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# Cytogenetic Profile of Variant Philadelphia Translocations in Chronic Myeloid Leukemia

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***Abstract-*** Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterised by the presence of the Philadelphia (Ph) chromosome which is due to the reciprocal translocation, t(9;22)(q34;q11.2). The translocation results in the *BCR-ABL1* fusion gene, encoding a constitutively active tyrosine kinase protein which causes the genesis of CML. About 5-10% of newly diagnosed Ph-positive CML patients have a variant translocation involving chromosomes 9 and 22, and one or more than one other chromosomes. The objectives of this study are to identify the chromosomes involved, breakpoints and additional chromosomal aberrations in variant Ph translocation. Conventional cytogenetic analysis is performed as a routine diagnostic test for all patients with hematological malignancies in our Cytogenetic Laboratory. Based on cytogenetic findings, 42 newly diagnosed CML patients with variant Ph translocation were selected for this study. Thirty nine patients (93%) had simple variant translocation and three patients (7%), complex variant translocation. Besides chromosomes 9 and 22, a total of 16 different chromosomes were involved in variant Ph translocation. The most frequently involved chromosomes were chromosomes 1 and 11. A total of 34 breakpoints were identified. Additional chromosomal abnormalities such as trisomies, translocations, and deletions were also observed. This shows that variant Ph translocations are very heterogeneous at the genetics level, with the involvement of different chromosomes and a diversity of breakpoints.

***Index Terms***- Chronic myeloid leukemia (CML), variant Philadelphia (Ph) translocations

## I. INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm. It is characterized by the presence of the Philadelphia (Ph) chromosome (derivative chromosome 22) which is due to the reciprocal translocation t(9;22)(q34;q11.2) [1]. The translocation results in the fusion of the 3' portion of the Abelson gene (*ABL1*) at chromosome 9q34 and the 5' portion of the breakpoint cluster region gene (*BCR*) at chromosome 22q11.2. The *BCR-ABL1* fusion gene generated encodes an oncogenic protein tyrosine kinase which causes the genesis of CML by activating multiple signalling pathways that are involved in the cell cycle, adhesion and apoptosis (2). The translocation t(9;22) can be detected by routine karyotyping. About 5-10% of newly diagnosed CML patients have a variant translocation involving chromosomes 9 and 22, and one other chromosome (simple variant translocation), or more than one other chromosomes (complex variant translocation) [3]. Sometimes, the chromosome changes are submicroscopic, so the translocation can be masked and detected by fluorescence in situ hybridization (FISH) or molecular studies.

Variant Ph breakpoints usually occur in the G-light bands, within the cytosine (C) and guanine (G) richest regions of the genome. CG content is related to chromatin condensation and transcription activity. Open chromatin is transcriptionally active and likely to undergo breakage and repair, and hence resulting in illegitimate recombination and translocation (4). The objectives of this study are to identify the chromosomes involved, breakpoints, and additional chromosomal aberrations in newly diagnosed CML patients in chronic phase (CP) with variant Ph translocation.

## II. MATERIALS AND METHODS

**Patients**

Conventional cytogenetic analysis (CCA) is performed by our Cytogenetics Laboratory on all patients with hematological malignancy as a routine diagnostic test. Based on cytogenetic findings (from the year 2004 - 2013), a total of 42 newly diagnosed CML patients in CP with variant Ph translocation were included in this retrospective study. The median age was 51.5 years (range: 12 to 77 years). 31 patients (73.8%) were male and 11 patients ( 26.2%), female.

**Cytogenetic Analysis**

About 1-2 ml of bone marrow aspirate from patients with leukemia were collected in transport media containing RPMI 1640 and sodium heparin. CCA was performed on the bone marrow cells according to standard procedures. The bone marrow cells were cultured overnight without the addition of any mitogen. Chromosomes were G-banded and analysed using the Applied Imaging Cytovision System (Genetix, UK). Karyotype designation was according to the International System for Human Cytogenetic Nomenclature (ISCN 2009) [5] at the time of cytogenetic analysis.

