Brief Report
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Detection of an Insertion in the ATXN3 Gene in Chronic Myeloid Leukemia Cases Using Exome Sequencing

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Abstract

Background: Chronic myeloid leukemia (CML) is a common type of cancer. Leukemia is associated with diverse molecular and genetic changes, including loss and gain of chromosomes, gene deletions, duplications, point mutations, and gene fusions derived from chromosomal translocations. Advanced genetic tests are done to diagnose leukemia; however, many patients are not diagnosed because many of the symptoms are vague, unspecific, and referable to other diseases.

Method: Following the extraction of genomic DNA from CML patient, we performed exome sequencing. Variants were detected via GATK software. Novel alterations in CML cases were then visualized in the integrated genome browser. To enrich our findings, we included exome sequencing data pertaining to 11 individuals. The data was deposited in the ENA database in our analysis. Afterwards, we verified insertion in the ATXN3 gene through performing PCR reactions for both healthy and CML cases.

Results: We identified an alteration in the genomic sequence of the ATXN3 gene in the CML cases.

Conclusion: A correlation existed between insertion in the ATXN3 gene and positive CML cases. These findings might be conducive to the detection of CML at the early stages of the disease.

Keywords: Chronic myeloid leukemia, Exome sequencing, ATXN3

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Introduction

Leukemia is associated with diverse molecular and genetic changes, including loss and gain of chromosomes, gene deletions, duplications, point mutations, and gene fusions derived from chromosomal translocations.[1] These genetic alterations lead to either the improper expression or the creation of a novel product with altered activity.[2] Diagnosis is usually based

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on repeated complete blood counts and a bone marrow examination after observing the symptoms. However, in rare cases, blood tests may not reveal leukemia; this is usually because the disease is in the early stages or has entered remission.[3] Advanced genetic tests are used to diagnose leukemia; nonetheless, many people are not diagnosed as many of the symptoms are vague, unspecific, and referable other diseases. One of the most useful applications of NGS is the early detection of cancer. The cost of sequencing is decreasing exponentially; therefore, it is expected to be employed as the primary tool for the early detection of chronic myeloid leukemia (CML). Whole exome sequencing allows for the identification of a large number of mutated genes in CML harbour prognostic and predictive significance as they contain ASXL1 and TP53, both found to be mutated at the CP and BC stages.[4]

Menezes and colleagues described the mutations in genes such as TP53, NPM1, IKZF1, RUNX1, and ASXL1 in CML progression.[5] They obtained a total of 719,1839 and 869 single-nucleotide substitutions (SNSs) and small insertions and deletions (in/dels) for CP, CCyR, and BC, respectively. They further reported that TP53 mutation (p. G244S) was present in all disease stages. This indicates its potential in the initiation of these types of cancer. Interestingly, ASXL1, UBE2G2, ZEB2, and IKZF3 were present only in the CP and BC stages. Additionally, mutations in ARSD, FRG2Cand AADACL3 might contribute to leukemic transformation.[6] Mutations in ASXL1 during CML progression were described for the first time by Boultwood et al.[7] ASXL1 has since been observed as a commonly affected gene in similar frequencies. Taken together, these data corroborate that ASXL1

