An acquired stable variant of a dicentric dic(9;20) and complex karyotype in a Syrian childhood B-acute lymphoblastic leukemia case

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Abstract

Background: About 25 years ago, the acquired chromosome abnormality dicentric dic(9;20)(p11 ~ 13;q11) was seen described as a non-random aberration in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Yet, about 200 cases were reported. However, dicentric dic(9;20) is a subtle abnormality which easily may be mixed up with monosomy 20 and/or del(9p). The dicentric dic(9;20) can be found as a sole chromosomal abnormality or can be masked within complex rearrangements; also, a dicentric dic(9;20) is often associated with mono- or biallelic loss of CDKN2A gene.

Case presentation: Here we report a case of 16-year-old male diagnosed with a de novo pre-B-ALL. Molecular approaches (array-based multicolor banding (aMCB) and array comparative genomic hybridization (aCGH)) were applied, and a unique complex karyotype involving six chromosomes was identified. It included three previously unreported chromosomal aberrations: dicentric dic(9;20;X), deletion del(7)(p22.2p15.2) and dicentric dic(7;13). The dicentric dic(9;20;X) also led to monoallelic loss of tumor suppressor gene CDKN2A. After successful chemotherapeutic treatment the patient experienced a relapse with a secondary ALL without complex karyotype but a deletion del(19)(p13). Unfortunately, the patient died after 17 months of the initial diagnosis.

Conclusions: To the best of our knowledge, a comparable childhood ALL associated with such complex karyotype and deletion del(19)(p13) in secondary ALL was not previously reported. Thus, the complex karyotype with dicentric dic(9;20;X) seems to indicate for a poor prognosis.

Keywords: Acute lymphoblastic leukemia, Complex karyotype, Dicentric dic(9;20), Array-based multicolor banding (aMCB), Array comparative genomic hybridization (aCGH), Prognostic factors
**Background**

The stable chromosome abnormality dicentric dic(9;20)(p11 ~ 13;q11) was first reported as a non-random aberration in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) in 1995 [1, 2]. Even though dicentric dic(9;20) can easily be missed and/or mixed up with other rearrangements (like monosomy 20 and/or del(9p)) in banding cytogenetics, still, already 199 cases have been published [1–5].

The dicentric dic(9;20) is more common in pediatric ALLs (2%) than in adult cases (< 1%) and seems to be more frequent in females [3]. The median age at diagnosis is 3 years; the median leucocyte count is 20–30 × 10^9/l [6]; an event-free survival (EFS) and overall survival (OS) up to 5 years are reached by 62 and 82% of the patients, respectively. Accordingly, relapse cases are quite common and post-relapse treatment of many patients was successful [7].

All BCP-ALL cases reported had an immunophenotypes showing positive results for TdT, HLA-DR, CD10, CD19 and CD24, and negative for myeloid markers [1, 2, 4, 5]. The prognostic impact of dicentric dic(9;20) is still unclear, but most reported patients have attained complete remission; thus, such patients are suggested to have a good prognosis [1, 2, 4, 5]. Interestingly, unrecognized dicentric dic(9;20) cases may also be included in cases with monosomy 20 as sole abnormality in ALL; thus, it is noteworthy that the latter is considered to be a favorable prognostic marker [8, 9].

Dicentric dic(9;20) can occur as a sole cytogenetic abnormality, or in the context of a more complex karyotype [7]. Common additional genetic changes in ALL with dicentric dic(9;20) are deletions involving chromosome 13q and gains of chromosomes X, 8 and 20 [3, 4, 7]. Based on data obtained by fluorescence in situ hybridization (FISH) it is known that dicentric dic(9;20) can occur in the presence of the BCR-ABL1 and ETV6-RUNXI fusion genes [7]. Furthermore, for the CDKN2A (cyclin-dependent kinase inhibitor 2A) gene in 9p21, mono- or biallelic deletions were also repeatedly seen [10, 11].

We present here clinical, cytogenetic and molecular data of bone marrow cells obtained from a de novo childhood pre-B-ALL case with a complex karyotype and relapse, involving a variant dicentric dic(9;20).

