DA5 (5'-GATCCGTGCTCCAGGCCGTCG-3'), a 0.9 kb fragment was amplified from the genomic DNA, which carried the intronic sequence between cDNA nucleotide positions 1234 and 1235 (data not shown). Cloning of PCR product from normal genomic DNA yielded a 54 bp shorter fragment than the unretreated one (fig 2). The 54 bp fragment was not detectable on the gel system used. In the study of genomic DNA from the family members, the fragments from patients 1 and 2 were not digested with SacI, whereas cleaved bands were observed for the father and a second unaffected sib as well as normal subfamily 1 (data not shown). Half the PCR product from the mother was digested with SacI, indicating carriage of the mutation by one of the alleles. The Sac restriction site was present in the DNA from all of 10 unrelated Japanese females (20 alleles) investigated (data not shown). Cosegregation of the mutation with the disease provides evidence that it is directly causative. The present approach clearly offers advantage for carrier detection and prenatal diagnosis.

The affected leucine is within the SH2 domain. This is highly conserved in the SH2 domains of other non-receptor tyrosine kinases.2 SH2 domains have been shown to bind tyrosine phosphorylated ligands.3 While the mutation of the highly conserved leucine would therefore be expected to affect the conformation or function of Btk, further analysis of the protein is required before such a conclusion can be definitely drawn. While heterozygous mutations of the btk gene have been found in XL patients, 25,26 the three previously reported missense mutations resulting in Arg-288 to Trp, Arg-307 to Gly, and Tyr-293 to Cys within the SH2 domain in the mutation in our patient is a new missense mutation within the SH2 domain.

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YOSHIIYUKI OHASHI SHIGERU TSUCHIYA TATSUKE KONDO Department of Paediatric Oncology, Institute of Aging, Development, and Cancer, Tokushima University, 4-1 Seigo-machi, Daba-ku, Sendai 980, Japan.