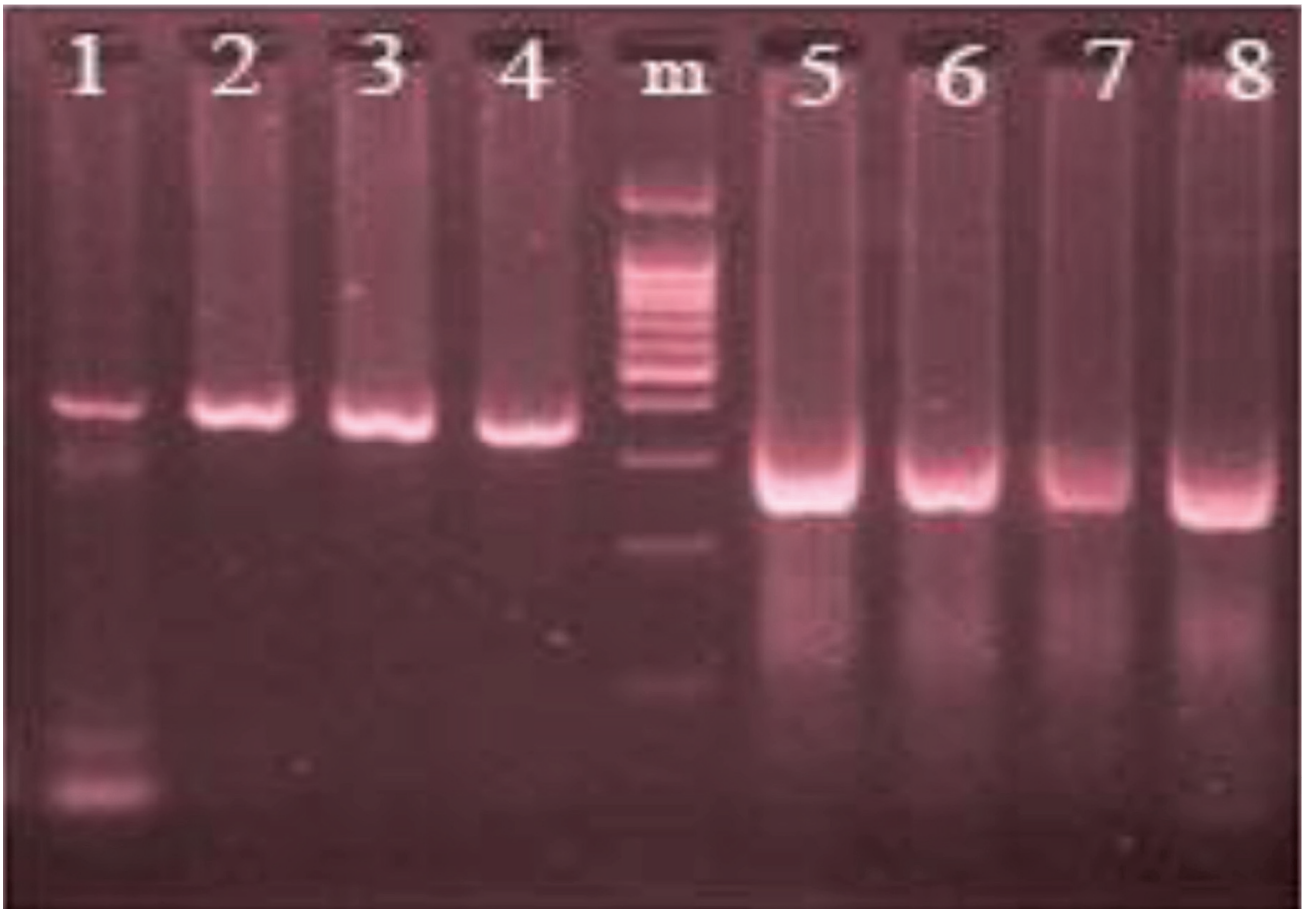

Figure 1. A sample image of an agarose gel run for the CML samples and controls. PCR products of ATXN3 gene for CML and control cases were resolved on a 2 % agarose gel. Lane m; 100 bp marker. Lanes 1,2,3, and 4 samples from CML samples. Lanes 5, 6,7, and 8 are control samples.
CML: chronic myeloid leukemia

mutations constitute an early event and might cooperate with other alterations such as BCR/ABL fusion.[8] It has also been reported that among the five members of the Ikaros family, IKZF1 is deleted and mutated in the acute lymphoblastic leukemia (ALL), the blastic phase of CML, and BCR/ ABL1-positive ALL. This further confirms the pathogenic contribution to leukemic transformation.[8] Despite all these advances in the identification of genetic alteration with defined roles in CML, there are still unknown events and alterations that require in-depth analysis. In order to find an alternative, easy marker to differentiate between blood samples collected from healthy and CML cases, we studied whole exome sequencing data and validated our findings through PCR reactions.

