

Constitutional Pericentric Inversion 9 in Korean Patients with Chronic Myelogenous Leukemia

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Background : Although the pericentric inversion of chromosome 9, inv(9)(p11q13), is generally considered a normal variation, it is also associated with solid tumors and several hematologic malignancies such as biphenotypic acute leukemia, ALL, AML, and myeloproliferative neoplasms. However, to the best of our knowledge, there have been no reports that suggest an association between CML and constitutional pericentric inversion of chromosome 9. The purpose of this retrospective study was to investigate the frequency and clinical features of CML patients with concomitant inv(9) and t(9;22)(q34;q11.2) variation at our institution.

Methods : We reviewed the bone marrow chromosome database entries between October 2006 and December 2008 to identify patients with concomitant inv(9) and t(9;22) variations. Laboratory and clinical data of the patients were obtained from the electronic medical record system.

Results : Among the 51 CML patients, 4 (7.8%) had concomitant inv(9) and t(9;22) variations.

Conclusions : Although the association between inv(9) variation and CML is still controversial, we believe that hematologists should consider the role of constitutional inv(9) variation in CML patients to avoid overlooking the impaired engraftment potential of hematopoietic stem cells harboring inv(9). Therefore, we suggest that more effort should be invested to develop cytogenetic tests for detecting constitutional inv(9) variation in CML patients. (*Korean J Lab Med 2010;30:218-23*)

Key Words : CML, Constitutional inv(9)

INTRODUCTION

Pericentric inversion of chromosome 9, inv(9)(p11q13), is inherited as a non-pathogenic variation in normal population (0.8–2%); however, this variation is also reported to be associated with predisposition to the development of solid tumors or acute leukemias (ALs). Keung et al. [1] reported

that the prevalence of constitutional inv(9) variation in 799 adult AL cases was similar to that observed in the general population (0.8%). Huh et al. [2] reported that the incidence of constitutional chromosomal aberration in hematologic malignancies was similar to that of benign hematologic disorders (2.4% and 2.8%, respectively), and the inv(9) variation was frequently observed in both groups. However, the association between constitutional inv(9) variation and CML or ALL patients with Philadelphia (Ph) chromosome has been rarely documented [3]. To our knowledge, there have been no reports on the association between CML and constitutional pericentric inv(9) variation in any ethnic group. In this study, we investigated the frequency and clinical features of Korean CML patients with concomitant inv(9) and t(9;22).

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MATERIALS AND METHODS

We retrospectively reviewed the bone marrow chromosome database at the Severance Hospital of Yonsei University (Seoul, Korea) between October 2006 and December 2008. After selecting the Ph⁺ karyotypes, we excluded the repeated results from the same patients and identified cases with inv(9) variation among the Ph⁺ cases. Clinical and laboratory data were obtained by reviewing the U-Severance electronic medical record system.

RESULTS

Our database search revealed 103 Ph⁺ karyotypes. After excluding repeated results from the same patients, we identified 70 patients, including 51 patients of CML, 16 of ALL, and 3 of biphenotypic acute leukemia (BAL), with a t(9;22)(q34;q11,2) variation or *BCR/ABL1* rearrangement. Among the 70 patients who underwent bone marrow and chromosome analysis, 4 CML patients had concomitant inv(9) and t(9;22) variation or *BCR/ABL1* rearrangement. Among the other 19 Ph⁺ ALL or BAL patients, none had a pericentric inversion in chromosome 9.

