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ADA-SCID with 'WAZA-ARI' mutations that synergistically abolished ADA protein stability

Adenosine deaminase (ADA) deficiency is a systemic purine metabolic disorder in which toxic levels of ADA substrates, particularly deoxyadenosine (dAdo), primarily affects lymphocyte development and functions (Hershfield & Mitchell, 2001). The affected patients present with varying degrees of immunodeficiency, such as severe combined immunodeficiency (ADA-SCID), delayed-onset ADA deficiency, and late-onset ADA deficiency. 'Partial ADA deficiency' has also been identified in healthy individuals with abolished ADA activity in erythrocytes but at greater levels in other cells (0·9–70% of normal) (Daddona *et al*, 1983; Hirschhorn & Ellenbogen, 1986). To date, more than 70 *ADA* mutations have been identified, including deletions, missense, nonsense, and splicing mutations (Hershfield, 2003). Correlation between the effect of mutations on ADA activity and clinical phenotype has been demonstrated by systematic expression studies of mutant *ADA* cDNAs in *Escherichia coli* (Arredondo-Vega *et al*, 1998; Hershfield, 2003). Here, we report an ADA-SCID patient with two mutations on the same allele, each of which retained detectable levels of ADA activity.

A Japanese 1-month-old boy was referred to our hospital because of poor sucking and failure to thrive. His elder brother had died of recurrent pneumonia at 4 months of age. His non-consanguineous parents and his younger sister were all in good health. Based on the history of his affected sibling, initial investigations were performed soon after birth. Although the initial haematological examination showed no lymphopenia (absolute lymphocyte count: $2{\cdot}79 \times 10^9$/l), profound lymphopenia (absolute lymphocyte count: $0{\cdot}13 \times 10^9$/l) was noticed when he was 40 d old. T-cell receptor excision circles were undetectable (Morinishi *et al*, 2009). After obtaining informed consent for genetic analysis under a protocol approved by the Institutional Review Board of Hokkaido University Graduate School of Medicine, we performed genetic analysis of T-B-SCID (SCID with a virtual lack of circulating mature T and B lymphocytes)-related genes, such as *ADA*, *RAG1*, *RAG2*, and *DCLRE1C*, and found mutations in *ADA* gene. He was diagnosed with ADA-SCID based on his clinical severity, immune dysfunction, and the presence of *ADA* mutations. He died of respiratory distress 22 d after unrelated umbilical cord blood stem cell transplantation.

Direct sequence analysis of the patient's genomic DNA demonstrated three base changes in the *ADA* gene: 355C>T (Q119X) in exon 4, 102A>T (R34S) in exon 3, and 715G>A (G239S) in exon 8 (Fig. 1A,B). Studies of his family members demonstrated that his mother was heterozygous for 355C>T encoding Q119X, whereas his father and younger sister were heterozygous for 102A>T and 715G>T encoding R34S and G239S, respectively (Fig. 1A,B, data not shown). Q119X has been identified as one of the mutations for ADA-SCID (Ariga *et al*, 2001a), while G239S is a 'partial mutation' observed in a patient with partial ADA deficiency (Ariga *et al*, 2001b), There have been no reports of R34S mutation in ADA deficiency. To further determine whether the father's R34S and G239S mutations were both on the same allele, the reverse transcription polymerase chain reaction (RT-PCR) products consisting of full-length *ADA* cDNA were cloned and analysed for their nucleotide sequences. Only two clones were found: one with wild-type sequence and the other containing both R34S and G239S mutations. These results indicated that ADA-SCID in the patient was caused by compound heterozygous mutations: Q119X inherited from his mother and R34S/G239S from his father (Fig. 1B).