**Case presentation**

On 30 Jun 2016, a 16-year-old male patient without any known medical background presented with a 1 month history of fatigue and fever without sweating. He had no familial history of malignancies and no social and environmental history or exposure to toxins and animals. Initial laboratory evaluation of peripheral blood (PB) revealed white blood cells (WBC) of 52.2 × 10^9/l (88% were blasts). He was treated with Predlon 60 mg/day per 10 days. Afterwards, physical examination and ultrasound at our hospital showed no splenomegaly, however, several lymphadenopathies (sternocleidomastoidal (1 cm) and right of subaxilla (1 cm)), normal heart rate (90/min) and his blood pressure was 12/6. His PB showed: WBC 3.5 × 10^9/l (neutrophils 33%, lymphocytes 64%), Hb = 7.5 g/dl, and platelets = 49.4 × 10^9/l. Serum biochemistry analyses were: Calcium (Ca^2+) 9.9 mmol/l (normal value 8.5–10.3); LDH 229 U/l (normal level < 460); β2-microglobulin 3.32 mg/l (normal value 0.61–3.7); alanine aminotransferase level was 24 U/l (normal up to 40 U/l); aspartate aminotransferase level 17 U/l (normal up to 40 U/l); creatinine was 0.57 μmol/l (normal 45–120); Urea 38 mmol/l (normal 10–50); Sodium (Na+) 137 mmol/l (normal 135–148), Potassium (K+) 4.7 mmol/l (3.5–5.2), total protein 6.2 g/dl (normal 6.6–8.7), albumin 4.2 g/dl (normal 3.8–5.4). Bone marrow (BM) aspiration revealed hypercellularity with 90% of lymphoblasts. In cerebrospinal fluid aspiration no cells were found.

He was diagnosed as having pre-B-ALL according to the World Health Organization (WHO) classification. Thus, the patient was treated further according to GRALL 2003 chemotherapy protocol. Two days after initiating GRALL 2003 chemotherapy, the patient developed neutropenia, was given Neupogen and restarted chemotherapy protocol. Two days after initiating GRALL chemotherapy, the patient was treated further according to GRALL 2003 chemotherapy protocol. Two days after initiating GRALL chemotherapy, the patient was treated further according to GRALL 2003 chemotherapy protocol.

Approximately 2 months after relapse patient died due to respiratory and heart arrest, as well as neutropenia. No autopsy was performed. Patient’s father agreed with scientific evaluation of his case and the study was approved by the ethical committee of the Atomic Energy Commission, Damascus, Syria.

**Results**

GTG-banding was performed on BM sample according to standard procedures [12] prior and post chemotherapy. A minimum of 20 metaphase cells derived from unstimulated BM culture were analyzed. Karyotypes were classified according to the International System for Human Cytogenomic Nomenclature [13]. Prior to chemotherapy treatment GTG-banding revealed a karyotype 46, XY,der(X)t(X;?)(?;?),t(7;?)(?;?),+8,8,der(9)t(9;?)(?;?),+13[9]/47,XY, der(X)t(X;?)(?;?),+8,8,der(9)t(9;?)(?;?)8/46,XY[3] (Fig. 1a). Further FISH analysis including home-made whole chromosome painting (WCP) probes for chromosomes 1, 2, 3, 5, 6, 7, 8, 9, 10, 13, 15, 16, 17, 19, 20, 21, 22 and X and array-based multicolor banding (aMCB) probes for...
chromosomes 7, 9, 13, 20 and X were done as previously reported (results are shown in Fig. 2) [14].

Besides, commercially available probes were applied: ZytoLight®SPEC CDKN2A/CEN9 (in 9p21.3 and 9p11q11 dual color probe) (Fig. 3) and ZytoLight®SPEC JAZF1 (7p15.2p15.1 Break Apart Probe) all from ZytoVision GmbH (Bremerhaven, Germany), LSI ETV6 (in 12p13.2 dual color break part probe) and LSI p53/ATM (in 17p13.1 and 11q22.3 dual color probe) all from Vysis (Abbott GmbH & Company, KG, Wiesbaden, Germany). A total of 10 metaphase spreads were analyzed, each, and (where applicable) 200 interphase nuclei were examined, using a fluorescence microscope (Axiolimger.Z1 mot, Zeiss) equipped with appropriate filter sets to discriminate between a maximum of five fluorochromes and the counterstain DAPI (Diaminophenylindol). Image capturing and processing were carried out using an ISIS imaging system (MetaSystems, Altussheim, Germany).

The final karyotype prior to chemotherapeutic was finally defined as:

46,XY,der(X)t(X;20)(p21;p12),der(7)dic(7;13)(p15.2q12.3), +8,der(9)(Xpter->Xp21::20p12->q11.2::9p13.2->9qter),-13[9]/46,XY[3].

