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RESEARCH **P**APER

Detection of polymorphism of ATM gene in Leukemia

POONAM SINGH, SANDEEP SHARMA **AND** UPASHNA UPADHYAY

Department of Molecular and Cellular Engineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, ALLAHABAD (U.P.) INDIA
Email : drpoonam.singh1@gmail.com

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The Ataxia telangiectasia mutated gene encodes the ATM protein, a key element in the DNA damage response (DDR) signalling pathway responsible for maintaining genomic integrity within the cell. In order to study the genetic changes of ATM gene in Lukaemia, we collected the blood samples of healthy persons for control and leukaemia persons for disease samples. We isolated DNA and performed PCR to analyse by Gene sequencing. As a result we found less number of sequencing result in nucleotide BLAST due to which we are unable to detect the compared result of mutation. Larger and/or combined association studies are needed to conclude the result.

Key words : ATM gene, Gel eelectrophoresis, Polymerase chain reaction, DNA sequencing

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INTRODUCTION

The Ataxia-telangiectasia-mutated (ATM) gene in humans was identifed as the basis of a rare autosomal disorder leading to cancer susceptibility and is now well known as an important esignal transducer in response to DNA damage (Johnson *et al*., 2007). ATM gene is large, spanning about 150 kbs of genomic DNA, and is located on chromosome 11q22- 23. It is expressed in many tissues throughout the body and has a key role in the DNA repair response and in conducting cell cycle arrest and apoptosis (Shiloh, 2003). The loss of ATM function leads to genome instability and an increased risk for cancer, neurodegeneration, and impaired glucose tolerance (Kastan and Bartek, 2004). MicroRNAs play critical roles in development, differentiation and disease progression and are predicted to target more than 60 per cent of mRNAs in humans (Brennan *et al.*, 2012).

RESEARCH METHODOLOGY

Sample collection:

48 healthy blood samples were collected from CytoGene students, relatives and friends and 48 disease samples were collected from different hospitals by the rules and guidelines of W.H.O.

Isolation of DNA from blood sample :

DNA from blood sample were isolated by DNA isolation kit .

RESEARCH FINDINGS AND ANALYSIS

Various blood samples were collected from healthy and leukemia infected persons. These samples were placed in EDTA tubes as to prevent it from coagulating. These samples were then used to extract DNA.

DNA extraction (Gel electrophoresis) :