To study the effect of R34S/G239S mutations on ADA activity, the cDNAs containing the base changes individually and in combination were recloned into the pZ plasmid and introduced into the bacterial ADA-defective *E. coli* strain, SØ3834. ADA activity of each mutant cDNA expressed in

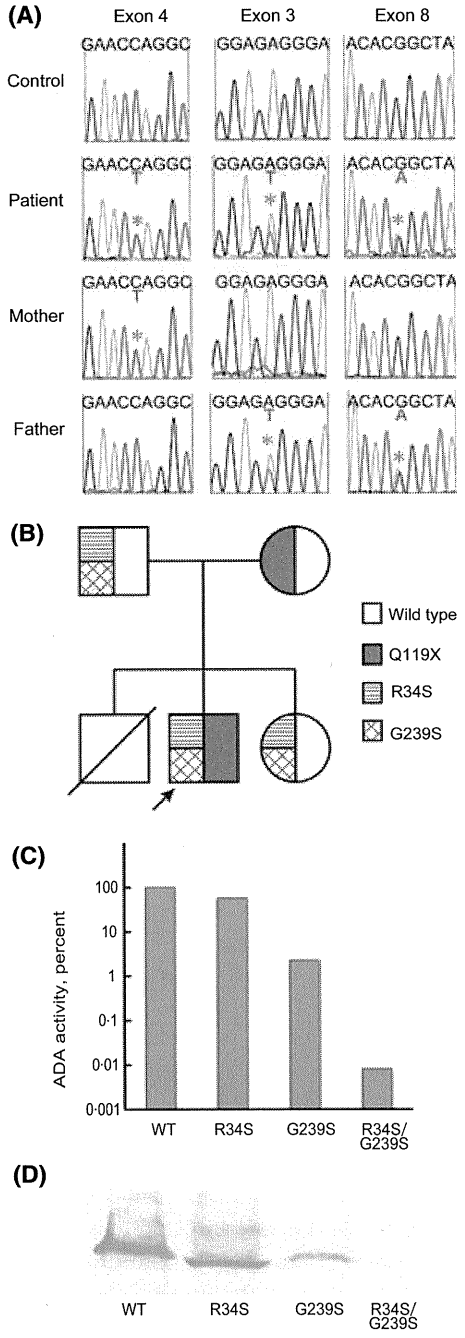

Fig 1. Studies of ADA sequence, activity, and expression. (A) Direct sequence analysis of the *ADA* gene in a control, the patient, mother, and father. The sites of mutations are indicated by asterisks. Left panels: Forward sequence of exon 4. 355C>T (Q119X) was present in the patient and his mother. Middle panels: Forward sequence of exon 3. 102A>T (R34S) was present in the patient and his father. Right panels: Forward sequence of exon 8. 715G>A (G239S) was present in the patient and his father. (B) Pedigree of the patient's family. Genotypes of the *ADA* are shown. An arrow indicates the patient. (C) ADA activity of mutants (R34S, G239S and R34S/G239S), expressed in *Escherichia coli*. Each mutant resulted in 56.4%, 2.2%, and 0.008% of the normal ADA activity, respectively. (D) Western blot analysis of ADA expression in lysates of SØ3834 in which wild-type, R34S, G239S, and R34S/G239S were expressed. R34S and G239S exhibited mildly and moderately reduced expression, respectively. R34S/G239S mutations in combination resulted in undetectable expression. WT, wild-type.

