Short Communication

A patient with Down syndrome with a *de novo* derivative chromosome 21

Zafer Cetin [a], Sezin Yakut [a], Ercan Mihci [b], Ayse Esra Manguoglu [a], Sibel Berker [a], Ibrahim Keser [a], Guven Luleci [a],*

[a] *Department of Medical Biology and Genetics, Faculty of Medicine, Akdeniz University, Antalya, Turkey*
[b] *Department of Pediatric Genetics, Faculty of Medicine, Akdeniz University, Antalya, Turkey*

ARTICLE INFO

ABSTRACT

Pure partial trisomy of chromosome 21 is a rare event. The patients with this aberration are very important for setting up precise karyotype–phenotype correlations particularly in Down syndrome phenotype. We present here a patient with Down syndrome with a *de novo* derivative chromosome 21. Karyotype of the patient was designated as 46,XY,der(21)(p13)dup(21)(q11.2q21.3)dup(21)(q22.2q22.3) with regard to cytogenetic, FISH and array-CGH analyses. Non-continuous monosomic, disomic and trisomic chromosomal segments through the derivative chromosome 21 were detected by array-CGH analysis. STR analyses revealed maternal origin of the *de novo* derivative chromosome 21. The dual-specificity tyrosine (Y)-phosphorylation regulated kinase 1A (*DYRK1A*) and Down Syndrome Critical Region 1 (*DSCR1*) genes that are located in Down syndrome critical region, are supposed to be responsible for most of the clinical findings of Down syndrome. However, our patient is the first patient with Down syndrome whose clinical findings were provided in detail, with a *de novo* derivative chromosome 21 resulting from multiple chromosome breaks excluding *DYRK1A* and *DSCR1* gene regions.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Trisomy 21 is one of the most common chromosomal abnormalities leading to intellectual disability and congenital malformations with a frequency of approximately 1/700 live births (Epstein, 2001). According to Jackson's checklist, characteristic clinical findings of Down syndrome include brachycephaly, inner epicanthic eye folds, oblique eye fissures, flat nasal bridge, protruding tongue, congenital heart disease, muscular hypotonia, short neck, short and broad hands, and a gap between the first and the second toes (Jackson et al., 1976).

An additional free chromosome 21 can be observed in most of the Down syndrome patients and only 2–3% of which are in a mosaic state. In the remaining 2–5%, Down syndrome results from Robertsonian translocation or rarely from a reciprocal translocation (Miller and Therman, 2001). Rare chromosome variants of Down syndrome are either due to inverted duplication of chromosome 21 which is known as 'mirror image duplication of chromosome 21', or due to recombinant duplication of chromosome 21 resulting from parental inverted or ring chromosome 21, or due to partial duplications of chromosome 21. These partial duplications are particularly important to narrow the critical region for development of Down syndrome phenotype and assignment of a specific clinical finding to a particular chromosomal region or gene/genes (Martinoli et al., 2010). Cytogenetic and molecular genetic studies on patients with partial trisomy of chromosome 21 indicate the presence of a Down syndrome critical region (DSCR) that has been narrowed down to a region within 21q22, about 2.3–5.4 Mb in length (Arron et al., 2006; Delabar et al., 1993; Sato et al., 2008). The dual-specificity tyrosine (Y)-phosphorylation regulated kinase 1A (*DYRK1A*) and Down Syndrome Critical Region 1 (*DSCR1)* genes that are located in this critical region are supposed to be responsible for most of the clinical findings of Down syndrome. We present here the first patient with Down syndrome whose clinical findings were provided in detail with a *de novo* derivative chromosome 21 resulting from serial events of chromosomal rearrangements that does not contain *DYRK1A* and *DSCR1* gene regions.

2. Material and methods

2.1. Patient

The patient was born to healthy non-consanguineous parents (maternal age 33 years, paternal age 35 years) after the diagnosis of mild degree intra-uterine growth retardation at 33 weeks of gestational age. He was referred to our Clinical Genetics Department at 9 months of age due to a facial appearance suggestive of Down syndrome. Patient's weight was 9350 g (3–10 centile), height was 67.5 cm (50–75 centile) and, head circumference was 42.5 cm (3–10 centile). Physical examination showed mild degree growth retardation, brachycephaly, flat facial profile, down slanting palpebral fissures, bilateral epicanthal folds, Brushfield spots, flat nasal bridge, hypertelorism, pseudostrabismus, malformed ears, an open mouth with downturned corners, micrognathia, short neck, short and broad hands, brachydactyly in both hands and feet, clinodactyly in the 5th fingers, and hyperextensibility of joints. Neurological examination was normal. Echocardiography showed subaortic ventricular septal defect (VSD). Abdominal ultrasound revealed a minimal hepatomegaly and a right kidney pelvicaliectasis. Otoacustic emission tests were normal. He was able to sit independently at 34 weeks of age but his Denver test revealed a delay both in expressive language and motor development. Follow-up at 2.5 years of age revealed moderate developmental delay and he had been enrolled in a special early intervention program.

