Identification of Novel Mutations in Adenosine Deaminase Gene in Korean Leukemia Patients

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Leukemia is the abnormal increase of hematopoietic progenitor cells in tissues, resulting in anemia, increased susceptibility to infection and impaired blood clotting. The adenosine deaminase (ADA) gene is an important druggable target for the treatment of leukemia patients. Genetic and molecular analyses were performed to determine the effects of ADA gene mutations in 20 leukemia patients in the Korean population. To analyze the relationship between genotype and phenotype, the ADA genomic DNAs - including 1,092 bp of 12 exons and partial intron sequences flanking each exon - were sequenced and compared. In this study, the known mutations in other diseases, more than 50 mutations already reported in patients with severe combined immunodeficiency disease (SCID) and autism, were not found, but two novel mutations in leukemia patients were discovered. They include one nonsense mutation (A>C at nt position 478, F101P) and one missense mutation (G>A at nt position 778, E260K). One missense mutation (G>A at nt position 22, D8Y) was also detected in 20 normal control patients (allelic frequency of 7.5%). Interestingly, subjects in the Korean population retained 2 bp insertion at the intron 6 (IVS6-52insGC), something that has never been shown in other populations. The genetic study to find out the correlation between the mutant alleles and leukemia patients revealed no association statistically (p>0.05). The mutation found in leukemia needs further study to determine its possibility as a molecular marker for the diagnosis of leukemia.

Key words : Adenosine deaminase, mutation, leukemia

Introduction

Adenosine deaminase (EC 3.5.4.4; ADA) is a ubiquitous enzyme that catalyzes the irreversible deamination of adenosine and deoxyadenosine in the purine catabolic pathway [5] and mainly participating the development and function of lymphocyte [4]. Partial sequence of ADA gene was isolated from a human T-cell lymphoblast cDNA library [16] and a full-length ADA cDNA encoding a 363-amino acid protein with a molecular mass of 40 kD was also isolated [15]. In 1985, Valerio et al determined that the ADA gene spans 32 kb and contains 12 exons [14]. By means of in situ hybridization to high resolution spreads of somatic and pachytenes chromosomes, the ADA gene was confirmed to localized on 20q12-q13.11 [8]. Mutations at the ADA gene locus, in humans result in a spectrum of phenotypes.

Mutations in ADA gene causing the complete absence of enzyme activity in all cell types are associated with the fatal infantile onset syndrome of severe combined immunodeficiency (SCID). Mutations causing the absence of enzyme activity in erythrocytes but with retention of variable amounts of enzyme activity in other cell types (partial ADA deficiency) are usually associated with grossly normal immune function. Mutations resulting in partial ADA deficiency but with extremely low residual enzyme activity usually present as a late-onset milder and slowly progressive immunodeficiency [1,4,6,7,12]. Mutations in ADA gene have also been reported ADA activity was reduced in sera of autistic children compared with normal controls [2,10]. In general, the degree of enzyme deficiency, accumulation of the toxic metabolite deoxyATP and urinary excretion of the substrate deoxyadenosine grossly correlate with severe of disease. Consistent with this diversity, >50 variants have been identified in ADA gene, including deletions, nonsense, nonsense and splicing mutations, but most variants are heteroallelic, and most alleles are rare [1].

The correlation between ADA genotypes and SCID phenotype is well known but never examined this concept in leukemia population. The objectives of this study are to identify the genetic variants of ADA gene in Korean population and to determine whether the ADA gene polymorphisms are associated with leukemia.
Materials and Methods

Study subjects

A total of 40 individuals were sampled throughout the leukemia patients and non-patients as controls. Of these, 20 were verified as leukemia patients as follows. Seven patients with acute lymphoblastic leukemia (ALL), 11 patients with acute myeloid leukemia (AML), one patient with chronic myeloid leukemia (CML), and one patient with hairy cell leukemia (HCL). The other 20 were chosen from the people has no symptoms of leukemia. The controls with a family history of leukemia were excluded. Both parents of each study participant were reported to be of Korean descent. Without exception the participants reported both parents to be of Korean descent. This study was approved by the Scientific-ETHical Review Board of participating institution approved the experimental protocols.

PCR and Sequencing

For mutation analysis, genomic DNA was isolated from peripheral leukocytes from each patient by the QiAamp DNA blood Mini kit (Qiagen, CA, USA) according to the manufacturer's specification. DNA was amplified by PCR using sense and antisense primer set designed to amplify the exons 1, 2, 3, 4-5, 6, 7-9 and 10-11 from genomic DNA. Total 8 sets of primers were used from previous report (Jiang et al. 1997) [9] and sequenced using cycle sequencing with a PCR products were amplified from 50 ng of genomic DNA in a 25 μl reaction system containing 2.0 mM MgCl₂, 1.0 mM dNTPs, 0.25 units of AmpliTaq Gold DNA polymerase (Applied Biosystems, CA, USA), and 1X PCR buffer II; the amplification protocol was: denaturation 10 min at a 95°C, then 40 amplification cycles of 95°C for 30 sec, 65°C for 30 sec, and 72°C for 45 sec, followed by an extension of 10 min at 72°C. The PCR products were purified using Microcon PCR filters (Millipore, FL, USA) and were directly sequenced in both directions using BigDye Terminator kits (Applied Biosystems, CA, USA) and run on ABI 377 sequencing apparatus (ABI, MI, USA). Sequences were inspected using DNAsStar (DNAsStar Inc., CA, USA), unambiguously aligned using Clustal-X (UCD, Belfield, Ireland) [13] and visually inspected. PCR product includes full sequence of each exon and partial flanking intron sequences.