Fig. 1 GTG-banding revealed a complex karyotype in BCP-ALL (a), and a karyotype 46,XY,del(19)(p13) after relapse to secondary ALL (b).
Genomic DNA was extracted from BM cells prior to chemotherapy treatment and aCGH was performed using the Agilent Sure Print G3 Human Genome Microarray 180 K as previously described [14]. Array-CGH revealed four losses of copy numbers in:

- 7p22.3 to 7p15.2 at positions 109,626 to 26,260,755 including five COSMIC census cancer genes;
- 7p14.2 to 7p11.2 at positions 35,292,065 to 56,174,888 including 3 COSMIC census cancer genes;
- 9p24.3 to 9p13.2 at positions 207,437 to 37,270,400 including 10 COSMIC census cancer genes, and
- 13q12.3 to 13q24 at positions 32,035,219 to 115,059,020 including 10 COSMIC census cancer genes.

Besides, four gain of gains of copy numbers were identified by array-CGH in:

- whole chromosome 8, including 34 COSMIC census cancer genes;
- 20p13 to p11.1 at positions 60,747 to 25,713,574 including 2 COSMIC census cancer genes;
- 20q11.2 to 20q11.2 at positions 29,467,937 to 29,948,374 (no COSMIC census cancer gene identified), and
- 20q13.13 to 20q13.13 at positions 46,828,431 to 48,880,347 (no COSMIC census cancer gene identified) (Tab. 1).

Immunophenotyping was performed on BM specimen prior to chemotherapy treatment using a general panel of antibodies against antigens specific for different blood cell lineages and blood cell types [15]. Those antibodies were against: CD1a, CD2, CD3, CD4, CD5, CD8, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD23, CD32, CD33, CD34, CD36, CD38, CD41a, CD45, CD56, CD57, CD64, CD79a, CD103, CD117, CD123, CD138, CD209, CD235a and CD243; In addition to antibodies to Kappa and Lambda light Chains, slgD, slgM, and HLADr. All antibodies were from BD Biosciences. Samples analyzed on a BD FACSCalibur™ flow cytometer. Auto fluorescence, viability, and isotype controls were included. Flow cytometric data acquisition and analysis conducted by BD Cellquest™ Pro software. Interpretations of FCM results were according to [16].

FCM analysis of BM specimen prior to chemotherapy treatment characterized this case as Pre-B-ALL according to WHO classifications. The abnormal cell population (51%) was positive for CD45dim, CD34, HLADr, CD19, CD10, cCD79a, and expressed CD13 and CD33 heterogeneously. Blast cell population was negative for CD3, CD117, CD14, CD64, CD7, CD2 and CD5.

**Fig. 2** aMCB results are shown. The normal chromosomes are depicted on the left side and the derivative of the corresponding chromosomes on the right side of normal chromosomes. The unstained regions when using chromosome-specific aMCB-probe sets on the derivative chromosomes are shown in gray. Der = derivative chromosome

**Fig. 3** FISH result of CDKN2A showed monoallelic deletion on the der(9)(Xpter->Xp21::20p12->q11.2::9p13.2->9qter)
**Table 1** Summary of CNAs detected by aCGH

| Chr. | Start – End band | Genomic position: start-end GRCh37/hg19 | Variant type | Size (Mb) | COSMIC census cancer gene(s) within the region |
|------|------------------|-----------------------------------------|--------------|----------|------------------------------------------------|
| 7    | p22.3p15.2       | 109,626-26,260,755                      | loss         | 26.1     | **CARD11, PMS2, RAC1, MACC1, HNRNPA2B1** |
|      | p14.2p11.2       | 35,292,065-56,174,888                    | loss         | 20.8     | **SFRP4, IKZF1, EGFR** |
| 8    | p23.3p11.1       | 176,452-43,399,198                      | gain         | 43.2     | **ARHGEF10, PCM1, LEPROT1, WRN, NRG1, NSD3, FGFR1, ANK1, KAT6A, KIBB** |
|      | q11.1q24.3       | 46,939,154-146,294,098                  | gain         | 99.3     | **TCEA1, PLAG1, CHCHD7, PREX2, NCOA2, HEY1, CNBD1, NBN, RUNX1T1, CDH17, COX6C, PABPC1, UB5, EIF3E, RSPO2, CSMD3, RAD21, EXT1, MYC, NDRG1, FAM135B, RECQL4** |
| 9    | p24.3p13.2       | 207,437-37,270,400                      | gain         | 37.1     | **JAK2, CD274, PDCD1LG2, PTPRD, NF1B, PSIP1, MLLT3, CDKN2A, FANCG, PAX5** |
| 13   | q12.3q24         | 32,035,219-115,059,020                  | loss         | 83.0     | **BRCA2, NBEA, LHFPL6, FOXO1, LCP1, RB1, CYSLTR2, GPCS, SOX21, ERCC5, SIRPA, CRNKL1** |
| 20   | p13p11.1         | 60,747,257-713,574                      | gain         | 25.6     |                                                     |
|      | q11.2            | 29,467,937-29,948,374                   | gain         | 0.5      | n.a.                                              |
|      | q11.3            | 46,828,431-48,880,347                   | gain         | 2.05     | n.a.                                              |

After chemotherapy and relapse GTG-banding revealed a karyotype of 46,XY[18],46,XY,del(19)(p13)[2] (Fig. 1b).