1. Patient 1

A 33-yr-old Korean man was admitted to the Severance Hospital with left upper quadrant discomfort in June 2008. The initial complete blood count (CBC) results showed Hb level of 9.1 g/dL; platelet count of 810,000/ μ L; and white blood cell (WBC) count of 355,400/ μ L with 49% segmental neutrophils, 1% lymphocytes, 19% myelocytes, 4% metamyelocytes, 14% band forms, 4% eosinophils, 4% basophils, and 5% immature cells. Bone marrow aspiration and biopsy analysis revealed a hypercellular marrow with a significantly large number of granulocyte precursors and megakaryocytes and a high myeloid/erythroid (M/E) ratio (30:1). The initial chromosome analysis showed the 46,XY,inv(9)(p11q13)c,t(9;22)(q34;q11,2) karyotype in all 20 metaphase cells (Fig. 1). FISH with *BCR/ABL1* probe (Abbott Molecular/Vysis, Des Plaines, IL, USA) revealed the “nuc ish (*ABL1* \times 3),(*BCR* \times 3),(*ABL1* con *BCR* \times 2)[91/252]/(*ABL1* \times 2),(*BCR* \times 2),(*ABL1* con *BCR* \times 1)[29/252]/(*ABL1* \times 2),(*BCR* \times 3),(*ABL1* con *BCR* \times 2)[15/252]” karyotype, which was consistent with the abnormal pattern (1 or 2 fusions) in 53.6% of the nuclei examined. Gene rearrangement analysis was positive for *BCR/ABL1* (b3a2 type) (Fig. 2). The patient was receiving imatinib mesy-

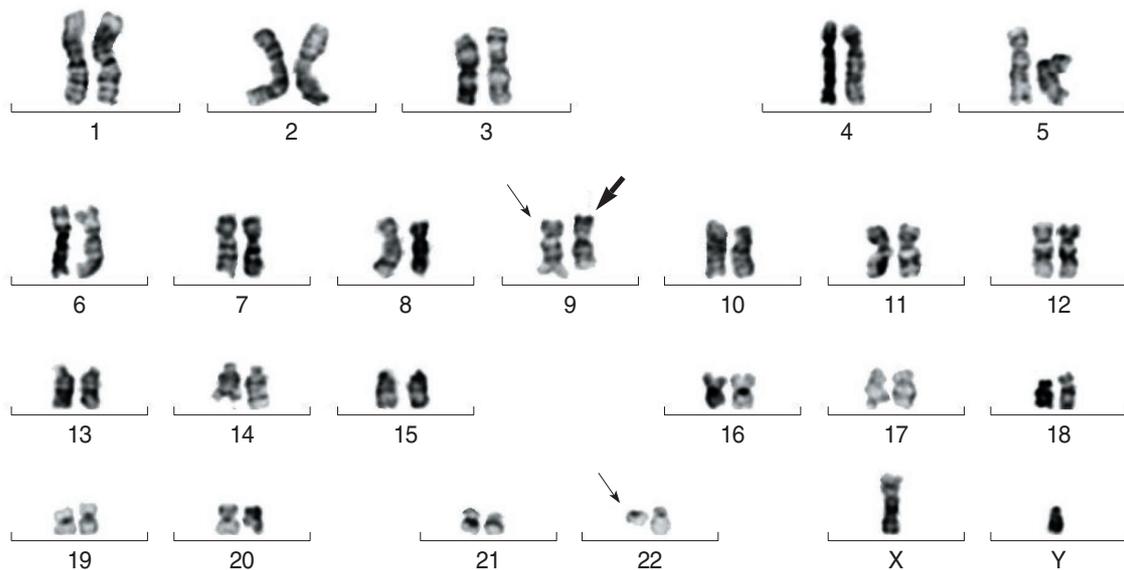


Fig. 1. The full karyogram of bone marrow cells of patient 1 is as follows: 46,XY,inv(9)(p11q13)c,t(9;22)(q34;q11.2). The bold arrow denotes inv(9)(p11q13) and the single arrows denote t(9;22)(q34;q11.2).

late (IM) after initial diagnosis, but we had to stop the IM therapy after 1 month due to thrombocytopenia. His follow-up bone marrow examination revealed 20–30% cellularity without hematologic relapse of CML. However, his second chromosome analysis still revealed the 46,XY,inv(9)(p11q13)c,t(9;22)(q34;q11.2) karyotype in 19 out of 20 analyzed cells.

