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Imatinib resistance in a novel translocation der(17)t(1;17)(q25;p13) with loss of *TP53* but without *BCR/ABL* mutation in chronic myelogenous leukemia

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Abstract

We describe here two novel translocations, t(7;14)(p22;q13) and der(17)t(1;17)(q25;p13), in a 41-year-old man with an accelerated phase (AP) of chronic myelogenous leukemia (CML). Chromosome analysis initially showed 46,XY,t(7;14)(p13;q22),t(9;22)(q34;q11.2)[20]. In three years, the karyotype evolved to 45,X,-Y,der(7)t(7;14)(p13;q22),t(9;22)(q34;q11.2),-14, der(17)t(1;17)(q25;p13),+der(22)t(9;22)[20], accompanied with a resistance to imatinib mesylate. The *TP53* was deleted from the der(17)t(1;17)(q25;p13), but there was no mutation of *TP53* in the remaining allele. Mutations in the *BCR/ABL* kinase domain could not be detected as well. Morphologically, dysplastic changes including pseudo-Pelger-Huët anomaly appeared in the bone marrow cells. These findings suggest that the t(7;14)(p22;q13) had a crucial role in the progression to CML-AP, and that the resistance to imatinib may be due to the additional cytogenetic abnormalities including der(17)t(1;17)(q25;p13), but not to *BCR/ABL* mutations.

1. Introduction

Chronic myelogenous leukemia (CML) is consistently associated with t(9;22)(q34;q11.2), resulting in the formation of the Philadelphia (Ph) chromosome [1,2]. During the clinical course from chronic phase (CP) to accelerated phase (AP) and blast crisis (BC), additional chromosome abnormalities appear besides the Ph chromosome in 60-80% of patients [1-3]. The most common additional changes are an extra Ph (+Ph), i(17q), +8 and +19, and less frequently, –Y, +21, +17 and -7. These unbalanced aberrations occur in more than 5% of CML with secondary changes and are called “major evolutionary route” [3]. Furthermore, several balanced translocations, such as t(3;21)(q26;q22) and t(15;17)(q22;q12-21), are infrequently found in CML-AP/BC [3]. However, the clinical significance of other additional cytogenetic abnormalities in CML-AP/BC remains to be completely elucidated. We describe here two novel additional translocations, t(7;14)(p22;q13) and der(17)t(1;17)(q25;p13) with loss of *TP53,* in CML-AP. These translocations are supposed to play an important role in the progression of the disease.

2. Materials and methods

2.1. Case History

A 41-year-old man was admitted to our hospital for general malaise in May 2004. Peripheral blood showed hemoglobin 64 g/L, platelets 383 x 10^9/L and white blood cells 275.3 x 10^9/L with 11% myeloblasts, 2% promyelocytes, 13% myelocytes, 8% metamyelocytes, 10% band forms, 34% segmented neutrophils, 9% eosinophils, 9% basophils, 3% monocytes and 1% lymphocytes. Bone marrow was markedly hypercellular marrow with myeloid hyperplasia (Fig. 1A): 7.2% myeloblasts, 3.2% promyelocytes, 74.4% other myeloid cells, 5.8% eosinophils, 5.6% basophils, 2.2% monocytes, 1.2% lymphocytes and 0.4% erythroblasts.

There was no apparent dysplastic change in myeloid cells. Surface marker analysis revealed that the blasts were positive for CD4, CD13, CD33, CD34, CD41 and HLA-DR. We diagnosed the disease as CML-AP in the World Health Organization classification.

He received the treatment with 600 mg of imatinib mesylate and achieved a complete hematologic response (CHR) in July 2004. The treatment with 300 to 400 mg of imatinib was continued for further three years. However, only minor cytogenetic response was shown by interphase fluorescence *in situ* hybridization (FISH) during the clinical course (Table 1). In April 2007, peripheral blood showed hemoglobin 82 g/L, platelets 208 x 10^9/L, and white blood cells 2.3 x 10^9/L. Bone marrow was normocellular with eosinophilia (Fig. 1B): 6.2% myeloblasts, 13.6% promyelocytes and 19.0% eosinophils. Dysplastic changes including pseudo-Pelger-Huët anomaly of neutrophils and micromegakaryocytes were observed (Figs. 1C and 1D). At this time, he finally underwent myeloablative cord blood transplantation (CBT) from an HLA two loci-mismatched unrelated male donor. He obtained complete chimerism and maintained hematological and cytogenetic complete remission after CBT for eight months.