## III. RESULTS

The cytogenetic findings of the 42 newly diagnosed CML patients with variant Ph translocation are shown in Table 1. Out of 42 newly diagnosed CML patients, 39 (93%) patients had simple variant translocation (SVT) and three patients (7%) had complex variant translocation (CVT). In addition to chromosomes 9 and 22, the other chromosomes involved in variant translocations are chromosomes 1, 2, 3, 4, 5, 7, 8,10, 11,12, 14, 16, 17, 21, 22, and Y (Table 1). The chromosomes most frequently involved were chromosome 11 (9 patients,

Patient No. 16-24) [21.4%] followed by chromosome 1 (4 patients, Patient No. 1-4) [9.5%]. Although the Ph chromosome was present, it was not possible to identify the third chromosome involved in the translocation in two patients (Patient No. 38 and 39). In these two patients the segment 22q11.2 was apparently deleted. Fig. 1 shows the karyotype of a patient with SVT involving three chromosomes. Three patients (Patient No. 40, 41, and 42) had CVT. Patient No. 40 had CVT involving four chromosomes, t(9;22;3;10). Patient No. 41 and No. 42 had CVT involving five chromosomes, t(9;22;7;3;16) and t(2;9;22;16;5) [Fig.2] respectively.

A total of 34 breakpoints involved in variant Ph translocation were identified (Table 2). Recurring breakpoints were most frequently observed at 11q13 (4 patients; Patient No. 19, 20, 23, and 24) followed by 1q21 (3 patients; Patient No. 1-3). Recurring breakpoints were also found at 8q22, 11p15, 12q13, 14q32, and 21q22 (2 patients, each breakpoint). Besides the variant translocation, four patients (Patient No. 15, 17, 33 and 34) had additional chromosomal aberrations. Patient No. 17 had trisomies of chromosomes 8 and 12. Patient No. 15 and 33 had an additional translocation, t(1;17) and t(6;11) respectively. Patient No. 34 had additional chromosomal material on 19q.

## IV. DISCUSSION

Variant Ph translocations are observed in about 5-10% of CML patients at diagnosis, and their occurrence is not associated with disease evolution. A majority of our newly diagnosed CML patients (93%) showed a 3-way translocation involving three different chromosomes (SVT). Three patients had CVT (7%) involving four or five chromosomes. In addition to chromosomes 9 and 22, a total of 16 different chromosomes were involved in variant Ph translocation. The two most frequently involved chromosomes in our study were chromosome 11 (21.4%) and 1 (9.5%). Marzocchi *et al.*, (2011) [6] and Chauffaille *et al*., (2014) [7] reported that the most frequently involved chromosome was 17. Other studies have reported the involvement of chromosomes 6, 13, 15, 18, 19, 20 and X, in variant Ph translocation (6, 8), although this was not observed in our study. This could be due to the small sample size in our study. A total of 34 breakpoints were identified. The recurring breakpoints observed in our CML patients were 1q21; 8q22, 11p15, 11q13; 12q13, 14q32, and 21q22. Reid *et al*., (2003) [9] listed nine recurring breakpoints in variant Ph translocation (3p21, 3q21, 6p21, 11q13, 12p13, 17p13, 17q21, 17q25, and 22q13), and most of these breakpoints occur in regions of known oncogenes, fragile sites, or typical secondary breakpoints in other cancers.

Besides the variant Ph translocation, additional chromosomal abnormalities such as trisomies, translocations and deletions were also observed in our patients. Deletions of the derivative chromosome 9 are found at a higher frequency in variant translocation compared to t(9;22) standard translocation, probably due to genomic instability [3]. Marzocchi *et al*., (2011) [6] reported that the clinical characteristics and outcome of CML patients with variant Ph translocation are similar to those with standard Ph translocation when treated with imatinib mesylate as frontline therapy. Their data showed that variant translocations had no impact on cytogenetic and molecular response, regardless of the involvement of different mechanisms and chromosomes, or the presence of deletions in the imatinib era. The European LeukemiaNet Recommendations for the management of CML (2013) [10] stated that variant translocations and chromosome 9 deletions have no prognostic value when tyrosine kinase inhibitors are used as frontline therapy (11, 12), whereas the Ph chromosome with any clonal cytogenetic abnormalities such as trisomy 8, an additional Ph chromosome, isochromosome 17q, and trisomy 19 confer an adverse prognostic value (12, 13).