2.2. Conventional cytogenetic analysis

Short term Phytohemagglutinin (PHA) stimulated peripheral blood lymphocyte cultures of both parents and the proband was performed according to the standard procedures. Karyotyping was performed using the image analyzer (Applied Imaging, San Jose, CA). At least twenty metaphase plates were evaluated from each individual by using G-banding (GTG) at 550 band level. Centromeric heterochromatin regions and nucleolus organizing regions were evaluated by CBG and NOR banding respectively in the proband.

2.3. Fluorescence in situ hybridization (FISH) analysis

FISH analysis was performed by using Texas Red labeled whole chromosome painting probe specific for chromosome 21 (WCP21) (Aquarius ™probes-Cytocell®, Oxon, UK), SpectrumGreen and SpectrumOrange labeled ETV6(TEL)/RUNX1(AML1) Probe Set (Vysis Inc, Downers Grove, Illinois, USA) and Texas Red labeled DSCR specific probe (Aquarius ™probes-Cytocell®, Oxon, UK) according to manufacturer's instructions. Subtelomeric FISH analyses were performed on the proband's metaphase plates using Chromoprobe Multiprobe-T Device including VIJ2yRM2029 probe specific for subtelomeric regions of chromosome 21 (Cytocell Technologies Ltd., UK) according to manufacturers' instructions to evaluate the positions of the subtelomeric sequences on the derivative chromosome 21. Ten metaphase plates of the proband were analyzed for each FISH probes. Images were recorded by using a Zeiss Axioplan epifluorescence microscope equipped with a CCD camera (Photometrics Sensys) and analyzed by using MacProbe v4.3 software.

2.4. Quantitative fluorescent polymerase chain reaction (QF-PCR)

Genomic DNA samples were obtained from peripheral blood samples of the proband and the parents by using salting out protocol. Aneufast™ multiplex QF-PCR kit including STR markers for D21S1008 (21q22.1), D21S1411 (21q22.3), D21S1412 (21q22.2), D21S1414 (21q21), D21S1435 (21q21), D21S1437 (21q21.1), D21S1446 (21q22.3-ter) loci was used to evaluate the parental origin of the derivative chromosome 21. PCR reactions were carried out in a 15 μl final PCR volume, containing 10 μl Multiplex PCR mix and 5–10 ng genomic DNA. The DNA was denatured for 15 min at 95 °C, followed by 28 cycles of 95 °C for 40 s, 60 °C for 1 min 30 s,

and 72 °C for 40 s, with a final extension of 60 °C for 30 min. Auomated capillary electrophoresis was performed by using ABI3130, (Applied Biosystems, Foster City, CA) and GeneMapper v3.5 software was used to analyze the results. Peak area ratios between 0.8 and 1.4 were considered normal, whereas ratios ≤0.6–≥1.8:1 were interpreted as trisomy. The presence of a single peak was considered as uninformative.

2.5. Array-CGH analysis

Microarray analysis was performed in duplicate using genomic DNA sample obtained from peripheral blood lymphocytes of the patient, according to the manufacturer's protocol for Cytogenetics Whole Genome 2,7M oligonucleotide catalog array (Affymetrix Inc., Santa Clara, CA, USA).The array which is spanning both coding and non-coding sequences with average genome coverage of approximately 600–1000 bases, consisted of approximately 2,700,000 oligonucleotide probes and including 400,000 single nucleotide polymorphisms (SNPs) was used for the detection of loss of heterozygosity (LOH) and uniparental disomy (UPD). The microarray image data were scanned by GeneChip® 7G High Resolution Scanner using the Command Console software (Affymetrix Inc., Santa Clara, CA, USA). Data were analyzed using Chromosome Analysis Suite Version 1.0 (Affymetrix Inc., Santa Clara, CA, USA). Furthermore, Copy Number State (CNS) at given loci was evaluated according to following criteria. CNS 0: homozygous deletion (nullisomy), CNS 1: heterozygous deletion (monosomy), CNS 2: disomy, CNS 3: heterozygous duplication (trisomy).

