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Research Article

GATA2 Haploinsufficiency Caused by G492A Mutation Associated with Familial AML

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Abstract

Myelodysplastic Syndromes and Acute myeloid leukaemia are hematopoietic disorders causing blood cancer mostly in old-aged persons. In the present study we collected blood from acute myeloid leukaemia patient who had a history of thrombocytopenia for 10 years with, affected familiy members. The DNA was extracted and GATA2 exon 4 was amplified by Polymerase chain reaction. The PCR product of 291 bp was subjected to Sanger sequencing. Present case exhibits a heterozygous mutation which was found at 164 codon position Alanine (GCC) to Threonine (ACC) and c.492 bp position (G/A) within exon 4 of GATA2 gene. This may be the first case of mutation associated with familial AML in India as literature survey has not revealed such a single point mutation (G492A) was not reported earlier in India.

Keywords: AML; PCR; Thrombocytopenia; DNA; GATA2 Gene

Introduction

Myelodysplastic Syndromes (MDS) and Acute myeloid leukaemia (AML) are hematopoietic disorders mostly affecting old-aged persons. There are several subtypes of MDS, and some subtypes of MDS may eventually turn into AML, a cancer of the blood in which immature cells called blasts increases in number and grow uncontrollably. Occurrence of MDS or AML is rare but mostly associated with genetic abnormalities. Mutations in RUNX1 or CEBPA, and *GATA2* genes have been associated with MDS and AML disease condition.

A *GATA2* gene in a human synthesizes a protein called GATA binding protein 2 [1] that is a part of a family of GATA transcription factors [2]. Other transcription factors are GATA1, GATA3, GATA4, GATA5 and GATA6. Three GATA family transcription factors including GATA1, GATA2 and GATA3 are involved in various aspects of haematopoiesis [3]. All GATA protiens contain zinc fingers motifs in their DNA binding domain, regulates of gene expression in hematopoietic cells [4]. As other transcription factors, GATA2 binding protein regulates haematopoiesis for stem cells development [5] and therefore highly expressed in most of the hematopoietic progenitor cells including early erythroid cells, megakaryocytes mast cells and also in non-hematopoietic embryonic stem cells. GATA2 factor also interacts with other proteins as co factors to control transcription [6-8].

The *GATA2* gene is located on chromosome 3 at position 21.3. Inactivation of *GATA2* gene allele in mouse induces defects of haematopoiesis. However, the consequences of GATA2 haplodeficiency upon haematopoietic stem cells (HSCs) equilibrium is more unusual in human than mice. So far, nearly 100 GATA2 mutations have been identified in either germ cells or somatic cells. These include complete gene deletions and frame-

shift mutations, at the transcription initiation site to the end of the second zinc finger. In addition, 11 frame shift insertions or deletions and 54 mutations causing amino acid substitution in two zinc finger domains. Two individual mutations in intron 5 enhancer, are reported to affect GATA2 transcription factor binding [9]. These single amino acid substitutions may result in the mutated protein with altered function, the functional effects of heterozygous perturbation are mainly because haplo-insufficiency in *GATA2* gene.

The effect of gene deletions and frame-shift mutations lead them to virtually the similar phenotypes as in case of amino acid substitution shows substantial effect on DNA binding property of the zinc finger domains and making them non-functional (Dickinson., *et al*. 2011). Various mutations in *GATA2* gene have been indicated the cause of primary immunodeficiency in patients with autosomal dominant MonoMAC Syndrome (Autosomal dominant syndrome) associated with monocytopenia, and cases of dendritic cell, monocyte, B-lymphocyte, Natural Killer lymphoid deficiency and leukemia (Dickinson., *et al.* 2011). Mutations in *GATA2* gene have also been associated with familiar MDS/AML and lymphedema, deafness and myelodysplasia [10]. Present study describes GATA2 mutations in a 49-year-old woman having thrombocytopenia for 10 years and she has significant family history of thrombocytopenia, with affected children, mother and siblings.