2.2. Chromosome analyses, spectral karyotyping and FISH analyses

Chromosome analyses were performed by the G-banding technique on short-term culture of the cells obtained from bone marrow. Karyotypes were described according to ISCN 2005 [4]. Spectral karyotyping (SKY) was carried out with a SkyPaint kit (Applied Spectral Imaging, Migdal Ha'Emek, Israel). FISH analyses with LSI BCR/ABL ES Dual Color Translocation Probe and LSI p53/CEP17 Dual Color Probe (Abbott Molecular/Vysis, Des Plaines, IL, USA) were performed on five metaphase spreads and 100 interphase nuclei before CBT.

2.3. BCR/ABL and TP53 mutation

Total RNA was extracted from bone marrow cells of the patient before CBT. Expression of the *BCR/ABL* fusion transcript was examined by reverse transcription polymerase chain

reaction (RT-PCR) analysis. Total RNA was also used for mutation screening of the *BCR/ABL* kinase domain, including M244V, L248V, L248R, G250E, Q252H, Q252R, Y253F, Y253H, E255K, E255V, E279K, F311L, T315A, T315I, F317L, M351T, F359I, F359V, V379I, L387M, H396P, H396R, S417Y, E459K and F486S, by PCR-Invader assay (BML, Inc., Saitama, Japan). Mutation of exons 5 to 8 in the *TP53* gene was screened by fluorescence-based polymerase chain reaction-single-strand conformation polymorphism (F-SSCP) [5].

3. Results

Chromosome analysis of the bone marrow cells at the initial diagnosis of CML-AP (May 2004) showed 46,XY,t(7;14)(p22;q13),t(9;22)(q34;q11.2)[20], whereas the karyotype before CBT (April 2007) evolved to 45,X,-Y,der(7)t(7;14)(p22;q13),t(9;22)(q34;q11.2),-14,der(17)t(1;17)(q25;p13),+der(22)t(9;22)[20] (Fig. 2). SKY analyses confirmed these karyotypes (Fig. 3).

For further characterization of t(9;22) and der(17)t(1;17), we next performed FISH and molecular analyses on cells before CBT. As expected, two *BCR/ABL* fusion signals were observed on double Ph chromosomes. RT-PCR analysis detected the p210-type *BCR/ABL* fusion transcript (b3a2 type). Furthermore, 25 types of mutations in the *BCR/ABL* kinase domain, covering ATP binding loop, T315, M351 and A-loop, were screened [6], but there was no mutation in the kinase domain. On the other hand, *TP53* signal was deleted from the der(17)t(1;17). Mutations of the *TP53* gene in the remaining allele could not be detected as well (data not shown). Results of G-banding and FISH with *BCR/ABL* during the clinical course are summarized in Table 1.

4. Discussion

We have identified two additional translocations, t(7;14)(p22;q13) and der(17)t(1;17)(q25;p13), in CML-AP. To our knowledge, both translocations have never been described in the literature [7]. The t(7;14)(p22;q13), which had already appeared at the initial diagnosis, is supposed to have a crucial role in the progression to CML-AP. On the other hand, as shown in Table 1, the evolved clone with the der(17)t(1;17) gradually proliferated in spite of the treatment with imatinib and the acquisition of CHR, indicating the drug resistance to imatinib. In the present case, there was no well-characterized mutation in the *BCR/ABL* kinase domain. It has been reported that patients with clonal evolution are more likely to have *BCR/ABL*-independent mechanisms of resistance to imatinib although *BCR/ABL* is active and remains a good therapeutic target in many resistant patients [8]. Therefore, in this case, the resistance to imatinib may be due to the additional karyotypic abnormalities besides the Ph chromosome, but not to *BCR/ABL* mutations. The der(17)t(1;17), along with a concurrently found “major route” abnormality +Ph, appear to be implicated in the drug resistance and further development of the disease.

Chromosome bands 7p22 and 14q13 are recurrently involved in simple variant or complex three-way translocations in CML. That is, five cases with t(7;22)(p22;q11), six cases with t(7;9;22)(p22;q34;q11), and four cases with t(9;22;14)(q34;q11;q13) have been reported [7]. These results suggest that genes located at 7p22 and 14q13 may have some role in the progression of CML. It is possible that the fusion gene was generated on the der(7)t(7;14)(p22;q13) in the present case. Genes located at 7p22 include the ubiquitin-specific protease gene *USP42*, fused with *RUNX1* by t(7;21)(p22;q22) in acute myeloblastic leukemia and the ETS family gene *ETV1*, fused with *EWS* by t(7;22)(p22;q12) in Ewing sarcoma [9,10]. These genes might be associated with t(7;14)(p22;q13).