3. Results

3.1. Conventional cytogenetics

Analyses of G-banded metaphases that were obtained from peripheral blood lymphocytes of the patient, showed additional material on the short arm of one of the chromosome 21 (Fig. 1a). CBG banding on metaphase plates of the proband showed two C bands on the derivative chromosome 21 (Fig. 1b) and one of them was located at the subtelomeric region of the short arm. No satellites were observed on the derivative chromosome 21 by using NOR staining technique. His karyotype based on conventional cytogenetics was 46,XY,der(21)(p13)[20]. Parental karyotypes were observed as normal.

3.2. Fluorescence in situ hybridization (FISH) analysis

FISH analysis by using WCP21 (Aquarius ™probes-Cytocell®, Oxon, UK) showed that both normal and derivative chromosome 21 have been totally painted with this probe, indicating the presence of an intrachromosomal rearrangement (Fig. 2a). There were three FISH signals that were observed by using DSCR specific probe (Cytocell Technologies Ltd., UK). One of them was on the normal chromosome 21 and the others were on the derivative chromosome 21. One of the signals on the derivative chromosome 21 was found to be diminished (dim) in comparison to the other two signals (Fig. 2b). This finding showed that duplication included only a part of the DSCR but did not cover all DSCR genes. FISH analysis with LSI ETV6(TEL)/RUNX1(AML1) dual color probe set (Abbott Molecular Inc., USA) showed one RUNX1(AML1)(21q22.12) signal on normal and one signal on derivative chromosome 21, indicating that this locus was not present in the duplicated part of the chromosome 21 (Fig. 2c). Subtelomeric FISH analysis showed two subtelomeric FISH signals on the derivative chromosome 21 which were located on both ends of the derivative chromosome 21. According to FISH results, his karyotype was designated as: 46,XY,der(21)(p13) dup(21)(q22.2q22.3).ish der(21)(p13)dup(21)q22.2q22.3)(WCP21+,VIJ2yRM2029+,KCNJ6 dim, DSCR4 dim,DSCR8 dim,RUNX1+,DYRK1A+,KCNJ6+,DSCR4+,DSCR8+, VIJ2yRM2029+).
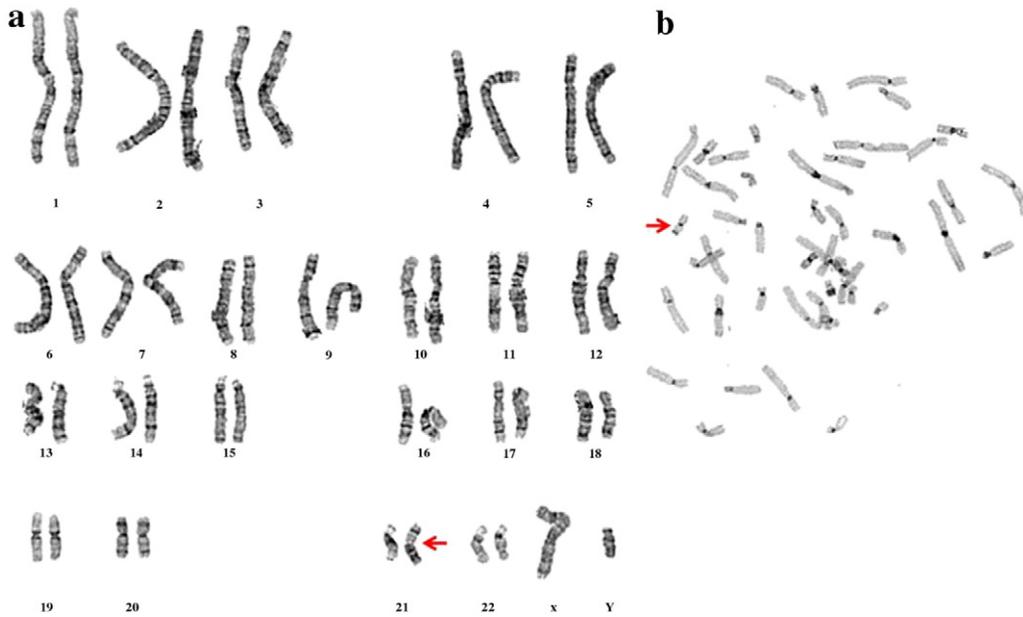
Fig. 1. a) GTG banded karyotype of the patient showing additional material on the short arm of one chromosome 21. b) CBG banded metaphase plate showing two C bands indicating that the derivative chromosome 21 had two centromeric heterochromatic regions.