Fig. 1 and 2 showing isolated DNA that was run on

Fig. 1: Blood sample were collected in EDTA tubes

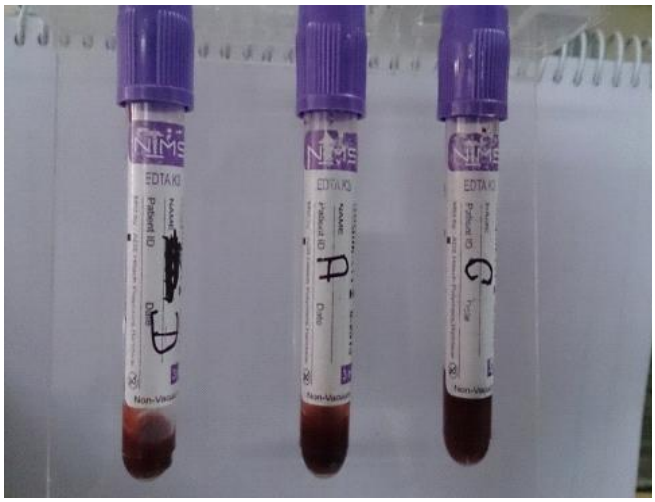

Fig. 2: Blood samples obtained from patients

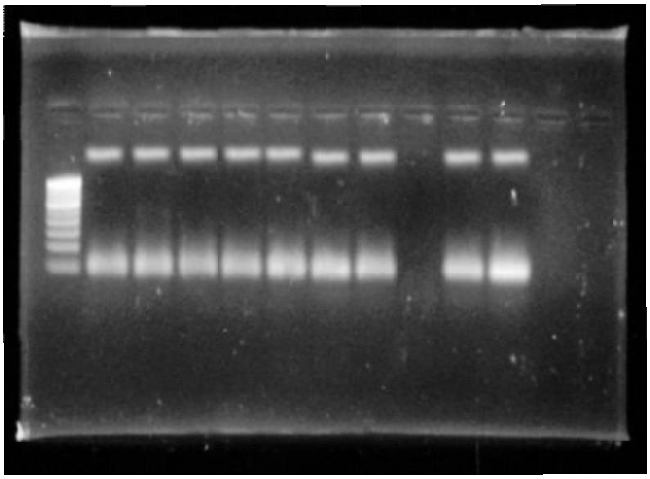

Fig. 3: Showing ladder in lane 1, and control samples in lane 2, 3, 4 and 5, respectively

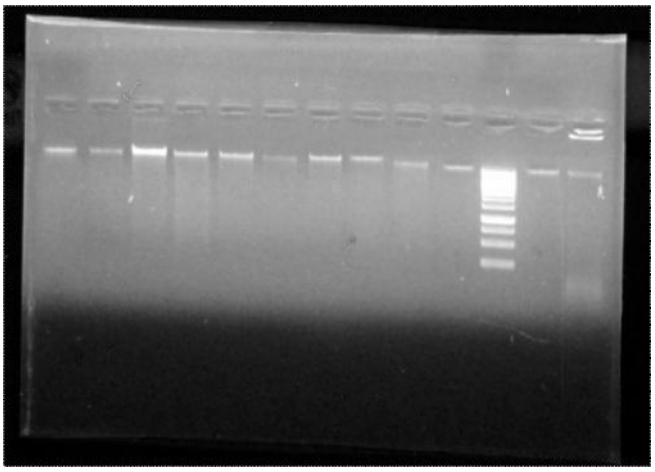

Fig. 4: Showing ladder in lane 1 and disease samples 1, 2, 3 and 4 in lane 2, 3, 4 and 5, respectively

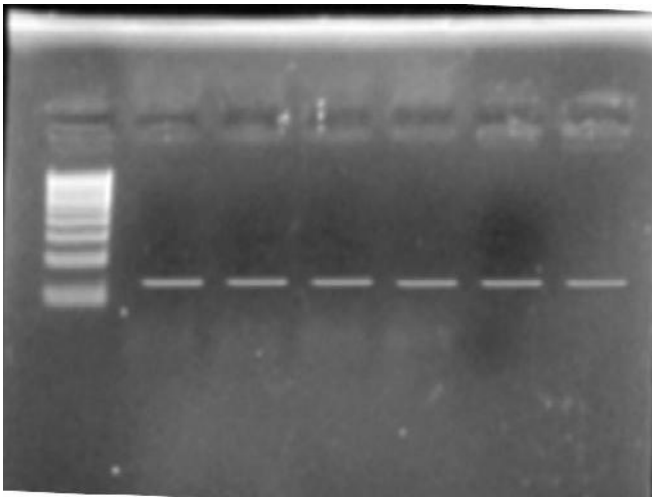

Fig. 5: Shows ladder in lane 1, and control samples in lane 2, 3, 4 and 5, respectively