The unbalanced translocation der(17)t(1;17)(q25;p13) resulted in loss of the short arm of

chromosome 17 (17p) including the *TP53* gene. At the time of the der(17)t(1;17) appearance, dysplastic changes including pseudo-Pelger-Huët anomaly of neutrophils were detected in the bone marrow cells, while nuclear segmentation of neutrophils was normal at the initial diagnosis. Sessarego et al. [11,12] reported a possible correlation between unbalanced translocations leading to partial 17p deletion and the appearance of the pseudo-Pelger-Huët anomaly, which was found only in the AP/BC but not CP of CML. These findings indicate that the der(17)t(1;17) was also closely correlated with pseudo-Pelger-Huët anomaly and disease progression. The der(17)t(1;17) may look as a substitute for i(17q), a classical anomaly associated with BC [3].

Mutations of the *TP53* gene are often detected in CML-AP/BC, and are generally accompanied with loss of one *TP53* allele, which results in complete loss of function of the *TP53* gene [13,14]. However, no mutation of exons 5 to 8, covering “hot spots” for mutation in human tumors, in the remaining *TP53* allele was detected in the present case. Nakai et al. [14] reported that about half of the cases with loss of 17p did not show *TP53* inactivation, suggesting that loss of a 17p precedes *TP53* mutation. Mutations of the *TP53* gene may have not appeared yet because the clinical stage of the present case remained in AP but not progressed to BC. Further studies for additional cases will be required to clarify the pathological roles of t(7;14)(p22;q13) and der(17)t(1;17)(q25;p13) in CML.

Acknowledgements

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Table 1. Summary of cytogenetic analyses

Date	*Disease Status*	*Karyotypes*	*Number of cells with BCR/ABL fusion signals (zero/one/two signals)*
May 2004	CML-AP	46,XY,t(7;14)(p22;q13),t(9;22)(q34;q11.2)[20]	0/100/0
May 2006	CML-AP	46,XY,t(7;14)(p22;q13),t(9;22)(q34;q11.2)[18]/45,X,-Y,der(7)t(7;14)(p22;q13), t(9;22)(q34;q11.2),-14,der(17)t(1;17)(q25;p13),+der(22)t(9;22)[2]	49/48/3
January 2007	CML-AP	ND	28/8/64
March 2007	CML-AP	45,X,-Y,t(7;14)(p22;q13),t(9;22)(q34;q11.2)[2]/45,X,-Y,der(7)t(7;14)(p22;q13), t(9;22)(q34;q11.2),-14,der(17)t(1;17)(q25;p13),+der(22)t(9;22)[18]	15/23/62
April 2007	CML-AP	45,X,-Y,der(7)t(7;14)(p22;q13),t(9;22)(q34;q11.2),-14,der(17)t(1;17)(q25;p13), +der(22)t(9;22)[20]	2/16/82
July 2007	CR, after CBT	46,XY[20]	100/0/0
November 2007	CR, after CBT	46,XY[20]	ND

Abbreviations: CML, chronic myelogenous leukemia; AP, accelerated phase; CR, complete remission; CBT, cord blood transplantation; ND, not done. Numbers of *BCR/ABL* fusion signals were examined by fluorescence *in situ* hybridization on 100 interphase nuclei.