3.3. Quantitative fluorescent polymerase chain reaction (QF-PCR)

D21S1411, D21S1414, D21S1437 STR markers were evaluated as uninformative. D21S1412 (21q22.2) and D21S1008 (21q22.1) STR markers showed normal diallelic pattern having 1:1 peak ratio. These results support the findings obtained from FISH and array-CGH analysis. D21S1435 (21q21) and D21S1446 (21q22.3-qter) STR markers showed diallelic trisomic peak pattern having 1:2 peak
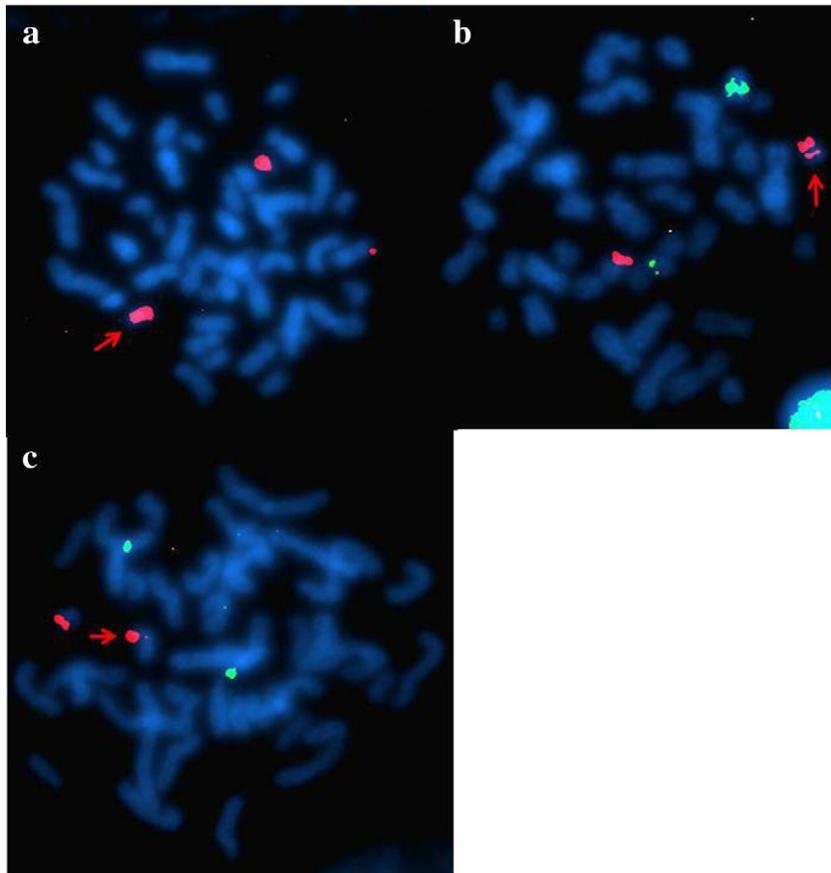

Fig. 2. Red arrows indicate the derivative chromosome 21 a) FISH with spectrum red labeled whole chromosome 21 painting probe showing that the derivative chromosome 21 has been totally painted, b) Green and red signals indicate 13q14.2 and 21q22.3 (Down Syndrome Critical Region) regions, respectively. FISH analysis showing two Down Syndrome Critical Region signals on derivative chromosome 21 which have different FISH signal intensities. c) Green and red signals indicates 12p13 (ETV6) and 21q22 (RUNX1) regions, respectively. FISH with LSI ETV6(TEL)/RUNX1(AML1) dual color probe set shows that there is one RUNX1 signal on both derivative and normal chromosome 21.

ratio, indicating that the derivative chromosome 21 originated from an intrachromosomal rearrangement of one of the maternal chromosome 21 (Fig. 3).

3.4. Array-CGH analysis

Array-CGH analysis showed non-continuous monosomic, disomic and trisomic chromosomal segments across the derivative chromosome 21 (Fig. 4). Array-CGH results were as follows: 46,XY,der(21)(p13). arr 21q11.2q21.3(14,312,385-27,731,477)x3,21q21.3q22.11 (29,390,126-30,535,119)x1,21q22.13(37,818,273-37,941,313)x1,21q22.2(39,102, 122-39,359,226)x3,21q22.2q22.3(39,951,929-46,589,274)x3. Genomic coordinates were mapped to hg18. *ETS2, AGPAT3* and *PRDM 15* genes were located in the breakpoint regions in the derivative chromosome 21. The genes located in the 21q22.2–q22.3 interval were also evaluated by using University of California Santa Cruz (UCSC) human genome browser. Repeated array-CGH analysis of the patient's DNA sample confirmed the first array-CGH analysis. Array-CGH analysis of the parents' DNA samples did not show any copy number variations on chromosome 21. According to array-CGH results

final karyotype was re-designated as 46,XY,der(21)(p13)dup(21) (q11.2q21.3)dup(21)(q22.2q22.3) (Fig. 5).