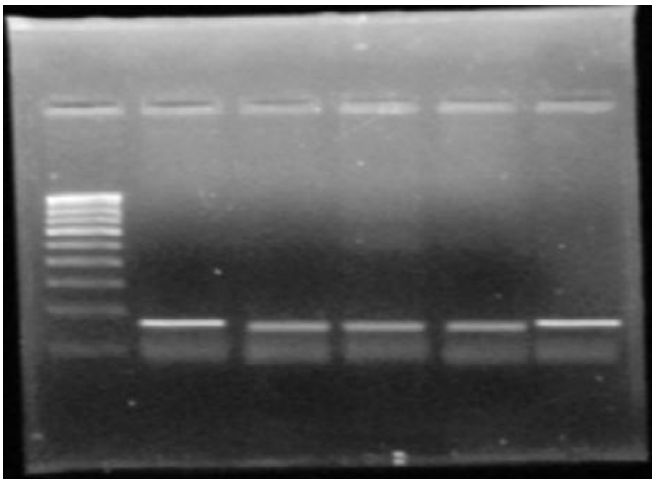

Fig 6: Shows ladder in lane 1 and disease samples in lane 2, 3, 4 and 5, respectively

a 0.8 per cent agarose gel at 100 V for 15 mins. The gel was stained with ethidium bromide and then visualized in a gel documentation system.

PCR :

Fig. 5 and 6 showing amplified DNA that was run on a 1.2 per cent agarose gel at 75V for 20 mins. The gel was stained with ethidium bromide and then visualized in a gel documentation system.

Sequencing result (Appendix 1) :

This is the sequencing result of ATM gene obtained by SANGER and SANGER METHOD.

After getting this result we did pairwise sequence alignment by EMBL-EBI and got which place is mutated.

ATM GENE control sequence

ATGGATGCTCATACGCTCGAGAGCTCTCCG
AGAGATCCTCTCGAAGGTTTGTTTCTAGAATT
AGCCTGGTTATCAGAAATTGTCCCAAGCAAT
AGATTACGACTGATGAAACAACATCGCACAT
GGGTTGAATAATTCTGAAAATGTACTGAAAG
AGAAATCTTCACATTTCAATGTTCTTCCTCTTG
TAGATAAAACAAAATTGTTCATGAATTATTAG
TGACACATGAGATAGACACATCGTCTCAATTGG
TATGATAAATGTTATTTAAACGAAGAACCGAAT
TCTTCACCAGACATATAGCATCATCCTAAATTAG
CGCTATGCTTACAACGTTTAGGTGCTAATCGA
CTGTTTAACAAGTTCAGTTATAGCAATAATGA
ATCAATAGACTTAACAGTTATCGGTCAGTGTT
GCGATCAGCTGCTGCATGGCGTCTATACGCG
CCGTAAAGCCGATTGGATAATAAATCCCCTGA
TAGATTCCATTTAAATCGTTTAATTTGTCTTCC
TACTGATTGGAAAATGTGTGGTTTAGAAACTT
CATTTTACTGGCTTTTACGAGCTGCTTCACAATC
AAACTGGTTTAGAGTATCAGAAATAGTTGCT
TCTCAAAGTGAACTTCTTATTGAAGAGCTGTA
TTCTGATACTGTACAATTCATCTCTTTAGCGC
GCTAGAGCCCCTTGGGCTGCTGATCTCTCCTA
CATGCGAATCAGGAGCAATGTTTACAATTGA
CATTAACTTTACTTTGTCATGCATTAAATTCTA
TGAATTTCTCATCTGCTACAATCCCCTAGCTCGT
ATCTTACTCATGATCAATTAAACTTTCAATCTAC
ATTGGATTCCATTGAACCATTTCTTACATTGTCA
ACTAGTTTTATTGATGTAGTATTATCAAAAAA
TATTTTTCCTAATCATGTTCAATGTCAATTAAG
AAGTTTCTTAATTCATACTTACCTTTATTTTGC
GGAAACTGCAACAACAAGCACAAATTAATTT
GCAACCGTCGG.

