effects in our patients.In the male, the CNVs probably interfered with spermatogenesis, with haploinsufficiency of SMYD3 emerging as a potential etiology for azoospermia. However, the spontaneous abortions experienced by the female were more likely caused by abnormal meiotic segregation due to the unbalanced translocation, rather than pathologic oocyte maturation (**Figure 7B**) [17]. The chromosome 1 pair and the single chromosome 21 form a trivalent during meiosis. The subsequent 2:1 or 3:0 segregation produces eight types of gametes, and only one of them has normal haploidy. Theoretically, the affected sister therefor has only a 1/8 chance to produce normal oocytes.

Conclusion

In this study, we have reported two novel karyotypes of partial monosomy 1 and 21,45, XY,der(1)t(1;21)(q44;q21),-21 and 45,XX,der(1)t(1;21)(q44;q21),-21,which resulted in intellectual disability, facial malformation, and infertility. However, the infertility was due to azoospermia in the male patient and recurrent spontaneous abortions in thefemale patient, suggesting different etiologies. Database analysis combined with our genetic findings lead us to propose that in addition to resulting in intellectual disability, the deletion of SMYD3 may be an important candidate gene for future research on male infertility.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[1] Van Dyke DL, Weiss L, Roberson JR and Babu VR. The frequency and mutation rate of balanced autosomal rearrangements in man estimated from prenatal genetic studies for advanced maternal age. Am J Hum Genet 1983; 35: 301-308.

[2] Egozcue J, Marina S and Templado C. Meiotic behaviour of two human reciprocal translocations. J Med Genet 1981; 18: 362-365.

[3] Rothlisberger B, Kotzot D, Brecevic L, Koehler M, Balmer D, Binkert F and Schinzel A. Recombinant balanced and unbalanced translocations as a consequence of a balanced complex chromosomal rearrangement involving eight breakpoints in four chromosomes. Eur J Hum Genet 1999; 7: 873-883.

[4] Morel F, Douet-Guilbert N, Le Bris MJ, Herry A, Amice V, Amice J and De Braekeleer M. Meiotic segregation of translocations during male gametogenesis. Int J Androl 2004; 27: 200-212.

[5] Nielsen J and Rasmussen K. Autosomal reciprocal translocations and 13/14 translocations: a population study. Clin Genet 1976; 10: 161-177.

[6] Penehaszadeh VB and Coco R. Partial 9 trisomy by 3:1 segregation of balanced maternal translocation (7q+; 9q-). J Med Genet 1975; 12: 301-305.

[7] Scriven PN, Handyside AH and Ogilvie CM. Chromosome translocations: segregation modes and strategies for preimplantation genetic diagnosis. Prenat Diagn 1998; 18: 1437-1449.
[8] Firth HV, Richards SM, Bevan AP, Clayton S, Corpas M, Rajan D, Van Vooren S, Moreau Y, Pettett RM and Carter NP. DECIPHER: database of chromosomal imbalance and phenotype in humans using ensembl resources. Am J Hum Genet 2009; 84: 524-533.
[9] Zweig AS, Karolchik D, Kuhn RM, Haussler D and Kent WJ. UCSC genome browser tutorial. Genomics 2008; 92: 75-84.
[10] Hamamoto R, Furukawa Y, Morita M, Iimura Y, Silva FP, Li M, Yagyu R and Nakamura Y. SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells. Nat Cell Biol 2004; 6: 731-740.
[11] Suzuki S, Nozawa Y, Tsukamoto S, Kaneko T, Imai H and Minami N. Histone methyltransferase Smyd3 regulates early embryonic lineage commitment in the mouse. Reproduction 2015; 150: 21-30.
[12] de Almeida Nagata DE, Ting HA, Cavassani KA, Schaller MA, Mukherjee S, Ptaschinski C, Kunkel SL and Lukacs NW. Epigenetic control of Foxp3 by SMYD3 H3K4 histone methyltransferase controls iTreg development and regulates pathogenic T-cell responses during pulmonary viral infection. Mucosal Immunol 2015; 8: 1131-43.
[13] Liu C, Wang C, Wang K, Liu L, Shen Q, Yan K, Sun X, Chen J, Liu J, Ren H, Liu H, Xu Z, Hu S, Xu D and Fan Y. SMYD3 as an oncogenic driver in prostate cancer by stimulation of androgen receptor transcription. J Natl Cancer Inst 2013; 105: 1719-1728.
[14] Mazur PK, Reynoird N, Khatri P, Jansen PW, Wilkinson AW, Liu S, Barbash O, Van Aller GS, Huddleston M, Dhanak D, Tummino PJ, Kruger RG, Garcia BA, Butte AJ, Vermeulen M, Sage J and Gozani O. SMYD3 links lysine methylation of MAP3K2 to Ras-driven cancer. Nature 2014; 510: 283-287.
[15] Bai H, Li Y, Gao H, Dong Y, Han P and Yu H. Histone methyltransferase SMYD3 regulates the expression of transcriptional factors during bovine oocyte maturation and early embryonic development. Cytotechnology 2016; 68: 849-59.
[16] Zhou Z, Ren X, Huang X, Lu L, Xu M, Yin L, Li J and Sha J. SMYD3-NY, a novel SMYD3 mRNA transcript variant, may have a role in human spermatogenesis. Ann Clin Lab Sci 2005; 35: 270-277.
[17] Wallace BM and Hulten MA. Triple chromosome synapsis in oocytes from a human foetus with trisomy 21. Ann Hum Genet 1983; 47: 271-276.