4. Discussion

DYRK1A and *DSCR1* genes are both located within the DSCR, expressed at a high level in the certain regions of the brain including cortex, hippocampus and cerebellum, indicating a significant role in the development of the central nervous system (Fuentes et al., 1995; Okui et al., 1999; Rahmani et al., 1998). *DYRK1A* and *DSCR1* protein levels have been reported to be increased in a dose dependent manner at profoundly affected brain areas of patients with Down syndrome as compared to brains of controls (Dowjat et al., 2007; Fuentes et al., 2000; Guimerá et al., 1996; Liu et al., 2008). Protein expression pattern and localization at the DSCR make these proteins good candidates for the development of specific neurological phenotypes observed in Down syndrome.

Korbel et al. (2009) declared to provide strong evidence against the existence of a single DSCR and synergistic contribution of *DSCR1*, *DYRK1A* and *APP* genes which have been previously proposed as
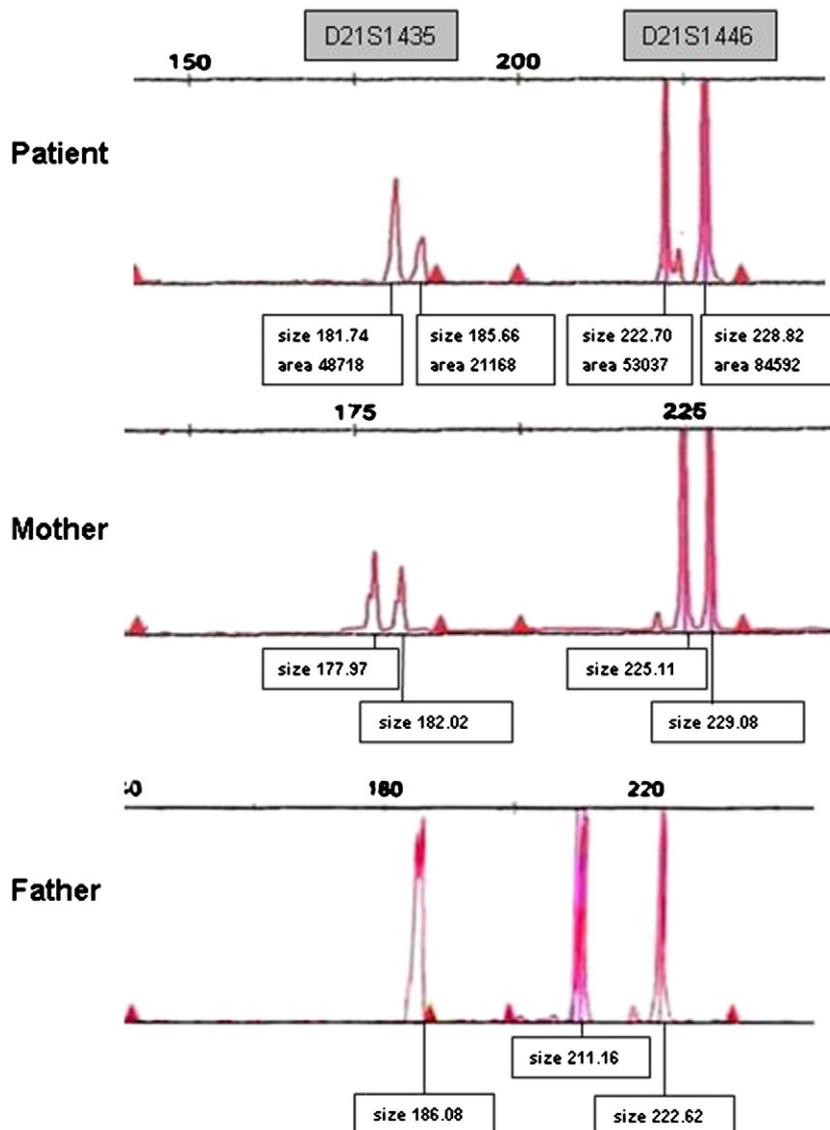