2. Patient 2

A 64-yr-old man with a history of CML since July 2007 was brought to our hospital with fever and skin rashes for 3 days. He was first diagnosed with CML and treated with IM for 7 months. His CBC on arrival (February 2008) showed Hb level of 7.4 g/dL, platelet count of 10,000/ μ L, and WBC count of 550/ μ L. Bone marrow biopsy examination showed a hypocellular marrow. M/E ratio was not evaluated due to the paucity of hematopoietic cells. The karyotype of all 20 G-banded marrow metaphase cells was 46,XY,inv(9)(p11q13)c (Fig. 3). However, FISH with *BCR/ABL1* probe (Abbott Molecular/Vysis) revealed the “nuc ish (*ABL1* \times 3), (*BCR* \times 3), (*ABL1* con *BCR* \times 2)[165/222]” karyotype, which was consistent with the abnormal signal patterns observed in 34.0% of the examined nuclei. In addition,

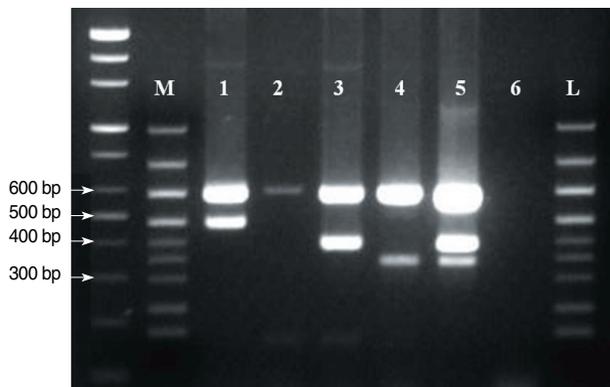


Fig. 2. Multiplex reverse transcriptase-PCR (RT-PCR) was performed using Seeplex Leukemia BCR/ABL kit (Seegene, Seoul, Korea). Lane M, molecular marker provided by manufacturer; Lane 1, sample from Patient 1 (b3a2 type); Lane 2, negative control; Lane 3, sample from Patient 3 (b2a2 type); Lane 4, positive control (e1a2 type); Lane 5, RT-PCR product of b2a2/e1a2 as a positive control; Lane 6, blank; Lane L, molecular size marker (100-bp ladder). Target and amplicon sizes are as follows: internal control (600 bp), b3a2 (476 bp), b2a2 (401 bp), and e1a2 (348 bp).

gene rearrangement analysis revealed a b2a2 type *BCR/ABL1* rearrangement. After 1 month, he was discharged from our hospital and referred to another regional institution.

3. Patient 3

A 45-yr-old man was brought to our hospital with epigastric discomfort and leukocytosis in December 2008. CBC showed Hb level of 10.1 g/dL; platelet count of 397,000/ μ L; and WBC count of 106,700/ μ L with 68% segmental neutrophils, 6% lymphocytes, 5% monocytes, 8% myelocytes, 4% metamyelocytes, 8% band forms, and 1% blasts. Bone marrow aspiration showed a hypercellular marrow with a significantly large number of myeloid precursors and megakaryocytes, which was consistent with the diagnosis of CML. Chromosome analysis revealed the 46,XY,inv(9)(p11q13)c,t(9;22)(q34;q11.2) karyotype in all 20 analyzed cells (Fig. 3). The FISH analysis for *BCR/ABL1* showed a “nuc ish (*ABL1* \times 3), (*BCR* \times 3), (*ABL1* con *BCR* \times 2)[165/222]” karyotype. Gene rearrangement analysis for *BCR/ABL1* was positive (b2a2 type) (Fig. 2). After being diagnosed with CML in chronic phase, he was discharged