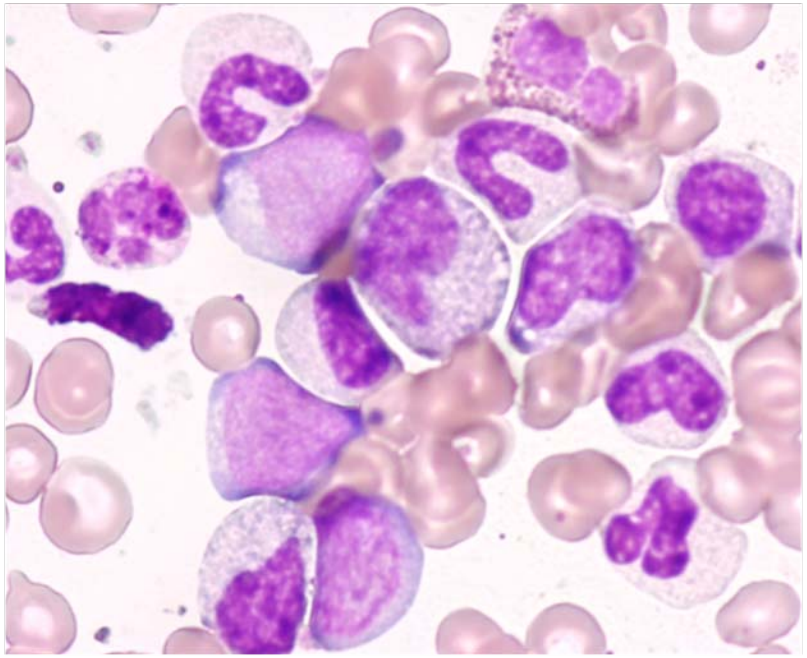
Figure legends

Fig. 1. Bone marrow smear at the initial diagnosis of CML (A) and before CBT (B to D) (x1000, May-Grünwald-Giemsa staining). (A) Myeloblasts and mature myeloid cells without apparent dysplastic changes, (B) myeloblasts, promyelocytes and increased number of eosinophils, (C) micromegakaryocytes (arrow) and megakaryocytes with non-lobulated nuclei, and (D) pseudo-Pelger-Huët anomaly, hypogranulation and ring-shaped nuclei of neutrophils, are shown.

Fig. 2. G-banded karyotype of the bone marrow cells at the initial diagnosis of CML. The karyotype is as follows: 46,XY,t(7;14)(p22;q13),t(9;22)(q34;q11.2). Arrows indicate rearranged chromosomes.

Fig. 3. Spectral karyotyping of the metaphase spread after spectrum-based classification before CBT. Chromosomes were assigned a pseudocolor according to the measured spectrum. The karyotype is confirmed as follows:
45,X,-Y,der(7)t(7;14)(p22;q13),t(9;22)(q34;q11.2),-14,der(17)t(1;17)(q25;p13),
+der(22)t(9;22). The grayscale images are reverse DAPI; the colored images, SKY. Arrows indicate rearranged chromosomes.

A



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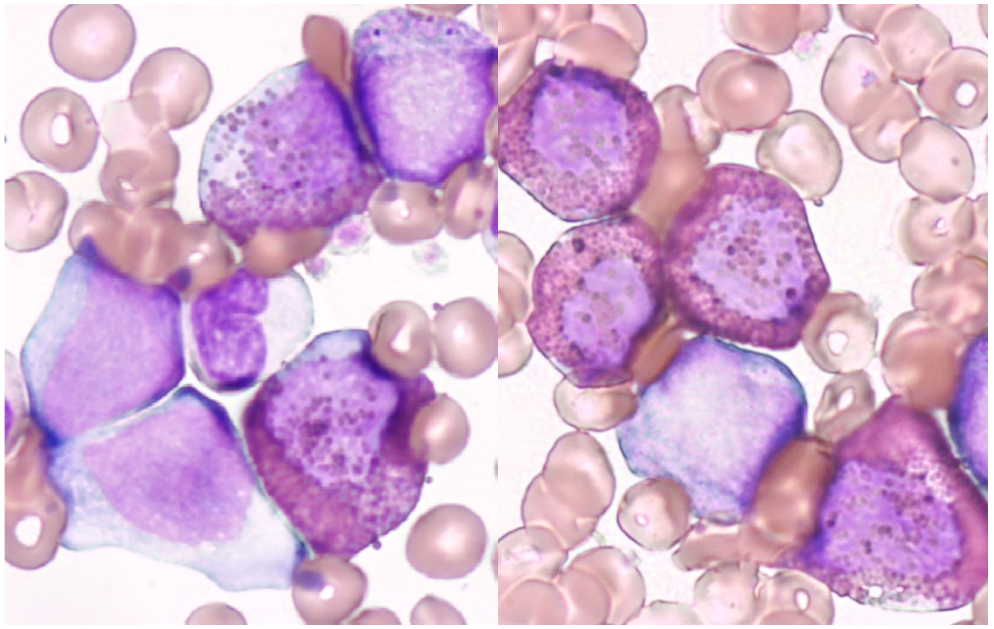