**Discussion and conclusions**

According to the literature, the dicentric dic(9;20) has been reported in 199 ALL cases listed in Mitelman database [3]. Dicentric dic(9;20) with trisomy of chromosomes 8 or 21 were seen in 10 and 7 ALL cases, respectively [3]. A translocation t(X;9) involving short and/or long arms of these chromosomes has been found in 11 ALL cases [3]. In addition, partial deletion of the short arm of chromosome 7 [del(7)(p14p11)], and derivative del(19)(p13) were previously reported in 2 and 102 ALL cases, respectively [3]. Interestingly, translocation t(X;20)(p21;p12), derivative del(7)(p22p15), dicentric dic(7;13) have never been described in ALL cases. To the best of our knowledge, a combination of all these complex rearrangements with new formation of dicentric dic(9;20) in one ALL case at diagnosis was not previous reported yet [3].

The dicentric dic(9;20) contains centromeres of both chromosomes 9 and 20, resulting in loss of 9p and 20q material [1, 2, 4, 5], which occurs at a low frequency in ALL cases (2% in children and < 1% in adult ALL patients), predominantly in females [3, 7].

The dicentric dic(9;20) can be found as a sole chromosomal aberration (~ 40% of the ALL cases) or with additional chromosomal aberrations (ACAs) (60% of the ALL cases) [17]. Strefford et al. [11] have suggested that the dicentric dic(9;20) is not associated with a recurrent gene rearrangement. While Coyaud et al. [18] noted that dicentric cases can masking a complex rearrangement. Our present case represents a novel formation of dic(9; 20) with loss 9p and 20q in a chromosomal aberration involving X-chromosome.

Notably, the dicentric dic(9;20)-positive leukemia is frequently associated with hetero- or homozygous loss of **CDKN2A** gene in 31% of all cases analyzed by FISH [17]. However, whether loss of function of this gene is pathogenetically and/or clinically important in dicentric dic(9;20)-positive ALL, remains to be elucidated, but is most likely valid [17]. Other common ACAs included gains of X and 21, both of which are frequent in other subtypes of BCP-ALL [6].

A complex karyotype has been generally classified as ≥ 3 unrelated chromosomal abnormalities in ALL cases with the absence of established translocations (t[9;22], t[v;11q23], t[1; 19], t[8;14], and t[14q32]) [19]. Moorman et al. [19] demonstrated that those ALL patients with complex karyotype ≥ 4 or more unrelated chromosomal abnormalities had a poor outcome in terms of OS and EFS, with most of the relapses occurring in the first 2 years after diagnosis. While, Motlofo et al. [20] showed that a complex karyotype was not associated with adverse prognosis in adult ALL patients treated with risk-adapted or subtype-oriented protocols.

In conclusion, we report the first pre-B-ALL case obtained complex karyotype with a new acquired stable variant of a dicentric dic(9;20) resulting from masked partial trisomy 20. In addition, monolellar deletion of tumor suppressor gene **CDKN2A** and subsequent deletion del(19p13) without all the previously observed changes in the secondary ALL were seen. Overall, such complex chromosomal changes seem to have adverse prognosis in pre-B-ALL.

**Abbreviations**

ACAs: Additional chromosomal aberrations; aCGH: array comparative genomic hybridization; aMCB: array-proven multicolor banding; ALL: Acute lymphoblastic leukemia; BM: Bone marrow; DAPI: 4',6- diamino-2-phenylindole; EFS: Event-free survival; FISH: Fluorescence in situ hybridization; HGB: Hemoglobin level; OS: Overall survival; PB: Peripheral blood; WBC: White blood cells; WCP: Whole chromosome painting; WHO: World health organization

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Authors’ contributions
AW, RJ, SA and WA performed banding cytogenetics; AA did the immunophenotyping; AW, SA, MO and TL performed the molecular cytogenetic analyses; JM, IC and MO performed the aCGH; AW, MO and TL drafted the paper and all authors worked on the final version of the paper. All authors read and approved the final manuscript.

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Availability of data and materials
All relevant data and material is included in this publication.

Ethics approval and consent to participate
Study procedures were reviewed and approved by the ethical committee of the Atomic Energy Commission, Damascus, Syria Review Board. Written informed consent was obtained from all subjects prior to participation.

