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Novel mutations of *FOXP3* in two Japanese patients with immune dysregulation, polyendocrinopathy, enteropathy, X linked syndrome (IPEX)

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EDITOR—Immune dysregulation, polyendocrinopathy, enteropathy, X linked syndrome (IPEX), also known as X linked autoimmunity-allergic dysregulation syndrome (XLAAD), is characterised by enteropathy and involvement of the endocrine system, such as insulin dependent diabetes mellitus (IDDM) and thyroiditis, which develop in association with autoantibodies in early infancy (MIM 304930, 304790).[1 2] IPEX has been mapped to chromosome Xp11.23-Xq13.3.[3 4] Recent studies have indicated that *FOXP3*, a member of forkhead/winged-helix proteins, is a causative gene for both IPEX and an equivalent mouse, *scurfy*.[5–8] Human *FOXP3* consists of 11 exons and encodes 431 amino acids containing a zinc finger (Zn) domain, a leucine zipper (Zip) motif, and a forkhead domain.[6 8] We have previously reported two unrelated Japanese patients with X linked autoimmune enteropathy associated with tubulonephropathy and endocrinopathy.[2 9 10] We report here novel mutations in the *FOXP3* gene of these patients.

Patients and methods

Clinical and laboratory findings of our patients have been previously reported.[2 9 10] Briefly, patient 1, a boy, now 11 years old, was diagnosed as having autoimmune thyroiditis, autoimmune haemolytic anaemia, and autoimmune enteropathy at the age of 2 weeks, 2 months, and 5 months, respectively. Renal tubular dysfunction was also noted. His maternal uncle and his older brother had died of a similar diarrhoeal disease complicated by IDDM, suggesting X linked inheritance of the disease.[2] He has been treated with a combination of tacrolimus (0.3 mg/day) and betamethasone (0.3-0.5 mg/day).[9] Hypocalcaemia and hypokalaemia often develop in spite of supplementation with calcium, potassium, and vitamin D, which suggests renal damage resulting from either the underlying disease or a side

Table 1 Primers used in the analyses of the FOXP3 gene

	Forward primer	*Reverse primer*
Exon 1	5'-AGTATCTCATACCGCCCTAGCACACGTGTGA-3'	5'-ACAGTAAAGGTCGGCACCTGTAGGTCCAGGTA-3'
Exons 2–3	5'-AGTGCAGAGTATTTGAATTAGACACAGAACAGTG-3'	5'-AGAATAGCCTACACTGCTCACAGCCAAGGATCTG-3'
Exons 4–5	5'-AGAGGCCTTGTGGGCCAAGCTCCAGAGCCCA-3'	5'-GAGCTGAGATCTGCACCCTAGACCTCTCCCCA-3'
Exon 6	5'-GCATGTGTTAAGGGAACGAGGGGTGT-3'	5'-GGTTTTGCGCACTATCCCTA-3'
Exon 7	5'-GGGATAGTGCGCAAACC-3'	5'-CAGCAGTCTGAGTCTGCCACCA-3'
Exon 8	5'-GGCGACAGAGCAAGACTCAGTA-3'	5'-CACCCAGAGCCTGTCAGGATTAGGA-3'
Exon 9	5'-GACGGTGAGATCTCAGGCCTGTAGACTCACCTTG-3'	5'-AACCCACTCTGAGGGCACTCAGAGGGAGACA-3'
Exons 10–11	5'-AGGTCATAGCCCCTCTAAACCCCAAGTTTG-3'	5'-ACCTCTGCCTCCCACCAGTTTGGCCCCTGTTCG-3'