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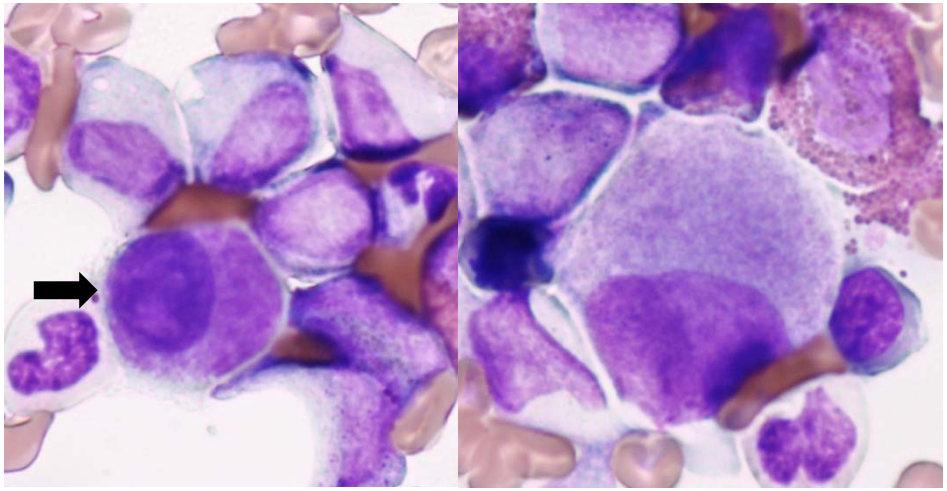

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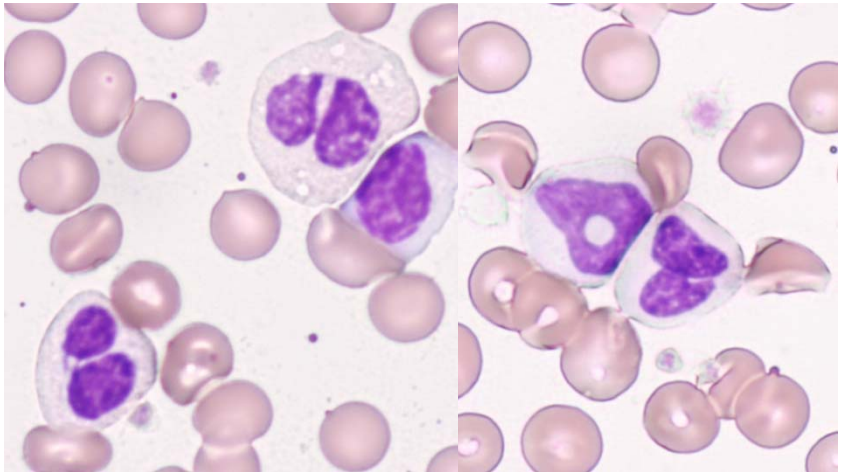