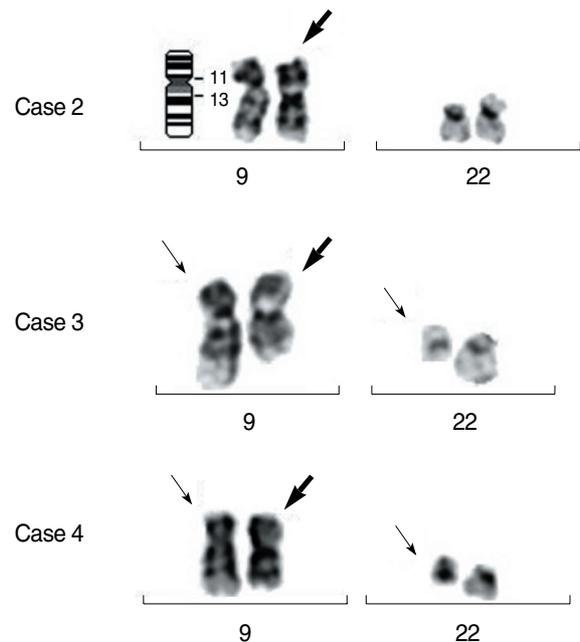


Fig. 3. The partial karyogram of bone marrow cells of patients 2, 3, and 4. The bold arrows denote inv(9)(p11q13) and the single arrows denote t(9;22)(q34;q11.2).

and treated with hydroxyurea on an outpatient basis.

4. Patient 4

A 40-yr-old woman was diagnosed with CML in chronic phase. Her chromosome analysis result in our database showed a 46,XX,t(9;22)(q34;q11.2),i(17)(q10) karyotype in 16 out of the 22 metaphase cells analyzed. However, when we reviewed our chromosome database, we detected an additional inv(9) variation in her karyogram (Fig. 3). Therefore, the revised karyotype of this patient was as follows: 46,XX,inv(9)(p11q13)c,t(9;22)(q34;q11.2),i(17)(q10)[16]/46,XX,inv(9)(p11q13)c[6]. Despite regular administration of IM, she developed neutropenic fever and septic arthritis. We suspected IM resistance because her condition deteriorated without any hematologic response and discontinued the IM therapy. HLA-matched transplantation was considered as an alternative treatment regimen.

DISCUSSION

There have been very few reports on the inv(9) variation as an acquired chromosomal abnormality in hematologic malignancies [4, 5], and such variations are generally regarded as a constitutional abnormality with familial inheritance. Keung et al. [1] have reported con-

stitutional pericentric inversion in chromosome 9 at a frequency of 0.8–2% in normal population and at a similar frequency in AL patients. Other studies reported little difference in the incidences of constitutional chromosome aberration between patients with hematologic malignancies and the general population; however, this may either be due to the low incidence of inv(9) [6–8] or the exclusion of inv(9) [9]. Although the association between inv(9) variation and CML is still a controversial topic, constitutional pericentric inv(9) may be important for predicting impaired engraftment potential of hematopoietic stem cells harboring inv(9) [10, 11].

A careful review of literature and Mitelman database revealed that concomitant inv(9) and t(9;22) variations occurred sporadically in 8 patients [2, 3, 12–17], which included 4 CML patients, 3 ALL patients, and 1 acute basophilic leukemia patient (Table 1) [7–13]. In this study, we analyzed the data of 70 Ph+ patients diagnosed with CML, ALL, and BAL (51, 16, and 3 patients, respectively). The inv(9) variation was not detected in any Ph+ ALL or BAL patient, but it was detected at a relatively high frequency (7.8%) in 4 out of 51 CML patients. The detection of inv(9) is considered important in other hematologic malignancies, including ALs [1, 18, 19]. However, there have been very few studies on inv(9) detection in CML, which indicates that this variation is either overlooked or underestimated. In the fourth case in our study, we

Table 1. Reported cases of concomitant inv(9) and t(9;22) variations in Ph+ leukemia patients