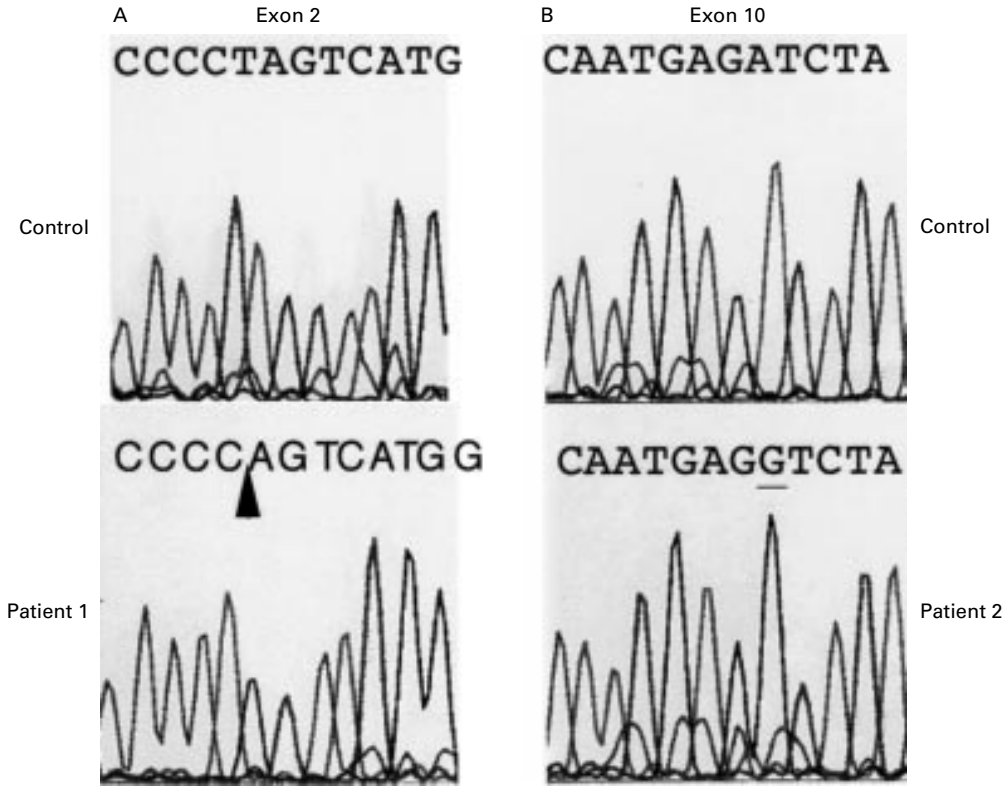

Figure 1 Sequence analyses of exon 2 of patient 1 (A) and exon 10 of patient 2 (B). Arrowhead indicates a deletion of T at nt 227 in patient 1. Underline indicates an A to G transition at nt 1087 in patient 2.

effect of tacrolimus. In addition, he is suffering from osteoporosis associated with multiple compression fractures of the vertebral bodies and steroid induced cataract. He has failed to gain weight and height, although his diarrhoea has been fairly well controlled.

Patient 2, a boy, was diagnosed as having autoimmune enteropathy, tubulonephropathy, IDDM, and thyroiditis in his early infancy. He died of sepsis before using immunosuppressants at the age of 3 years. His maternal uncle died of intractable diarrhoea.[9] Both of the patients showed extremely high levels of serum IgE.[10 11]

Genomic DNA was isolated from Epstein-Barr virus transformed cell lines in patient 1 and his family and from a necropsy specimen in patient 2 using a standard method. Each exon including the exon-intron junction was amplified by polymerase chain reaction (PCR) (94°C for five minutes, followed by 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for one minute) with primers listed in table 1. Additional primers were also used for sequence analyses.[8] PCR products were supplied for direct sequencing. Both PCR amplification and sequencing were performed using GeneAmp PCR System 2400 (Perkin Elmer). Sequence analyses were performed by Gene Analyzer 310 (ABI PRISM).

Results and discussion

Patient 1 showed a deletion of a single nucleotide T227 in exon 2, which results in a frameshift and generates a premature stop at codon 128 (fig 1A). Accordingly, the truncated protein in patient 1 lacks all of the domains and is apparently non-functional. This mutation was not observed in his healthy brother, sister, or father. His mother was found to be heterozygous for this mutation. Seven mutations of *FOXP3* have been reported in IPEX. Three cases carry single amino acid substitutions in the forkhead domain, whereas another has a single amino acid deletion in the Zip domain.[6–8] Deletion of the forkhead domain was reported in one case, which resulted from exon 9 skipping and a frameshift accompanied by premature termination.[8] The remaining two cases involve deletion of a stop codon which results in the addition of new residues.[6 7] Patient 2 showed an A1087G substitution in exon 10, which results in an Ile363Val substitution (fig 1B). Wildin *et al*[6] reported that no sequence variations are found in exons 10

and 11 in unaffected subjects, indicating that the Ile363Val substitution is not a polymorphism but a missense mutation. Ile363 is located in the middle of the second α-helix of the forkhead domain and is highly conserved among FOX family members,[13 14] suggesting that Ile363 is critical for the function of FOXP3.

IPEX patients, as well as *scurfy* mouse, show autoimmune and allergic features which are associate with skewed expression of Th2 type cytokines.[1–8 15–17] Our patients showed various autoantibodies against the intestine, renal tubules, thyroid, or red blood cells.[2 12] In addition, overexpression of Th2 type cytokines such as interleukin-4 has also been found in the peripheral blood mononuclear cells from patient 1.[11] Thus, it is predicted that *FOXP3* contributes to maintenance of immune tolerance and regulates cytokine expression. Clarification of its precise function in normal immune systems could provide new insights into the mechanism of autoimmunity and allergy.

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Maternal uniparental isodisomy 11q13→qter in a dysmorphic and mentally retarded female with partial trisomy mosaicism 11q13→qter

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EDITOR—Partial trisomy mosaicism describes the presence of a normal cell line together with an unbalanced translocation in a second cell line. Its incidence is not known. Only a few cases have been published,[1] almost all with developmental delay and a pattern of dysmorphism. The presence of a normal cell line points towards postzygotic formation, but the origin and mechanism of formation have so far only been investigated in one case of partial trisomy 16p mosaicism and in another case of partial trisomy 21q mosaicism.[2 3] In the former, a complex formation by trisomy first, translocation second, and uniparental disomy and partial trisomy third was inferred. In the latter, paternal meiotic origin of der(21;21)(q10;q10) mosaicism (46,XX/46,XX,der(21;21)(q10;q10),+21) in a girl with mild Down syndrome was described.

Here, we report on a 25 year old woman with mental retardation, dysmorphic features, partial trisomy 11q13→qter mosaicism (46,XX, der(19)t(11;19)(q13;p13.3)/46,XX), maternal uniparental isodisomy 11q13→qter in the