A TM GENE disease sequence :

ATGGATGCTCATACGCTCGAGAGCTCTCCG
AGAGATCCTCTCGAAGGTTTGTTTCTAGAATTA
GCCTGGTTATCAGAAATTGTCCCAATGAATAGA
TTACGACTGATGAAACAATTCATCGCACATG
GGTTGAATAATTCTGAAAATGTACTGAAAGA
GAAATCTTCACATTTCAATGTTCTTCCTCTTGTA
GATAAAACAAAATTGTTCATGAATTATTAGT
GACACATGAGATAGACAGATCGTCTCAATTG
CTATGATAAATGTTATTTAAACGAAGAACCGA
ATTCTTCACCAGACAATAGTGTACATCCTAAAT
TAGCGCTATGCTTACAACGTTTAGGTGCTA
ATCGACTGTTTAACAAGTTCAGTTATAGCAATA
ATGAATCAATAGACTTAACAGTTATCGGTCAGT
CGGATATAACATTAAAAGAGTTGCGATCAGC
TGCTGCATGGCGTCTATACGCGCCGTAAAGCCG
ATTGGATAATAAATCAAATGATATACCTCATTTA
AATCGTTTAATTTGTCTTCCTACTGATTGGAAAA
TGTGTGGTTTAGAAACTTCATTTTACTGGCTT
TTACGAGCTGCTTCACAATCAAACTGGTTTAGA
GTATCAGAAATAGTTGCTTCTCAAAGTGAACT
TCTTATTGAAGAGCTGTATTCTGATACTGTACA
ATTCATCTCTTTAGCGCGCTAGAGCCCCTTG
GGCTGCTGATCTCTCCTACATGCGAATCAGGAGC
AATGTTTACAATTGACATTAACTTTACTTTGT
CATGCATTAAATTCTATGAATTTCTCATCTGCTA
CAATCCCCTAGCTCGTATCTTACTCATGATCA
ATTAAACTTTCAATCTACATTGGATTCCATTGA
ACCATTTCTTACATTGTCAACTAGTTTTATTGAT
GTAGTATTATCAAAAAATATTTTTCCTAATCA
TGTTCAATGTCAATTAAGAAGTTTCTTAATTC
ATACTTACCTTTATTTTGCGGAAACTGCAACAA
CAAGCACAAATTAAT

After getting this result we did pairwise sequence alignment by EMBL-EBI and got which place is mutated.