Consent for publication
Written informed consent was obtained from the patient’s father for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests
The authors declare that they have no competing interests.

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References
1. Rieder H, Schnittger S, Bodenstein H, Schwonzen M, Wörmann B, Berkovic D, et al. Dic(9;20): a new recurrent chromosome abnormality in adult acute lymphoblastic leukemia. Genes Chromosomes Cancer. 1995;13:54–61.
2. Slater R, Smit E, Kroes W, Bellomo MJ, Mühlematter D, Harbott J, et al. A cytogenetic and immunophenotypic study of acute lymphoblastic leukemia patients with dic(9;20) or other balanced translocations of chromosome 9q22. Genes Chromosomes Cancer. 1999;26:91–8.
3. Mitelman F, Johansson B, Mertens F, editors. Mitelman database of chromosome aberrations and gene fusions in cancer (2019). http://cgap.nci.nih.gov/Chromosomes/Mitelman. Accessed 05.11.2019.
4. Clark R, Byatt SA, Bennett CF, Brama M, Martineau M, Moorman AV, et al. Monosomy 20 as a pointer to dicentric (9;20) in acute lymphoblastic leukemia. Leukemia. 2000;14:241–6.
5. Herve MA, Maben KD, Bernstein J, Breitfeld PP, Neiman RS, Vance GH. Dicentric (9;20)(p11;q11) identified by fluorescence in situ hybridization in four pediatric acute lymphoblastic leukemia patients. Cancer Genet Cytogenet. 1996;92:111–5.
6. Johansson B, Mertens F, Mitelman F. Clinical and biological importance of cytogenetic abnormalities in childhood and adult acute lymphoblastic leukemia. Ann Med. 2004;36:492–503.
7. Gibbons B. dic(9;20)(p11–13;q11). Atlas Genet Cytogenet Oncol Haematol. 1999;3(3):144.
8. Betts DR, Kingston JE, Doery EL, Young BD, Webb D, Katz FE, et al. Monosomy 20: a nonrandom finding in childhood acute lymphoblastic leukemia. Genes Chromosomes Cancer. 1990;2:182–5.
9. Silengo M, Vassallo E, Barisone E, Miniero R, Madon E. Monosomy 20 in childhood acute lymphoblastic leukemia. Cancer Genet Cytogenet. 1992;59:177–9.
10. Andersson A, Olsson T, Lindgren D, Nilsson B, Ritz C, Edén P, et al. Molecular signatures in childhood acute leukemia and their correlations to expression patterns in normal hematopoietic subpopulations. PNAS. 2005;102:19069–74.
11. Srefford JC, Worley H, Barber K, Wright S, Stewart AR, Robinson HM, et al. Genome complexity in acute lymphoblastic leukemia is revealed by array-based comparative genomic hybridization. Oncogene. 2007;26:4306–18.
12. Al-Achkar W, Wafa A, Nweder MS. A complex translocation t(5;9;22) in Philadelphia cells involving the short arm of chromosome 5 in a case of chronic myelogenous leukemia. J Exp Clin Cancer Res. 2007;26:411–5.
13. McGowan-Joran B, Simons A, Schmid M, Schwalbe H, et al. Monosomal karyotype in adult patients with acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) U.K. ALL XII/Eastern Cooperative Oncology Group (ECOG) 2993 trial. Blood. 2007;109:3189–97.
14. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. Blood. 2008;111:3941–67.
15. Forestier E, Gauthier F, Andersson MK, Auclair K, Borghs G, Golovleva I, et al. Clinical and cytogenetic features of pediatric dic(9;20)(p13.2;q11.2)-positive B-cell precursor acute lymphoblastic leukemias: a Nordic series of 24 cases and review of the literature. Genes Chromosomes Cancer. 2008;47:149–58.
16. Coyle A, Strukul S, Prade N, Familiard J, Eichner R, Quelen C, et al. Wide diversity of PAX5 alterations in B-ALL: a Groupe francophone de Cytogenetique Hematologique study. Blood. 2010;115:3089–97.
17. Moorman AV, Harrison CJ, Buck GA, Richards SM, Secker-Walker LM, Martineau M, et al. Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALL XIII/eastern cooperative oncology group (ECOG) 2993 trial. Blood. 2007;109:3189–97.
18. Motlló C, Ribera JM, Morgades M, Granada I, Monserinos P, Gonzalez-Campos J, et al. Prognostic significance of complex karyotype and monosomal karyotype in adult patients with acute lymphoblastic leukemia treated with risk-adapted protocols. Cancer. 2014;120:3958–64.

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