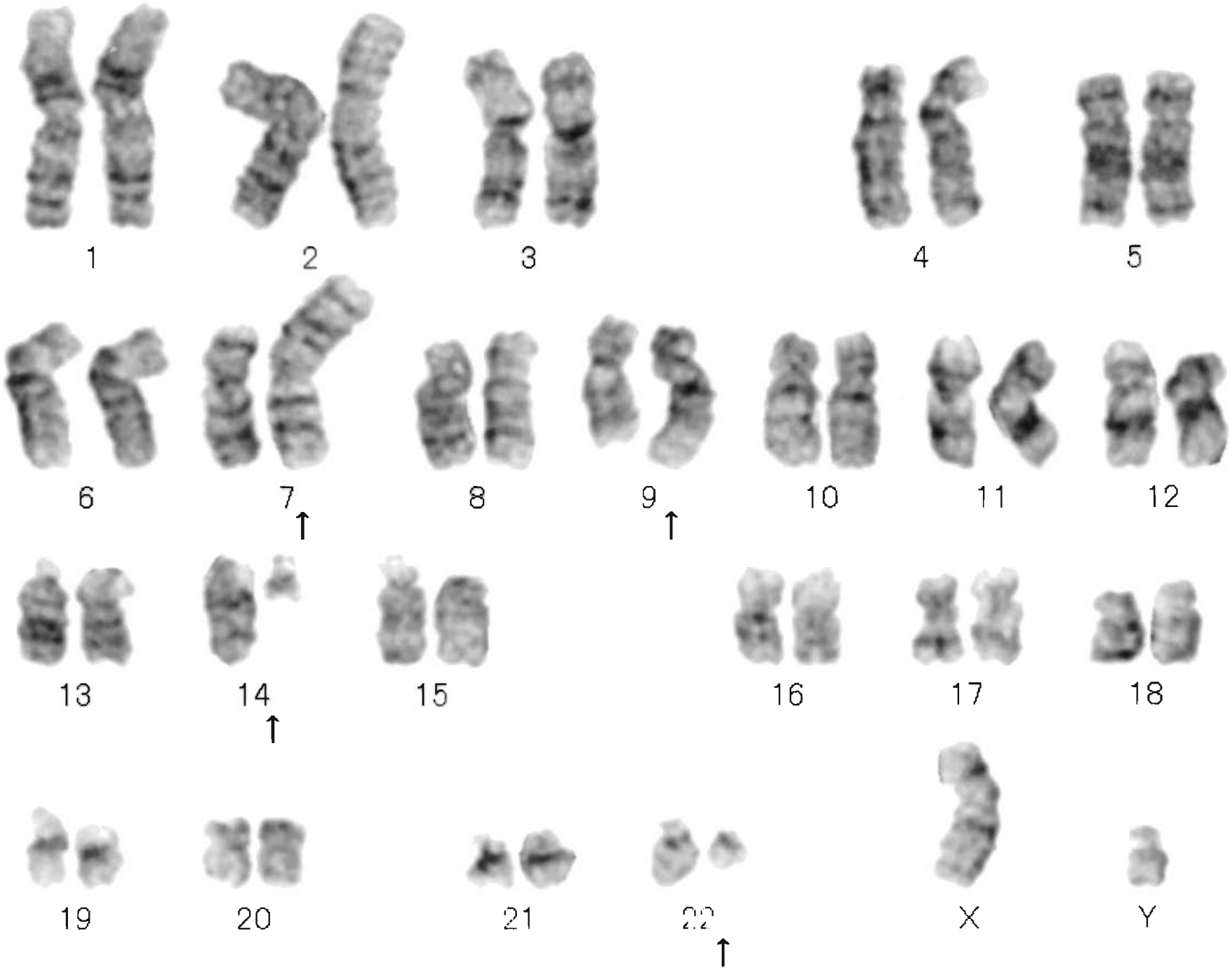
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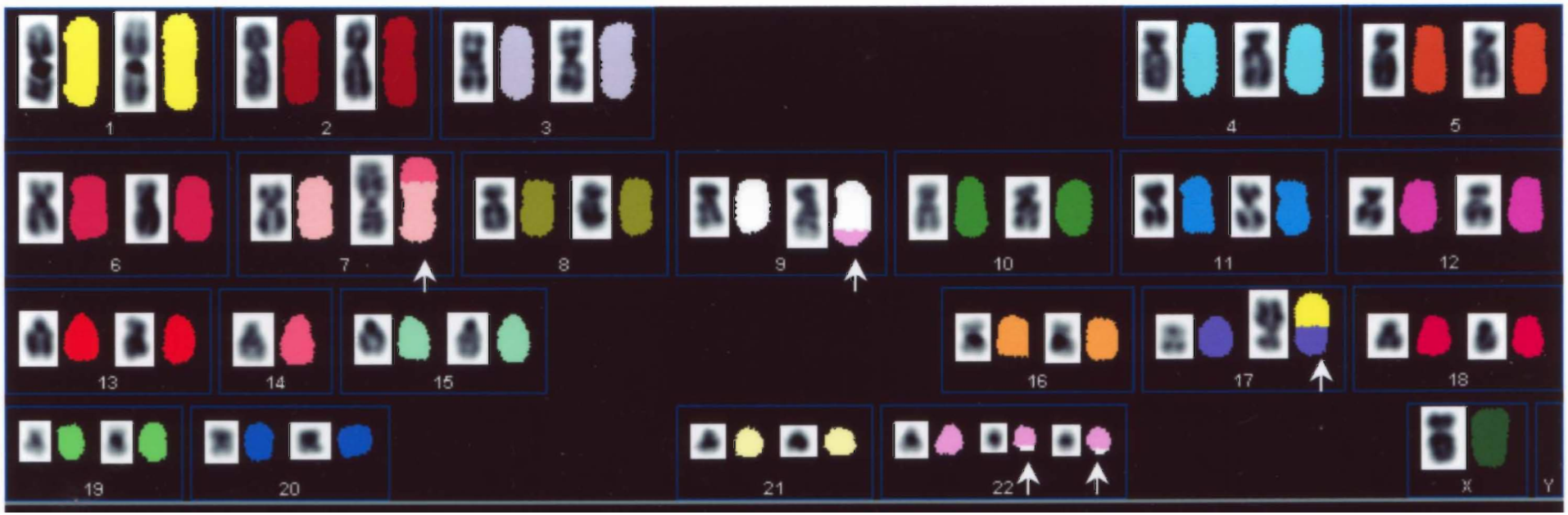
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