Case No.	Sex/Age/Country	Diagnosis	Karyotypes	References
1	F/55/South Africa	CML, aberrant translocation	46,XX,inv(9)(p11q13),t(9;22)(p11q11)/46,XX,t(9;22),i(17)(q10)	[7]
2	F/35/Korea	CML	47,XX,dup(1)(q21q44),+6,inv(9)(p11q12),t(9;22)(q34;q11)	[8]
3	F/55/Singapore	ALL, B-lineage	45,XX,add(7)(p13),inv(9)(p11q13),t(9;22)(q34;q11),-21	[9]
4	M/7/Italy	ALL, T-lineage	47,Y,del(X)(q21),del(3)(q23),del(5),inv(9)(p13q13)c,t(9;22)(q34;q11),add(10)(q26),+mar/47,idem,del(7)(p13)	[10]
5	M/4/United States	ALL	71,XXY,+Y,inv(9)(p11q13),t(9;22)(q34;q11)x2,+10,-16,+21	[11]
6	F/26/United States	Acute basophilic leukemia	46,XX,inv(9)(p11q13)c,t(9;22)(q34;q11)/46,XX,inv(9)c,t(9;22)(q13;q11)	[12]
7	M/24/Korea	CML	46,XY,inv(9)(p11q13)c,t(9;22)(q34;q11.2)	[13]
8	M/52/Korea	CML	46,XY,der(9)inv(9)(p11q13)ct(9;22)(q34;q11.2),der(22)t(9;22)	[13]
9	M/33/Korea	CML	46,XY,inv(9)(p11q13)c,t(9;22)(q34;q11.2)	Present study
10	M/64/Korea	CML	46,XY,inv(9)(p11q13)c	Present study
11	M/45/Korea	CML	46,XY,inv(9)(p11q13)c,t(9;22)(q34;q11.2)	Present study
12	F/40/Korea	CML	46,XX,inv(9)(p11q13)c,t(9;22)(q34;q11.2),i(17)(q10)	Present study

Abbreviations: Ph+, Philadelphia positive; F, female; M, male.

identified a previously undetected *inv(9)* variation as an additional chromosomal abnormality during retrospective review. Other laboratories may similarly overlook this variation partly due to neglect of its importance, and underestimate the frequency of *inv(9)*. In fact, in a study investigating the association between constitutional chromosomal abnormality and hematologic malignancy, the frequency of *inv(9)* in CML patients was observed to be 2.6% (2/77) [2]. The discrepancies in the frequencies may partly be due to the fact that the *inv(9)* variation confers an atypical chromosome morphology and complicates karyotypic analysis. Further, because the overall number of cases was limited in both the present and previous studies, the difference in frequency may not be significant [2]. More case studies are required for further elucidation of the *inv(9)* prevalence and clinical significance.

With rapid progress in pharmacogenetics, it is now possible to develop tailored pharmacotherapy, even for neoplastic diseases. IM has been used in tailored pharmacotherapy for the treatment of CML, clonal hypereosinophilic disorder, gastrointestinal stromal tumor, etc. Some patients develop resistance after IM therapy; however, this effect can be overcome with a standard dose or high-dose imatinib therapy in most CML patients. For patients with mutations that confer resistance to imatinib, new generation tyrosine kinase inhibitors like nilotinib and dasatinib may be effective. Thus far, no pharmacologic therapy has been effective for mutations like T315I, and stem cell transplantation is the only available treatment option.

A majority of CML patients showed significant improvement with the use of new tyrosine kinase inhibitors, but a small fraction of patients manifested multiresistance to these drugs. In such cases, additionally detected chromosomal abnormalities, whether acquired or constitutional, may partly influence the success of drug therapy or transplantation. We have suggested this possibility in the present study and our previous report [20], and emphasized the need for identifying additional chromosomal abnormalities in patients with drug resistance.

Being a retrospective analysis, the present study was limited by a small number of patients of a single ethnic group. Hence, we could not obtain data concerning further prognosis of transplantation in CML patients with *inv(9)*. The underscoring finding of our study was that the frequency of detecting constitutional pericentric *inv(9)* in Korean CML patients was not lower than that in normal population. This result suggested that constitutional chromosomal abnormalities, including *inv(9)*, should be more cautiously in CML patients of not only Korean population but of other ethnic groups as well. We think that the prevalence of constitutional *inv(9)* variation among CML patients is similar or slightly higher than that in the general population, although further studies in this regard are required. Therefore, on the basis of the results of our study and literature review, we propose that cytogenetic analysis should involve detection of constitutional *inv(9)* variation in CML patients.

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