Materials and Methods:

Blood sample was collected in an EDTA coated blood collecting vacutainer tube from a 49-year- acute myeloid leukemia (AML) patient who had a. history of thrombocytopenia for 10 years with a significant family history of thrombocytopenia,. The DNA was extracted by Qiagen kit method (QIAprep Spin Miniprep Kit). Agarose gel electrophoresis and UV spectrophotometer were used to determine the DNA quality and the quantity. As described by The PCR product of 291 bp was amplified by Polymerase chain reaction (PCR), using forward primer (5'- GTGAGCCCCTTCTCCAAGAC-3') and reverse primer (5' GTACTTGACGCCGTCCTTGT-3'). The PCR mix contained 1X PCR buffer, 1.5 mM $MgCl_2$, 10 mM dNTPs, 10 pM each of forward and reverse primer, 2 Unit Taq DNA polymerase, 80ng genomic DNA and distilled water to make a final volume of 10 µl. The PCR reaction included the following steps: predenaturation for 6 minutes at 95°C followed by 35 cycles of 50 seconds at 95°C, 45 seconds at 60°C, 50 seconds at 72°C and final extension for 12 minutes at 72°C for utilization of extra dNTPs in mixture.

PCR products purification prior to sequencing using ExoSAP-IT treatment was done. After purification PCR product was sequenced by Applied Bio systems 3130XL Automated Sequencer using the ABI Big Dye Ver 3.1. Sequence data analysis comparison was performed using the Codon Code Aligner 4.0.4 Software.

Protein Structure Modeling

GATA2 sequence was submitted to Robetta full length chain modeling for the structure prediction and has generated 5 models [11]. The steriochemical quality was of the GATA2 protein models were validated by Ramchandran plot analysis [12]. The best protein model was selected based on the number of amino acids in the generously allowed and disallowed regions in the Ramachandran plot. Protein models were visualized by pymol software (Pymol).

Results and Discussion

Recurrent mutations have been suggested to be a cause of hematopoietic disorders including MDS and AML. In the current investigation, a heterozygous mutation was found at 164 codon position Alanine (GCC) to Threonine (ACC) and c.492 bp position (G/A) in a 49-year-old woman as shown in the sequencing electropherogram figure 1. The size of PCR product was 291bp. The DNA Sanger sequencing revealed a heterozygosity at c.492 position (G/A) in a 49-year-old woman whereas in control sample doesn't have any variation in nucleotide position which was normal for GATA2. The heterozygosity at c.492 position was found to be missense mutation because of change in amino acid from Alanine to Threonine (GCC>ACC) within exon 4 of *GATA2* gene. Similar case of a 49-year-old woman was diagnosed with myelodys-plastic syndrome was died within a few days [13]. However, her genetic testing was not performed to know about the mutation status in candidate gene. Recently, a 50-year-old pAML patient with history of thrombocytopenia and a significant family history of thrombocytopenia was reported [14]. Sequencing of *GATA2* gene revealed two heterozygous mutations (p. Thr358Lys (c.1074 C > A) and p. Leu359Val (c.1076 T > G) in second zinc finger domain of *GATA2* gene. A novel heterozygous c.1061C>T (p. Thr354Met) mutation in the *GATA2* gene was reported which involved in transmission of MDS/AML in three families. A GATA2 (p. Thr355del) mutation was detected at an adjacent codon in a fourth MDS/AML family [15-17].