## V. CONCLUSION

In our retrospective study of 42 newly diagnosed CML patients with variant Ph translocation, 93% had SVT and 7% had CVT. In addition to chromosomes 9 and 22, a total of 16 different chromosomes were involved in variant translocation. A total of 34 different breakpoints were also identified. Recurring breakpoints were most frequently observed at 1q21 and 11q13. Additional chromosomal aberrations were also present in some patients. This shows that variant Ph translocations are very heterogeneous genetically with the involvement of different chromosomes and a diversity of breakpoints. However, studies have reported that variant Ph translocations are of no prognostic value compared to standard Ph translocation in CML patients in the imatinib era.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest

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**Table 1: Cytogenetics Findings in variant Philadelphia translocation in chronic myeloid Leukemia**

| Patient No. | Age | Sex | Karyotype |
|---|---|---|---|
| 1 | 38 | M | 46,XY,t(1;9;22)(q21;q34;q11.2)[6]/46,XY[2] |
| 2 | 28 | F | 46,XX,t(1;9;22)(q21;q34;q11.2)[20] |
| 3 | 48 | M | 46,XY,t(1;9;22)(q21;q34;q11.2)[3]/46,XY[9] |
| 4 | 59 | F | 46,XX,t(1;9;22)(p13;q34;q11.2)[20] |
| 5 | 33 | F | 46,XX,t(3;9;22)(q29;q34;q11.2)[20] |
| 6 | 51 | M | 46,XY,t(4;9;22)(p16;q34;q11.2)[20] |
| 7 | 59 | M | 46,XY,t(5;9;22)(q13;q34;q11.2)[20] |
| 8 | 62 | M | 46,XY,t(5;9;22)(q35;q34;q11.2)[20] |
| 9 | 77 | M | 46,XY,t(7;9;22)(p11.2;q34;q11.2)[12] |
| 10 | 34 | F | 46,XX,t(8;9;22)(q22;q34;q11.2)[3]/46,XX[4] |
| 11 | 28 | M | 46,XY,t(8;9;22)(q24;q34;q11.2)[20] |
| 12 | 67 | M | 46,XY,(8;9;22)(q22;q34;q11.2)[10] |
| 13 | 66 | F | 46,XX,t(9;22;10)(q34;q11.2;q22)([5] |
| 14 | 57 | M | 46,XY,t(9;22;10)(q34;q11.2;q24)[6] |
| 15 | 64 | M | 46,XY,t(1;17)(q35;q25),t(9;22;10)(q34;q11.2,q24)[10] |
| 16 | 26 | M | 46,XY,t(9;22;11)(q34;q11.2;q24)[14] |
| 17 | 64 | F | 48,XX,t(9;22;11)(q34;q11.2;q25),+8,+12[14]/ 48,XX,t(9;22;11)(q34;q11.2;q25)[10] |
| 18 | 57 | F | 46,XX,t(9;22;11)(q34;q11.2;p15)[9]/46,XX[6] |
| 19 | 58 | M | 46,XY,(9;22;11)(q34;q11.2;q13)[8] |
| 20 | 26 | M | 46,XY,(9;22;11)(q34;q11.2;q13)[10] |
| 21 | 32 | M | 46,XY,t(9;22;11)(q34;q11.2;p15)[14]/46,XY[6] |
| 22 | 25 | M | 46,XY,t(9;22;11)(q34;q11.2;p11.2)[20] |
| 23 | 57 | M | 46,XY,t(9;22;11)(q34;q11.2;q13)[13] |

