A Novel In-Frame 231bp Deletion Mutation in ABL1 Kinase Activation Loop

Abstract

Tyrosine kinase domain (TKD) mutation is one of the most common causes for tyrosine kinase inhibitors' resistance in patients with chronic myeloid leukemia (CML). Mutations in the exon 7 of ABL1 gene are one of the most common TKD mutations, especially in the Indian population, but they are frequently underreported, and their clinical significance is not clear. We are reporting a novel ABL1 exon 7 mutation in a previously diagnosed and treated patient CML who presented at the blast crisis stage. Cytogenetic studies showed multiple copies of Philadelphia (Ph) chromosome along with isochromosome 17. Kinase domain mutation studies showed a novel 231bp in-frame deletion mutation (p. 372_448del) in the activation loop of BCR-ABL1 chimeric protein. The given mutation would result in a complete loss of activation loop, including DFG domain-regulating activation status of the catalytic domain. This mutation, along with cytogenetic abnormalities, could have contributed to progression to blast crisis.

Keywords: ABL1 exon 7 mutation, chronic myeloid leukemia, novel mutation, tyrosine kinase inhibitor resistance

Introduction

Tyrosine kinase inhibitors have become an integral part of the standard treatment protocol for chronic myeloid leukemia (CML) and are usually associated with excellent outcomes.[1] However, a subset of patients exhibit imatinib resistance, and ABL1 kinase domain mutation is one of the most common causes for the same.[2‑4] Although point mutations involving different domains of ABL1 kinase are reported frequently, large deletion mutations involving ABL1 exon 7 are also common, especially 185bp deletion involving exon 7 (c. 1086_1270del, p.R362fs*21) which has been reported in up to 25% of CML, at different time points.[5‑6] Pathogenic potential of these mutations remains unexplained; however, affected domains are critical for ABL1 kinase activity, and loss of these domains may potentially affect the activity of enzymes and/or interfere with imatinib binding.[6‑8] We are reporting a novel 231bp deletion mutation involving exon 7–8 resulting in the loss of the regulatory activation domain.

Case Report

A 38-year-old male patient, previously diagnosed with CML, presented with cough, fever, altered sensorium, and other features suggestive of sepsis. He was diagnosed with CML in 2008 and was treated with imatinib (400 mg BD); however, no records containing documentation of treatment responses were available. Since the last 6 months, imatinib was stopped, and the patient was treated with hydroxyurea. Physical examination revealed pallor and massive splenomegaly. Peripheral blood smear examination revealed leukocytosis (white blood cell count: 242,6 × 10^9/L) with 30% blasts. Chromosomal analysis revealed two clones: smaller clone (two metaphases) showed t(9;22) (Ph chromosome) as the sole anomaly, while larger clone (18 metaphases) showed the presence of additional chromosomal abnormalities: isochromosome 17q and three copies of Ph chromosome. Tyrosine kinase domain mutation analysis was performed using peripheral blood specimen.

RNA extraction was performed with Qiagen RNA blood mini kit (Qiagen, Germany), and cDNA was prepared using high-capacity cDNA reverse transcriptase kit (Applied Biosystems, Foster City, CA) and reverse transcribed using oligo-d(T) primers. PCR amplification was performed to amplify exon 7–8 of ABL1 kinase activation loop. PCR primers (p. 372_448; p. 501_571) were designed for the amplification of ABL1 kinase activation loop. The PCR products were sequenced and analyzed for mutations. A novel 231bp in-frame deletion mutation in ABL1 kinase activation loop was identified (p. 372_448del).

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transcription kit (Applied Biosystems, CA). Complete
ABL1 kinase domain of BCR-ABL1 fusion transcript was
amplified by the nested polymerase chain reaction (PCR)
technique as described by Alikian et al.[8] PCR products
were sequenced using BigDye Terminator v3.1 cycle
sequencing reaction, and products were analyzed on
ABI3500 Genetic analyzer (Applied Biosystems, Foster
City, USA). Sequences were compared with ABL1
reference sequence (NM_005157).

Sequencing revealed the presence of a 231 bp deletion
mutation (CDS: c.1113-1343del231, p. 372-448del), an
in-frame mutation causing deletion of 77 amino
acids [Figure 1], resulting in a complete loss of activation
loop along with the adjoining regions of C-terminal lobe.
No other pathogenic variants were identified. This mutation
was not reported either in COSMIC database or as an
alternative transcript in NCBI or Ensembl databases (as
assessed in October 2018).

The deleted sequence was flanked on the either end
by GU sequence, the most common splicing acceptor
site. The proximal end of the deleted region showed
AG sequence, a common splicing donor site (5'-GGU/
AGGGG… GU/GUAU-3'). These findings raised
a possibility of alternative splicing as a potential
mechanism for this mutation. Common exon 7 mutation
del185bp (c. 1086_1270 del185bp) also involves the
same region, and it is postulated that the given mutation
could be the result of alternative splicing.[5,6] Interestingly,
del231bp starts 27bp downstream of del185bp mutation,
at the next potential donor splice site (CCACAG/
AGAUCUGCUG-CCCGAAACUGCCUG)
AG sequence, a common splicing donor site (5'
AG
AG
GU
site.	Furthermore,	imatinib
can	bind	only
to	ABL1 protein
which	is	in	inactive	state.	del231bp	mutation
can	lead
to	the
to	switching	between	active	and	inactive	states	and
can	potentially	reduce	imatinib	binding,
thus	resulting	in	imatinib
to
in	matinib	resistance.

However,
the
hypotheses
are
based
on
the
protein
structure
prediction
models,
and
it
is
essential
that
these
findings
are
confirmed
with
additional
studies.

The presence of this mutation was associated with blastic
transformation. However, alteration of treatment and clonal
evolution in the form of acquisition of additional poor
risk abnormalities (isochromosome 17q and additional
copies of Ph chromosome) may have contributed to blastic
transformation. TKD mutation analysis was not performed
before blastic transformation and hence, it could not
be ascertained whether the given mutation was present
before or has developed during clonal evolution and was
responsible for disease progression.

Although exon 7-8 mutations are common in CML,
especially in the Indian population,[9] they are relatively
underreported. This is probably due to the inability of the
commonly used primer(s) to cover area during testing,[5,6]
lack of the understanding regarding the pathogenicity of
these mutations, their contribution to imatinib resistance,
and prognostic-therapeutic implications of detecting
these mutation(s).[9,10] Hence, a large study evaluating
prognostic and therapeutic significance of these mutations
is warranted.

![Figure 1: (a) Sanger sequencing showing the presence of deletion mutation. (b) Deletion mutation caused loss of 77 amino acids coded in exons 7 and 8](image)
Conclusion

We are reporting a novel 231bp deletion mutation in the ABL1 kinase activation loop domain (exons 7 and 8) of BCR-ABL1 fusion transcript in a patient of CML-blast crisis. This mutation caused in-frame deletion of 77 amino acids, resulting in loss of the activation loop and a part of the C-terminal lobe. This mutation may have arisen due to the alternative splicing, a mechanism similar to a common del185bp mutation.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that his name and initials will not be published and due efforts will be made to conceal his identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References