Results and Discussion

By direct sequencing analysis, all 40 Korean subjects consisting 20 patients with sporadic leukemia and 20 with non leukemia showed no statistical differences of the allele frequencies which were known in ADA gene. I initially found that three novel mutations from three sites on exon 5 (2) and 8 (2), and 2 bp insertion at intron 6 near exon 7 in Korean leukemia population (Fig. 1, Table 1). This was confirmed by reverse direction PCR reactions. These mutations are G to A transition at the nt 778, that results in a glutamic acid to lysine substitution at codon 260 (G778A, E260K) in exon 8 (Fig. 1A) in one ALL patient and one AML patient. The other mutation is a C to T transition at the nt 432 (C432T, F101F) in exon 5 of genomic DNA (Fig. 1B) in one AML patient, 2 bp insertion (IVS6-52insGC) was found all leukemia patients and also found all normal control samples (Fig. 1D). These mutations never showed in other populations. I also detected one missense mutations in normal control, a G to A transition at the nt 22 (G22A, missense) predicting

![Fig. 1. Determination of the ADA gene by sequence analysis. A) E260K, C/G heterozygote at 778 position in two leukemia patients. B) F101F, T/C heterozygote at 432 position in two leukemia patients. C) IVS6-52insGC, 2 bp insertion was found in all Korean samples, but this was not reported in other ethnic groups studied before. D) D8Y, G/A mutant allele at 22 position of ADA exon 1 was found in three non-leukemia patient samples only but not in patients. The location of the mutation is indicated by arrow.](https://example.com/fig1.jpg)
Table 1. The ADA mutations

<table>
<thead>
<tr>
<th>Exon</th>
<th>Size (bp)</th>
<th>Number of observed mutation</th>
<th>Mutation ID</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>2</td>
<td>Q8X, D8N</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>1</td>
<td>H15D</td>
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<tr>
<td>5</td>
<td>116</td>
<td>10</td>
<td>P126Q, V129M, G140E, R142Q, R142X, R149W, R149Q, L152M, R154H, R156C</td>
</tr>
<tr>
<td>6</td>
<td>128</td>
<td>3</td>
<td>V177I, A179D, Q199P</td>
</tr>
<tr>
<td>7</td>
<td>72</td>
<td>5</td>
<td>R211C, R211H, A215T, G216R, E217K</td>
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<td>8</td>
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<td>T231I, R235Q, R238P, Q254X</td>
</tr>
<tr>
<td>9</td>
<td>65</td>
<td>1</td>
<td>P274L</td>
</tr>
<tr>
<td>10</td>
<td>130</td>
<td>4</td>
<td>S294L, P197Q, L304R, M310T</td>
</tr>
<tr>
<td>11</td>
<td>103</td>
<td>2</td>
<td>A329V, E337del</td>
</tr>
<tr>
<td>12</td>
<td>14</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1092</td>
<td>47</td>
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</table>

an aspartic acid to tyrosine substitution at codon 8 (D8Y). This D8Y mutation was revealed in three non-leukemia patients as heterozygote (allelic frequency of 5%). As mentioned in other studies, the mutations found as heterozygote and showed low frequencies [1]. The variants showed statistically non significant signal of the associations between leukemia patients group (case) and non-leukemia group (control) (p>0.05).

The sequences from 12 exons showed very conservative patterns among the samples without any statistical significance. The whole ADA genomic DNA sequences were blasted to the sequences reported [3,14]. Most of the sequences were identical among the samples and there was no signal of the mutant alleles which were characterized as risk alleles in SCID patients [1,11] and in autism patients [2,10] (Table 1). These observations suggest that the known ADA mutations associated to the SCID are unlikely to affect genetic susceptibility to leukemia in Korean population.

In conclusion, the known about 50 mutations in the ADA gene are undetectable in Korean leukemia patients and non-leukemia patients groups. All previously reported ADA mutations in SCID, in consecutively ascertained child, this ADA mutation study is the first report related to leukemia. In this study I found four novel mutations, and the possible involvement of these novel variations to the development of leukemia in Korean population remains to be investigated.

References


추록 : 한국인 백혈병 환자에서 아데노신 디아미나제 유전자에 새로운 변이의 확인

박 기 호
(침례병원 소아과)

백혈병은 조혈모세포의 비정상적인 증식에 의해 인어나서 질환이고, adenosine deaminase (ADA) 유전자는 백혈병의 약물 작용점으로 중요합니다. 이러한 연구의 일환으로 한국인 백혈병 환자 20명의 ADA 유전자의 변이를 조사하기 위해 혈액 genomice DNA를 추출하여 염기서열을 결정하였다. 그 결과 nonsense 변이인 F101F 하나, missense 변이 E260K, D8Y 각각 하나, 그리고 외국에서는 보고되지 않은 것으로 정상인에서 IVS6-52에 GC가 도입된 것을 확인하였다. 백혈병 환자와 유전자 변이간에 동계학적인 차이점은 없지만 이러한 연구는 앞으로 백혈병의 진단 및 치료 개발에 도움이 될 것으로 사료된다.