Fig. 3. A representative figure of STR analysis. D21S1435 and D21S1446 STR markers showed diallelic trisomic peak pattern having 1:2 peak ratio indicating maternal origin of the derivative chromosome 21.
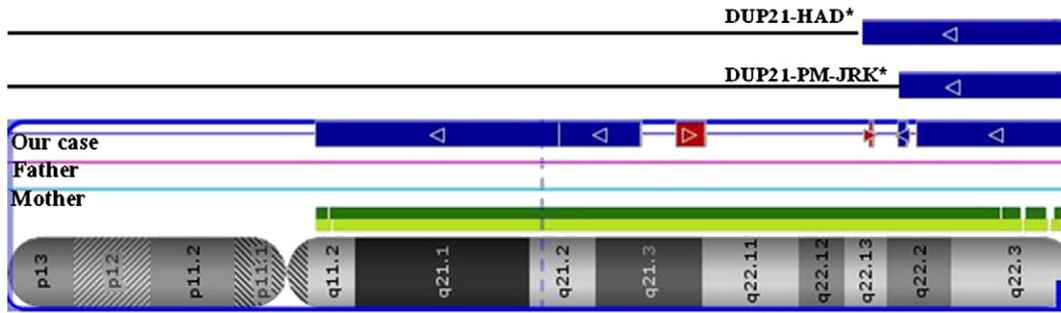
Fig. 4. Array-CGH partial ideogram of the chromosome 21 showing monosomic, disomic and trisomic regions across the chromosome 21. Gray line: patient, pink line: father, light blue line: mother. Blue and red blocks indicate the trisomic and monosomic regions, respectively. The other parts were disomic regions.*Patients previously reported by Korbel et al., (2009).

predominant contributors to many phenotypic traits in Down syndrome (Korbel et al, 2009). Some patients who are severely affected by Down syndrome have been reported to have segmental duplications that did not include DSCR1 gene. Also, in two patients, distal duplication of chromosome 21q (dup21HAD and dup21PM-JRK) were reported not to contain both *DSCR1* and *DYRK1A* genes. Our patient had duplication of the proximal and distal regions of the long arm of chromosome 21. Likewise, both the *DYRK1A* and *DSCR1* genes were also not duplicated. Our findings also support the findings of Korbel et al. suggesting *DSCR1* and *DYRK1A* might not be the part of the critical region that is causing the most of the Down syndrome associated features. In their study, additional copies of several non-continuous regions were detected in several patients by tilling array analysis, indicating a complex mechanism of formation such as inverted or direct duplications and deletions. In some patients, segmental trisomic, tetrasomic, monosomic and disomic regions have also been observed. In our patient,

non-continuous disomic, trisomic, and monosomic segments were observed by array-CGH, QF-PCR and FISH analysis. These results suggest that at least a part of the apparently simple duplications might be originated from complex intrachromosomal rearrangements including multiple chromosomal breaks. Korbel et al. found that around 33% of the breakpoints occur in the genes. In our patient, three breakpoints were found to be located in the *ETS2, PRDM15* and *AGPAT3* genes.

Two primary and well established recombination based mechanisms known as non-allelic homologous recombination (NAHR) and non-homologous end joining (NHEJ) might be major contributors in the development of genomic rearrangements. However, due to multiple breakpoints in complex chromosomal rearrangements, as in our patient, formation of these rearrangements cannot be simply explained by these recombination events. Recently, alternative mechanisms based on DNA replication errors called 'Fork Stalling and Template Switching' (FoSTeS) and 'Micro-homology-mediated break induced
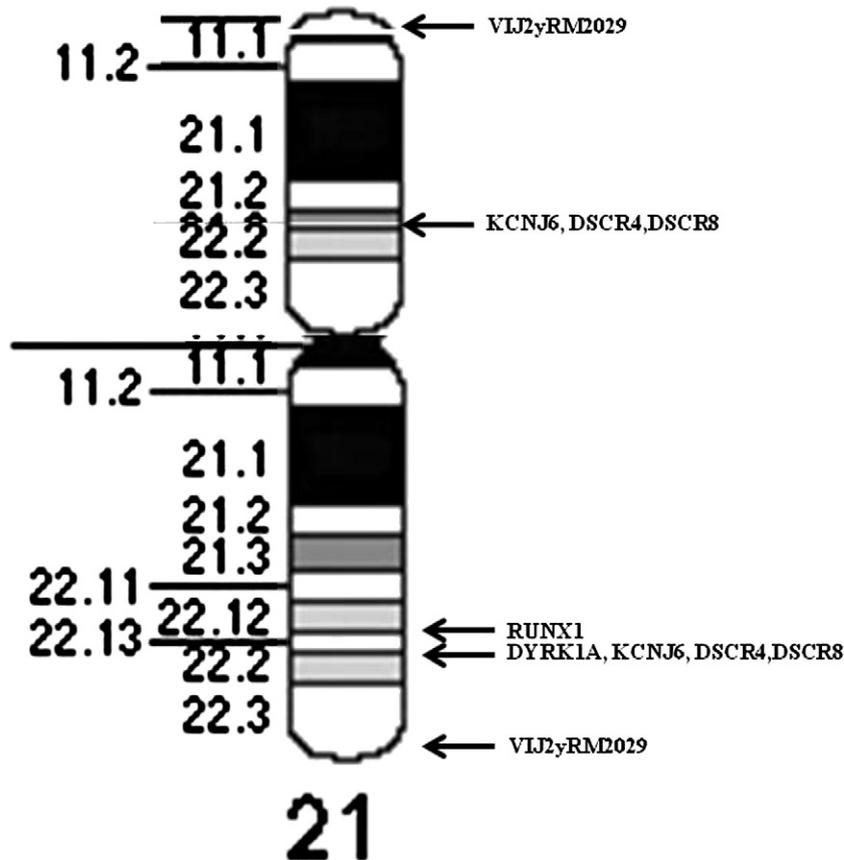