| | | | |
|---|---|---|---|
| 24 | 36 | M | 46,XY,t(9;22;11)(q34;q11.2;q13)[20] |
| 25 | 40 | M | 45,X,-Y,t(9;22;12)(q34;q11.2;q13)[15] |
| 26 | 61 | F | 46,XX,t(9;22;12)(q34;q11.2;q13)[7]/46,XX[8] |
| 27 | 22 | M | 46,XY,t(9;22;12)(q34;q11.2;q24.3)[15] |
| 28 | 61 | M | 46,XY,t(9;22;14;)(q34;q11.2;q32)[20] |
| 29 | 58 | M | 45,X,-Y,t(9;22;14;)(q34;q11.2;q32)[20] |
| 30 | 64 | M | 46,XY,t(9;22;14)(q34;q11.2;q24)[3]/46,XY[15] |
| 31 | 12 | F | 46,XX,t(9;22;17)(q34;q11.2;p13)[20] |
| 32 | 40 | M | 46,XY,t(9;22;17)(q34;q11.2;q24)[20] |
| 33 | 43 | M | 46,XY,t(6;11)(q11;q11.1),t(9;22;17)(q34;q11.2;q21)[21]/ 46,XY[3] |
| 34 | 52 | F | 46,XX,t(9;22;21)(q34;q11.2;q22)[11]/ 46,XX,t(9;22;21)(q34;q11.2;q22),add(19)(q13)[10] |
| 35 | 59 | M | 46,XY,t(9;22;21)(q34;q11.2;q22)[20] |
| 36 | 31 | M | 46,XY,t(9;22;22)(q34;q11.2;q13)[20] |
| 37 | 56 | M | 46,XY,t(Y;9;22)(p11.2;q34;q11.2)[10] |
| 38 | 65 | M | 46,XY,t(9;22; ?)(q34;q11.2,?),?del(22)(q11.2)[10] |
| 39 | 40 | M | 46,XY,t(9;22; ?)(q34;q11.2,?),add(9)(q34),?del(22)(q11.2)[5] |
| 40 | 45 | F | 46,XX,t(9;22;3;10)(q34;q11.2;p21;p11)[14] |
| 41 | 56 | M | 46,XY,t(9;22;7;3;16)(q34;q11.2,q22;p25;q24)[10] |
| 42 | 50 | M | 46,XY,t(2;9;22;16;5)(q11;q34;q11.2;p13;q31)[7]/46,XY[3] |

**Table 2: Breakpoints involved in Variant Phildelphia (Ph) Translocation in Chronic Myeloid Leukemia**

| Breakpoints in Variant Ph Translocation | | | |
|---|---|---|---|
| 1p13 | 5q35 | 11q13 | 16q24 |
| 1q21 | 7q11 | 11p15 | 17p13 |
| 2q11 | 7q22 | 11q24 | 17q21 |
| 3p21 | 8q22 | 11q25 | 17q24 |
| 3p25 | 8q24 | 12q13 | 21q22 |
| 3q29 | 10p11 | 12q24 | 22q13 |
| 4p16 | 10q22 | 14q24 | Yp11 |
| 5q13 | 10q24 | 14q32 | |
| 5q31 | 11p11 | 16p13 | |