##
Programme : needle
Rundate : 10 June 2014 19:56:31
Commandline : needle
-auto
-stdout
-asequence emboss_needle-I20140708-195630-0864-53790836-pg.asequence
-bsequence emboss_needle-I20140708-195630-0864-53790836-pg.bsequence
-datafile EDNAFULL
-gapopen 10.0
-gapextend 0.5
-endopen 10.0
-endextend 0.5

```
# -aformat3 pair
# -snucleotide1
# -snucleotide2
# Align_format: pair
# Report_file: stdout
########################################
#=======================================
#
# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 1033
# Identity:    984/1033 (95.3%)
# Similarity:  984/1033 (95.3%)
# Gaps:         36/1033 ( 3.5%)
# Score: 4818.5
#
#
#=======================================

EMBOSS_001    1
ATGGATGCTCATACGCTCGAGAGCTCTCCGA
GAGATCCTCTCGAAGGTTT                 50
|||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001    1
 ATGGATGCTCATACGCTCGAGAGCTCTCCGA
GAGATCCTCTCGAAGGTTT                 50


EMBOSS_001    51
GTTTCTAGAATTAGCCTGGTTATCAGAAAT
TGTCCCAAGCAATAGATTAC                100
|||||||||||||||||||||||||||||||||||..|||||||||
EMBOSS_001    51
 GTTTCTAGAATTAGCCTGGTTATCAGAAATTGT
CCCAATGAATAGATTAC                   100


EMBOSS_001    101
GACTGATGAAACAA—
CATCGCACATGGGTTGAATAATTCTGAAAATGTA
                                    148
|||||||||||||   |||||||||||||||||||||||||||||
EMBOSS_001    101
GACTGATGAAACAATTCATCGCACATGG
GTTGAATAATTCTGAAAATGTA              150


EMBOSS_001 149
CTGAAAGAGAAATCTTCACATTTCAATGT
TCTTCCTCTTGTAGATAAAAC               198
|||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001    151
CTGAAAGAGAAATCTTCACATTTCAATGT
TCTTCCTCTTGTAGATAAAAC               200


EMBOSS_001    199
AAAATTGTTCATGAATTATTAGTGACACAT
GAGATAGACACATCGTCTCA                248
|||||||||||||||||||||||||||||||||||||.|||||||||
EMBOSS_001    201
AAAATTGTTCATGAATTATTAGTGACACAT
GAGATAGACAGATCGTCTCA                250


EMBOSS_001    249
ATTGGTATGATAAATGTTATTTAAACGAAG
AACCGAATTCTTCACCAGAC                298
||||.|||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001                          251
ATTGCTATGATAAATGTTATTTAAACGAAGAAC
CGAATTCTTCACCAGAC                   300


EMBOSS_001   299 ATATAGCAT-
CATCCTAAATTAGCGCTATGCTTACAACGT TT
AGGTGCTA                            347
| ||||..| |||||||||||||||||||||||||||||||||||
   EMBOSS_001    301 A-
ATAGTGTACATCCTAAATTAGCGCTATGCTT
ACAACGTTTAGGTGCTA                   349


EMBOSS_001    348
ATCGACTGTTTAACAAGTTCAGTTATAGC
AATAATGAATCAATAGACTTA               397
|||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001    350
ATCGACTGTTTAACAAGTTCAGTTATAGCAA
TAATGAATCAATAGACTTA                 399


EMBOSS_001    398
ACAGTTATCGGTCAGT———————————
GTTGCGATCAGCTGC                     428
|||||||||||||||   |||||||||||||||
EMBOSS_001    400
ACAGTTATCGGTCAGTCGGATATAACATTAAAA
GAGTTGCGATCAGCTGC                   449


EMBOSS_001    429
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```
    TGCATGGCGTCTATACGCGCCGTAAAGCCG
ATTGGATAATAAATCCCCTG                478
    ||||||||||||||||||||||||||||||||||||...||
    EMBOSS_001     450
    TGCATGGCGTCTATACGCGCCGTAAAGCCGATTGG
ATAATAAATCAAATG                     499

    EMBOSS_001     479
    ATAGATTCCATTTAAATCGTTTAATTTGTCTTCC
TACTGATTGGAAAATG                    528
    |||.|...|||||||||||||||||||||||||||||||||
    EMBOSS_001     500
    ATATACCTCATTTAAATCGTTTAATTTGTCTT
CCTAC TGATTGGAAAATG                 549

    EMBOSS_001     529
    TGTGGTTTAGAAACTTCATTTTACTGGCTTT
TACGAGCTGCTTCACAATC                 578
    |||||||||||||||||||||||||||||||||||||||||
    EMBOSS_001     550
    TGTGGTTTAGAAACTTCATTTTACTGGCTTT
TACGAGCTGCTTCACAATC                 599

    EMBOSS_001     579
    AAACTGGTTTAGAGTATCAGAAATAGTTGCT
TCTCAAAGTGAACTTCTTA                 628
    |||||||||||||||||||||||||||||||||||||||||
    EMBOSS_001     600
    AAACTGGTTTAGAGTATCAGAAATAGTTG
CTTCTCAAA GTGAACTTCTTA              649

    EMBOSS_001     629
    TTGAAGAGCTGTATTCTGATACTGTACAAT
TCATCTCTTTAGCGCGCTAG                678
    |||||||||||||||||||||||||||||||||||||||||
    EMBOSS_001     650
    TTGAAGAGCTGTATTCTGATACTGTACAAT
TCATCTCTTTAGCGCGCTAG                699

    EMBOSS_001     679
    AGCCCCTTGGGCTGCTGATCTCTCCTACAT
GCGAATCAGGAGCAATGTTT                728
    |||||||||||||||||||||||||||||||||||||||||
    EMBOSS_001     700
    AGCCCCTTGGGCTGCTGATCTCTCCTACAT
GCGAATCAGGAGCAATGTTT                749

    EMBOSS_001     729
    ACAATTGACATTAACTTTACTTTGTCATGC
ATTAAATTCTATGAATTTCT                778
    |||||||||||||||||||||||||||||||||||||||||
    EMBOSS_001     750
    ACAATTGACATTAACTTTACTTTGTCATGCAT
TAAATTCTATGAATTTCT                  799

    EMBOSS_001     779
    CATCTGCTACAATCCCCTAGCTCGTATCTTA
CTCATGATCAATTAAACTT                 828
    |||||||||||||||||||||||||||||||||||||||||
    EMBOSS_001     800
    CATCTGCTACAATCCCCTAGCTCGTATCTTA
CTCATGATCAATTAAACTT                 849

    EMBOSS_001     829
    TCAATCTACATTGGATTCCATTGAACCATTTCTTA
CATTGTCAACTAGTT                     878
    |||||||||||||||||||||||||||||||||||||||||
    EMBOSS_001     850
    TCAATCTACATTGGATTCCATTGAACCATTT
CTTACATTGTCAACTAGTT                 899

    EMBOSS_001     879
    TTATTGATGTAGTATTATCAAAAAATATTT
TTCCTAATCATGTTCAATGT                928
    |||||||||||||||||||||||||||||||||||||||||
    EMBOSS_001     900
    TTATTGATGTAGTATTATCAAAAAATATTTT
TCCTAATCATGTTCAATGT                 949

    EMBOSS_001     929
    CAATTAAGAAGTTTCTTAATTCATACTTAC
CTTTATTTTGCGGAAACTGC                978
    |||||||||||||||||||||||||||||||||||||||||
    EMBOSS_001     950
    CAATTAAGAAGTTTCTTAATTCATACTTACC
TTTATTTTGCGGAAACTGC                 999

    EMBOSS_001     979AACAACAAGCACAAA
TTAATTTGCAACCGTCGG                  1011
    ||||||||||||||||||
    EMBOSS_001     1000 AACAACAAGCACAAAT
TAAT————————                       1019
```