Fig. 5. An ideogram of the derivative chromosome 21 and the locations of the FISH probes.

replication' (MMBIR) were proposed as underlying mechanisms (Hastings et al., 2009; Lee et al., 2007). Also, it has been suggested that both the recombination and the replication based mechanisms can be involved in formation of complex chromosomal aberrations. Details of these two mechanisms and several other proposed mechanisms are available in two recent reviews (Pellestor et al., 2011; Zhang et al., 2009). Non-continuous monosomic, disomic and trisomic segments with inverted orientations indicate complex intrachromosomal rearrangements resulting from both recombination and replication based mechanisms that could be the underlying mechanisms in our patient.

Array-CGH is a new technology that is widely used in research and routine diagnostic laboratories on clinical genetics. This technology provides the possibility of establishing specific phenotype-genotype correlations in patients with cytogenetic abnormalities. Also, the confirmation of the array-CGH results with further analysis such as FISH and QF-PCR as done in our study, is very important. Lyle et al. (2009) applied an array-CGH study to enable high resolution mapping of partial trisomy and monosomy of the chromosome 21 and to identify the chromosomal localizations of the genes responsible for the development of particular phenotypes (Lyle et al., 2009). Trisomic regions and the clinical findings such as malformed ears, bilateral epicanthal folds, brachycephaly, hyperextensibility of joints, flat facial profile, cardiac abnormalities and Brushfield spots observed in our patient were correlated with their results. Also, monosomic regions and clinical findings such as flat nasal bridge, broad mouth, brachydactyly, short neck, clinodactyly and mental retardation observed in our patient were correlated with their findings.

Congenital heart defects are among the most common major findings in Down syndrome with a frequency of 44–58% (Freeman et al, 2008; Jaiyesimi and Baichoo, 2007). Barlow et al., (2001), narrowed the DSCR to 5.5 Mb spanning from D21S3 through *PFKL* gene. They speculated that over-expression of *DSCAM* gene which constitutes a large part of the Down syndrome congenital heart defect (DSCHD) region may contribute to abnormal cushion development observed in DSCHD. They also emphasized that while trisomy of a gene located in DSCHD region is essential for the development of congenital heart defects including ventricular septal defects (VSD) and Tetralogy of Fallot (TOF), trisomy of the other genes such as *COL6A1* located in the subtelomeric region may also be contributing to the diversity of the congenital heart defect (Barlow et al., 2001). However, according to Kosaki et al., (2005), when *DSCAM* but not *COL6A1* was duplicated, cardiac defect was TOF, however, when both *DSCAM* and *COL6A1* were duplicated, in addition to TOF different defects including atrioventricular septal defects, and VSD might be observed (Kosaki et al., 2005). In our patient, echocardiography showed subaortic ventricular septal defect and array-CGH analysis showed that duplication covers both *DSCAM* and *COL6A1* genes.

In conclusion, our results show that at least a part of the apparently simple chromosomal duplications on chromosome 21 might have resulted from complex chromosomal rearrangements including three or more breaks and monosomic, disomic and trisomic regions across the long arm of the chromosome. Therefore, characterization of the chromosomal abnormalities by molecular genetic techniques is very important for exact genotype-phenotype correlations. In our patient, duplicated region did not cover both the *DYRK1A* and *DSCR1*

genes and this finding may exclude the hypothesis that there is a strong contribution of *DYRK1A* and *DSCR1* genes on Down syndrome phenotype.

References

Arron, J.R., et al., 2006. NFAT dysregulation by increased dosage of DSCR1 and DYRK1A on chromosome 21. Nature 441, 595–600.