Materials and Methods

To detect new alterations in the genomic regions of CML patients, we primarily obtained the exome sequences of a number of patients and analyzed them using GATK software. The bioinformatics analysis was enriched through including the exome sequencing of 11 patients from ENA; alterations were visualized in the integrated genome browser (IGB). Several regions common to all these cases were considered as novel genomic alterations in CML cases. To verify the existence of such alteration, we selected three genes for PCR reactions. We visualized and scored the results after running gel electrophoresis. The following sections provide more details.

The genomic DNA was isolated from the whole fresh and frozen bloods and collected in EDTA anticoagulant tubes by use of Wizard genomic DNA purification kits (Geneaid) according to the manufacturer's manual.

Following quality control and adjustment of DNA concentration, we sent the samples for exome sequencing. Library preparation, clustering, and sequencing was conducted by Novogene company (Hong Kong). We received the results of sequencing in one CML sample in common "fastq" format. The data were analyzed according to Sabri.[9]

In order to confirm the results obtained from exome sequencing and bioinformatics analysis, we performed PCR via specific primers designed for FAM46A, NOTCH4, and ATXN3 genes. Using Primer-BLAST and general primer design criteria, we designed the primers considering the variable sites on their nucleotide sequence. Primers were synthesized by Macrogen (Korea) through one of its representatives in Iran (Denazist Asia Company; http://denazist.ir). We resolved the products on 2% agarose gels and visualized them under UV light after staining with GEL RED.

Results

First, we extracted DNA samples from 70 patients and 30 healthy individuals. Exome sequencing was conducted by Novogen (Hong Kong) for one of the CML individuals. The raw data were deposited in ENA under the accession number PRJEB2708. We analyzed the data using standard protocols; genomic alterations such as insertions, deletions, SNPs events were further obtained (Supplementary File 1). Moreover, we obtained exome sequencing data from 11 individuals deposited in the ENA database (Supplementary File 2).

To find novel genomic alterations with a possible role in CML, we only considered the genes harbouring all the following criteria. Primarily, we only selected the Insertion/Deletions (in/dels) occasions for further analysis. The alteration should be novel (no ID in the dbSNP) and present in the exonic region of the genome. The size of in/dels should be detectable by simple gel electrophoresis following PCR reactions. Ultimately, the alteration should be present in almost all CML exome sequencing samples. We further filtered out all the poorly annotated genes, pseudo-genes, gene fragments, predicted genes, and chromosomal locations. Altogether, we identified alterations in 46 genes satisfying our criteria. By literature review, we also investigated whether these genes were identified as a marker for CML. Based on the literature review and in-depth pathway analysis, we provided a list of potential genes that could be affected in the CML

cases. Three of these genes, namely NOTCH4, ATXN3, and FAM46A were selected. PCR primers were designed such that the products would contain the altered sequences. Therefore, the samples of healthy individuals and patients could be distinguished based on the shift in their electrophoretic bands.

Therefore, we identified the genetic alterations by routine gel electrophoresis and visual inspections. We found that the PCR analysis of the alterations in the ATXN3 gene could be successfully used for identifying CML blood samples (Figure 1).

Discussion

Alterations in FAM46A and NOTCH4 did not significantly correlate with CML status; however, we observed a possible link between insertion in ATXN3 gene and the disease. According to GeneCards, ATXN3 is a coding for the components of proteasome pathway. Its malfunction is mainly associated with neuronal-related disorders such as spinocerebellar degenerations,[10] and Machado-Joseph disease.[11] However, its direct association with CML was not reported previously. The expression of these genes increased in hematopoietic stem cell isolated from CML patients in comparison with that of normal donors.[12] Therefore, the role of ATXN3 in CML should be studied in more details to determine whether its loss of function could contribute to different aspects of disease, including initiation, progress, or treatment.

Conclusion

This is the first report on the possible role of insertion in the ATXN3 gene and CML. We propose that ATXN3 gene be considered in the development of diagnostic and therapeutic strategies for monitoring the status of CML.

Conflict of Interest

None declared.

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