In the current study, we detected single point mutation (G492A) in 49-year-old woman diagnosed with acute myeloid leukaemia. However, this single point mutation (G492A) was not reported

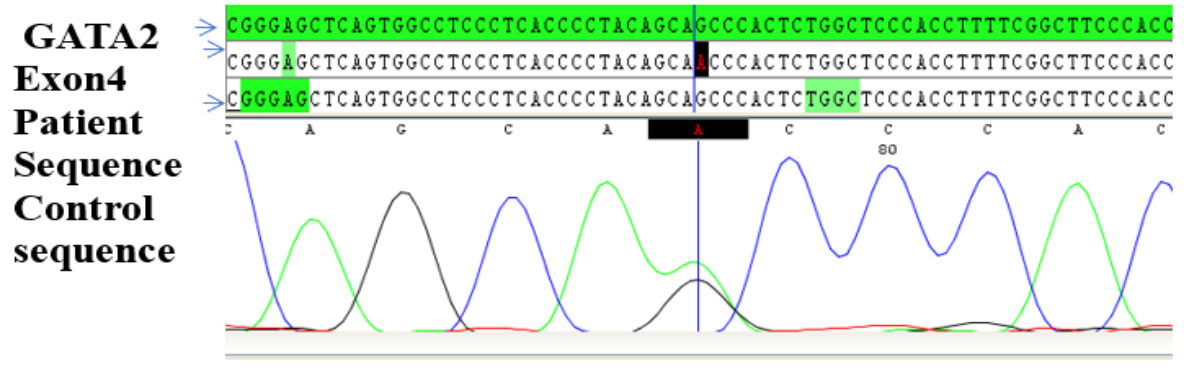

Figure 1: Identification of G492A mutation in familial AML patient. Electropherograms of sanger sequencing reactions showed a G492A mutation in GATA2 gene (exon 4) in a AML patient.

before especially in India. Therefore, it is first case of mutation associated with familial AML individuals exhibited variable clinical symptoms.

Structure analysis of the G492A mutation revealed that the mutant protein might be compromised. Robetta server has predicted 5 3-D models for the GATA2 protein and all of them contain two α-helical segmants, two β sheets and the major part of the protein contains loops (Figure 2A). Best model was selected based on the residues were fall in the most favorable allowed region in the Ramachandran plot. A79T was mutated using coot software to compare the structural alignment of wild type of GATA and A79T mutated protein (Figure 2B). Alanine 79 residue has present on the loop reagion that might interact with DNA binding part and/or another protein-protein interaction region. It is a significant change when GATA 2 get mutated from alanine to threonine (Figure 2C). Threonine is a polar residue and it is more reactive than alanine because of hydroxyl group on its side chain. From structural analysis the A79T is not making any interactions with in the protein, hence we postulate that it must be involving in the intermolecular interactions.

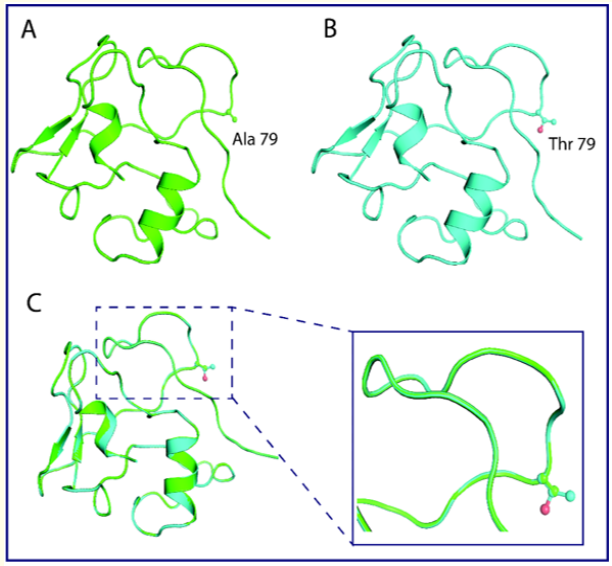

Figure 2: A. Robetta structure prediction of Human GATA2 protein (gree color) and Ala 79 residue shown as ball and stick. B. GATA2_A79T variant model (cyan color) and Thr 79 shown as ball and stick. C. Structural alignment of GATA2_WT-GATA2_A79T and close view of A79T mutation.

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