Barlow, G.M., et al., 2001. Down syndrome congenital heart disease: a narrowed region and a candidate gene. Genet. Med. 3, 91–101.

Delabar, J.M., et al., 1993. Molecular mapping of twenty-four features of Down syndrome on chromosome 21. Eur. J. Hum. Genet. 1, 114–124.

Dowjat, W.K., et al., 2007. Trisomy-driven overexpression of DYRK1A kinase in the brain of subjects with Down syndrome. Neurosci. Lett. 413, 77–81.

Epstein, C.J., 2001. Down syndrome (trisomy 21). In: Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D. (Eds.), The Metabolic and Molecular Bases of Inherited Diseases. McGraw-Hill, Inc., NewYork, pp. 1223–1256.

Freeman, S.B., et al., 2008. Ethnicity, sex, and the incidence of congenital heart defects: a report from the National Down Syndrome Project. Genet. Med. 3, 173–180.

Fuentes, J.J., Pritchard, M.A., Planas, A.M., Bosch, A., Ferrer, I., Estivill, X., 1995. A new human gene from the Down syndrome critical region encodes a proline-rich protein highly expressed in fetal brain and heart. Hum. Mol. Genet. 10, 1935–1944.

Fuentes, J.J., et al., 2000. DSCR1, overexpressed in Down syndrome, is an inhibitor of calcineurin-mediated signaling pathways. Hum. Mol. Genet. 9, 1681–1690.

Guimerá, J., et al., 1996. A human homologue of Drosophila minibrain (MNB) is expressed in the neuronal regions affected in Down syndrome and maps to the critical region. Hum. Mol. Genet. 5, 1305–1310.

Hastings, P.J., Ira, G., Lupski, J.R., 2009. A microhomology-mediated break-induced replication model for the origin of human copy number variation. PLoS Genet. 5, e1000327.

Jackson, J.F., North, E.R.I.I.I., Thomas, J.G., 1976. Clinical diagnosis of Down's syndrome. Clin. Genet. 9, 483–487.

Jaiyesimi, O., Baichoo, V., 2007. Cardiovascular malformations in Omani Arab children with Down's syndrome. Cardiol. Young 17, 166–171.

Korbel, J.O., et al., 2009. The genetic architecture of Down syndrome phenotypes revealed by high-resolution analysis of human segmental trisomies. Proc. Natl. Acad. Sci. U. S. A. 106, 12031–12036.

Kosaki, R., Kosaki, K., Matsushima, K., Mitsui, N., Matsumoto, N., Ohashi, H., 2005. Refining chromosomal region critical for Down syndrome-related heart defects with a case of cryptic 21q22.2 duplication. Congenit. Anom. (Kyoto) 45, 62–64.

Lee, J.A., Carvalho, C.M., Lupski, J.R., 2007. A DNA replication mechanism for generating non-recurrent rearrangements associated with genomic disorders. Cell 131, 1235–1247.

Liu, F., et al., 2008. Overexpression of Dyrk1A contributes to neurofibrillary degeneration in Down syndrome. FASEB J. 9, 3224–3233.

Lyle, R., et al., 2009. Genotype-phenotype correlations in Down syndrome identified by array CGH in 30 cases of partial trisomy and partial monosomy chromosome 21. Eur. J. Hum. Genet. 17, 454–466.

Martinoli, E., et al., 2010. A tandem duplication of chromosome 21 in a newborn showing a phenotype inconsistent with Down syndrome. Am. J. Med. Genet. A. 4, 1043–1045.

Miller, O.J., Therman, E., 2001. Human Chromosomes, 4th edition. Springer-Verlag, Inc., New York. (245 pp.).

Okui, M., et al., 1999. High-level expression of the Mnb/Dyrk1A gene in brain and heart during rat early development. Genomics 62, 165–171.

Pellestor, F., et al., 2011. Complex chromosomal rearrangements: origin and meiotic behavior. Hum. Reprod. Update 17, 476–494.

Rahmani, Z., Lopes, C., Rachidi, M., Delabar, J.M., 1998. Expression of the mnb (dyrk) protein in adult and embryonic mouse tissues. Biochem. Res. Commun. 2, 514–518.

Sato, D., et al., 2008. A girl with Down syndrome and partial trisomy for 21pter-q22.13: a clue to narrow the Down syndrome critical region. Am. J. Med. Genet. A 1, 124–127.

Zhang, F., Carvalho, C.M., Lupski, J.R., 2009. Complex human chromosomal and genomic rearrangements. Trends Genet. 25, 298–307.