The ATM protein is a serine/threonine kinase involved in DNA double-strand break repair (Lavin *et al.*, 2005 and Taylor and Byrd, 2005). Ataxiatelangiectasia is caused by the inheritance of biallelic deleterious mutations in the ATM gene and occurs in 1/40,000 to 1/

300,000 live births. Ataxia-telangiectasia is characterized by progressive cerebellar ataxia, oculomotor apraxia, telangiectasias of the conjunctiva and skin, immunodeficiency, sensitivity to ionizing radiation, and an increased rate of malignancies, particularly lymphoma and leukemia (Swift *et al.*, 1987).

Conclusion :

The Ataxia-telangiectasia-mutated (ATM) gene in humans was identifed as the basis of a rare autosomal disorder leading to cancer susceptibility and is now well known as an important esignal transducer in response to DNA damage. Ataxia telangiectasia mutated gene (ATM) is a newly identified member of PI3 kinase family that is mutated in human autosomal recessive disease, ataxia telangiectasia (AT) . ATM gene is large, spanning about 150 kbs of genomic DNA, and is located on chromosome 11q22- 23. Mutations in the ATM gene are the cause of a rare autosomal recessive syndrome, ataxia-telangiectasia (AT). The finding that ATM heterozygotes have an increased breast cancer risk was supported by some studies but not confirmed by others. In our study, the entire coding sequence of the ATM gene was prescreened for mutations by the protein truncation test to detect the chain-terminating mutations that are highly predominant in patients with AT. This review covers the role of ATM in DDR signalling and describes the interaction of the ATM kinase with other proteins in order to fulfil its various functions.

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