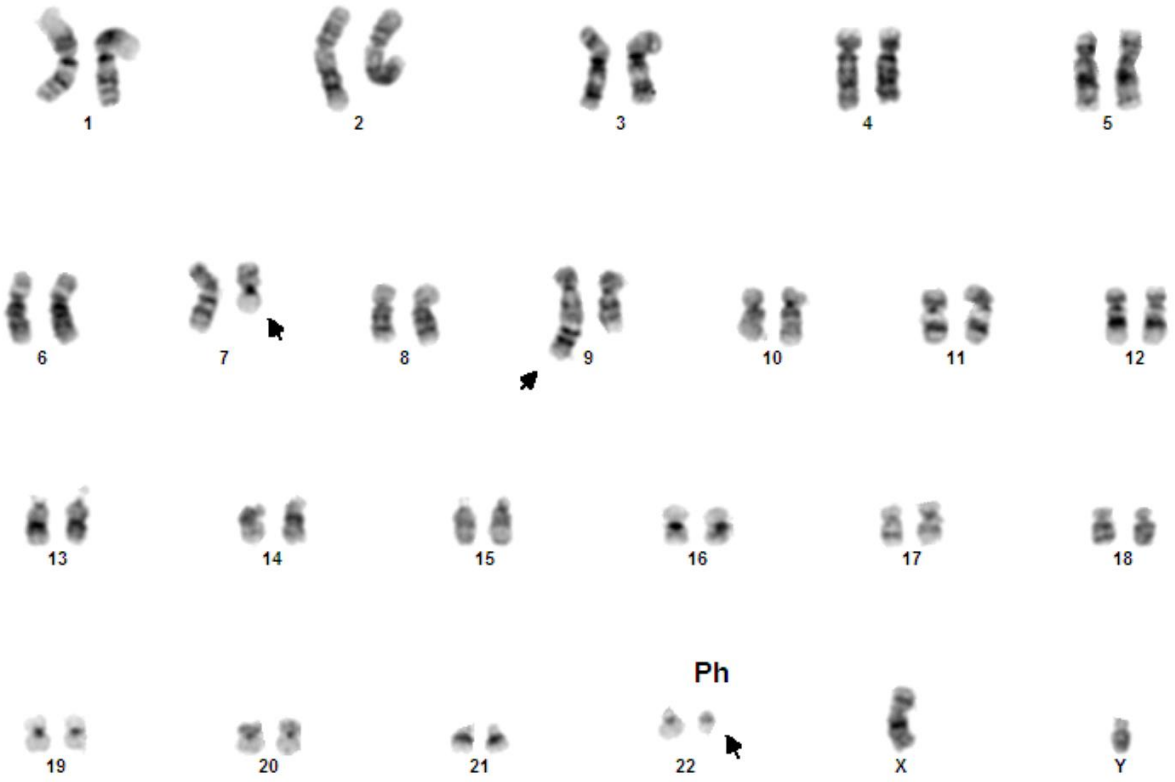


**Fig. 1 Karyotype of a chronic myeloid leukemia patient with simple variant translocation, 46,XY,t(7;9;22)(q11;q34;q11.2)**

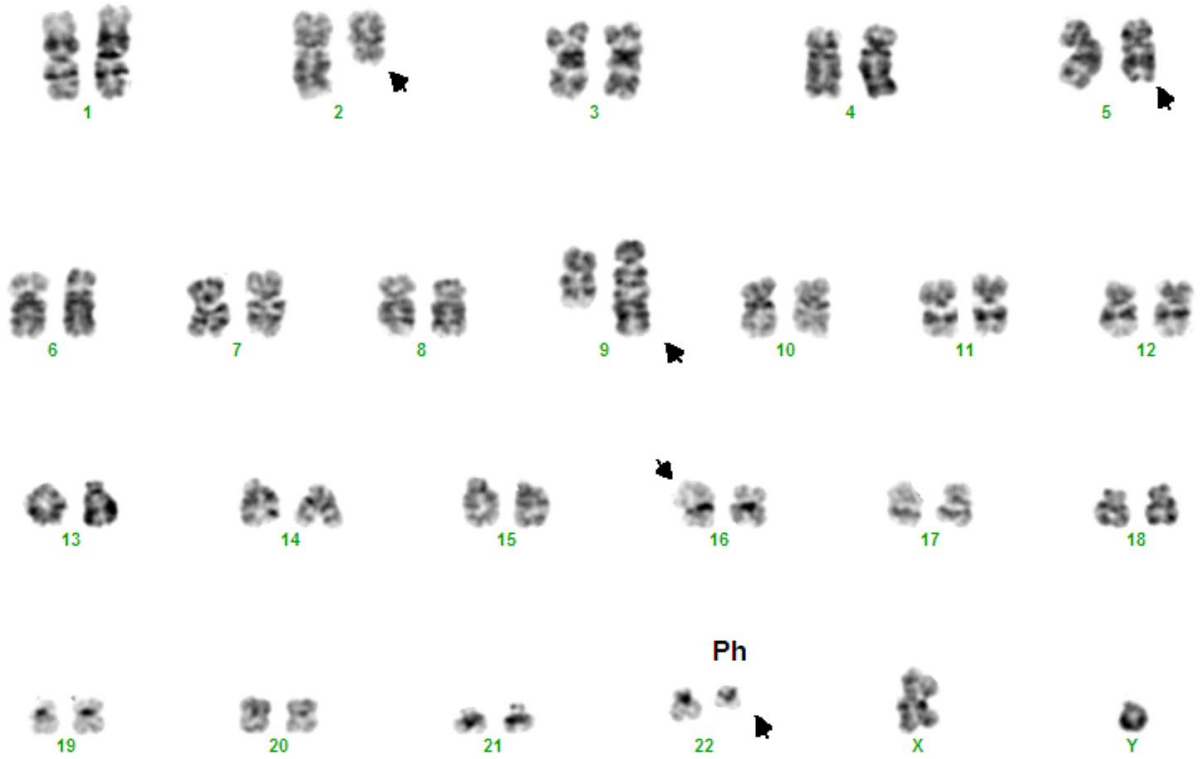


**Fig. 2 Karyotype of a chronic myeloid leukemia patient with complex variant translocation, 46,XY,t(2;9;22;16;5)(q11;q34;q11.2;p13;q31)**