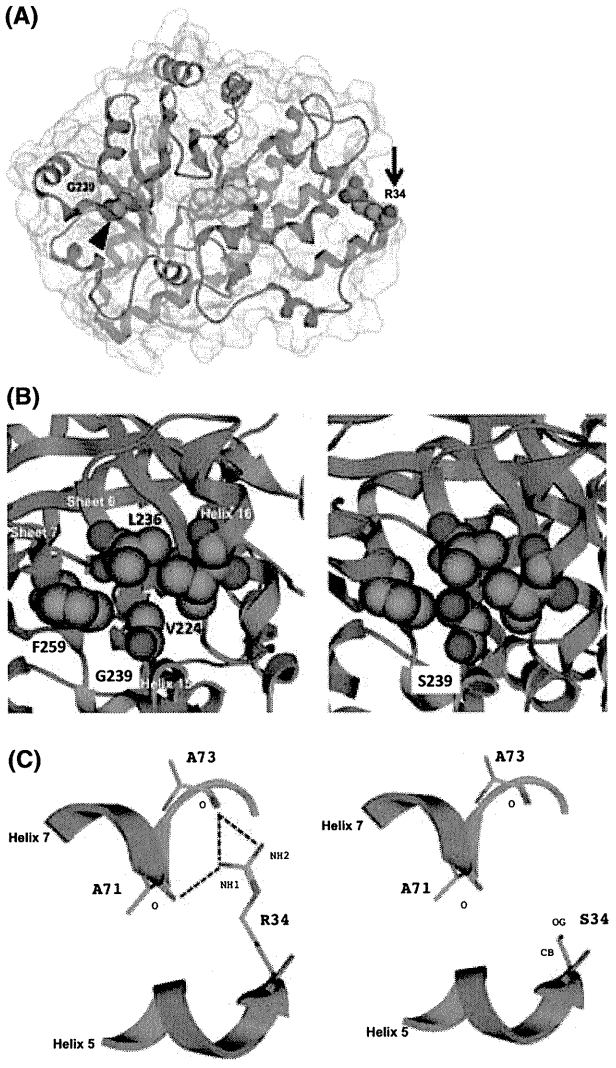

Fig 2. The effect of mutations on the tertiary structure of ADA. (A) Location of mutated residues, R34 and G239, are shown by an arrow and an arrowhead, respectively. The complexed ligand within the active site is represented by yellow spheres. (B) An enlarged view of regions neighbouring G239 (left) and S239 (right). Substitution of Gly to Ser, which has a hydrophilic side chain, weakens the hydrophobic force of the core. (C) An enlarged view of regions neighbouring R34 (left) and S34 (right). R34 is located in Helix 5. Substitution of Arg with Ser, which has a shorter side chain and lacks a positive charge, eliminates the hydrogen bonds, thereby affecting the ligand gating function of Helix 7. Hydrogen bonds are shown by dotted lines.

SØ3834 was quantitated as previously described (Arredondo-Vega *et al*, 1998). Each R34S and G239S mutation resulted in 56·4% and 2·2% of normal ADA activity, respectively. However, the combination of R34S/G239S mutations resulted in 0·008% of normal ADA activity, indicating that the two mutations have synergistic effects on the loss of this activity (Fig. 1C).

Western blot analysis of the *E. coli* transformed with each mutant cDNA as previously described (Arredondo-Vega *et al*, 1998) revealed reduced expression of R34S or G239S mutant

protein, whereas the combination of R34S/G239S resulted in no detectable protein expression (Fig. 1D). These results suggest that abolished ADA activity in R34S/G239S, is attributed to impaired protein expression. Direct sequence analysis of cDNA derived from his father demonstrated comparable signals of both wild-type and R34S/G239S mutant. In addition, sequence analysis of cloned RT-PCR products of *ADA* cDNA derived from his father showed wild-type and R34S/G239S mutant at a ratio of 7–5 (data not shown). These suggest that the lack of the mutant ADA protein expression is a result of protein instability, rather than mRNA instability.

Next, we analysed the tertiary structure of the mutant ADA based on the crystal structure of human ADA (PDB code: 3IAR) as previously described (Montano *et al*, 2007) (Fig. 2A). G239 is a part of the hydrophobic core formed by Helices 15 and 16 and Sheets 6 and 7. Substitution of this amino acid with Ser, which has a hydrophilic side chain, retains the overall structure but weakens the hydrophobic force of the core (Fig. 2B). R34 is a positively charged peripheral residue located in Helix 5. Helix 5 forms hydrogen bonds with Helix 7, which is enables for the substrate to enter the active site. Substitution of Arg with Ser, which has a shorter side chain and is uncharged, loses the hydrogen bonds. This causes a presumably unstable local structure, despite retained overall structure (Fig. 2C).

Jiang *et al* (1997) described the presence of two missense mutations on the same allele (L106V/Y97C), which synergistically abolished ADA activity independent of the protein instability. Therefore, the present report is the first to ADA-SCID in which two partial mutations on the same allele, R34S/G239S, synergistically abolished ADA protein stability. We named the combination of partial mutations that resulted in complete deficiency of the gene product 'WAZA-ARI' mutations, following a scoring system of Judo's competition. A 'waza-ari' is a half point; two 'waza-ari' scoring constitute the full point needed for win. In cases that lack correlation between genotype and phenotype, another mutation on the same allele should be assessed for 'WAZA-ARI' mutations.

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細網異形成症の診断と治療に関する調査研究

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