A new point mutation involving a highly conserved leucine in the Btk SH2 domain in a family with X linked agammaglobulinaemia

Two Japanese sibs with X linked agammaglobulinaemia (XLA) were shown to have a new missense mutation, a C to T transition at nucleotide position 1204, resulting in an amino acid change from Leu-358 to Phe in the SH2 domain of the Btk (Bruton's tyrosine kinase). The affected leucine in the patients is highly conserved in SH2 domains of various non-receptor tyrosine kinases.

XLA is an inherited immunodeficiency disorder characterised by recurrent bacterial infections, because of antibody deficiency. We investigated btk gene defects in two Japanese sibs with XLA, aged 22 (patient 1) and 12 years (patient 2), respectively. The clinical histories and immunological data of the patients have been described previously. To determine genetic defects in the patients, we first analysed an Epstein-Barr virus transformed B lymphoblastoid cell line, K4, derived from bone marrow of patient 1. The protein coding region of K4 btk cDNA was amplified in two overlapping regions using reverse transcription PCR as previously described. The 5′ half of the cDNA synthesised with primer A3 (5′-GGAATTTTGA-GCCTACTGAG-3′) was amplified using primers A1 (5′-ACTGAGCACACAGG-TGAAC-3′) and B2 (5′-GTGATGAT-GAAATTTGGA-3′). The primers B4 (5′-AGCTGGGATTTCTCTAG-3′), and B3 (5′-ATTGGGAGCTCAG-GATTCTTC-3′), described elsewhere, were prepared to amplify the remaining region of the molecule. Sequence analysis of the patient’s btk cDNA showed a C to T transition at nucleotide position 1204. This would be expected to result in an amino acid change at position 358 from leucine to phenylalanine (fig. 1).

In view of the mutation at a SacI restriction site (GAGCTC to GAGTTC), the region carrying the substitution in the genomic btk DNA was amplified by PCR and then digested with SacI. Using primers DA2 (5′-CCCTAGAGGCCATATTACCT-3′) and

![Figure 1](https://example.com/f1.png)  Sequence of PCR amplified btk cDNA from the patient (left) and a normal control (right). The base substitution mutation at position 1204 of C to T (*) is indicated by the arrow. This mutation resulted in a change of codon 358 CTC for leucine to TTC for phenylalanine.

![Figure 2](https://example.com/f2.png) Detection of the mutation by PCR and SacI digestion in btk genomic DNA from the family members. The pedigree of the family is shown at the top. PCR amplified btk DNA fragments were digested with SacI and electrophoresed on a 1% agarose gel. Digestion of a 0.9 kb PCR product with SacI yielded a 54 bp shorter fragment (open arrow) than the untreated one (closed arrow) in the normal, but not the mutant allele.
DA5 (5'-GATCGCTATCCAGGCCTGC-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). Cleavage by BglII of the PCR product from normal genomic DNA yielded a 54 bp shorter fragment than the untreated 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 subjects (fig 2). Half the PCR product from the mother was digested with SacI, indicating carriage of the mutation by one of the alleles. The SacI restriction site was present in the DNA from all of 10 unrelated Japanese females (20 alleles) investigated (data not shown). Co-segregation 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, and is highly conserved in the SH2 domains of other non-receptor tyrosine kinases. 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 Brk, 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 XLA patients,19 20 the three previously reported missense mutations resulting in Arg-288 to Trp, Arg-307 to Gly,11 and Tyr-369 to Phe within the SH2 domain of the mutation in our patient is a new missense mutation within the SH2 domain.

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Exclusion of retinoic acid receptor and a cartilage matrix protein in non-syndromic CL(P) families

We read with interest the report of Vintiner et al11 excluding linkage to RARA (17q21), D17S800 (1-12), D17S800 (1-25), and D17S800 (1-57) loci in eight multigeneration white families with autosomal dominant non-syndromic cleft lip with or without cleft palate (CL(P)). These candidate genes were chosen on the basis of their mapping close to previously reported non-syndromic cleft palate loci.12 16 17 We have also tested linkage to the same loci in ten non-syndromic CL(P) white families at these same loci, and can confirm the reported exclusions.18 19 The RARA locus was tested using Southern gel and Genus non-radioactive techniques.20 21 Tightly linked flanking short tandem repeat PCR markers at the RARA locus, Thra1, Mf1688 (D17S579), D17S800, and Hox2B and CRTL2 were amplified and separated on 8% sequencing gels,2 and visualised by silver staining using the GELCODE® system.22 Linkage was tested by using MLINK and LIPED, assuming a dominant mode of inheritance for CL(P) with a penetrance of 0-32 in males and 0-24 in females and with a 0-01 allele frequency. The lod scores were 1-14, -9-57, 9-72, -8-87, and -2-49 for RARA, Thra1, D17S579, D17S800, and Hox2B respectively. The summed lod scores were enough to declare the RARA locus unlinked to CL(P) in our families. However, haplotype analysis of flanking markers (Thra1, RARA, D17S800) excludes this region in these multigenerational families. Hox2B was tested as a candidate gene for clefting and was excluded. We have also tested linkage to the CRTL1 locus and the lod score of -2-1 at 0-01 also excluded this gene. We have previously reported exclusion of the F13A1 locus and the entire region spanning from F13A1 to TCCTE, which included the HLA region.19

Our findings and those of Vintiner et al suggest that RARA, CRTL1, and F13A1 do not have a major causal role in the aetiology of CL(P) in the 18 families tested. However, as previously suggested, we can not distinguish whether RARA plays a modifying role in the aetiology of CL(P).3

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Further report of a patient with humeroauricular synostosis and hydropneumone

A case of humeroauricular synostosis with lamboyd synostosis was published recently in this journal.1 We report on a male baby with clinical and skeletal abnormalities very similar to those previously reported.2 3

The proband was the first child of young and healthy non-consanguineous parents. He was born at term following a normal delivery because of cephalopelvic disproportion. Birth weight and length were 3490 g and 51 cm, respectively. Apart from the upper limb defects a and a midline capillary haemangioma, no other abnormality was found on physical examination.

At 11 months he measured 73-5 cm (50th centile), head circumference was 47 cm (50th centile), and weight was 8300 g (10th centile). Psychomotor development has been normal. Both upper limbs were short, the left one more markedly so than the right. Both shoulders had normal range of movement. The left upper limb was shorter than the right and kept in a fixed position; there were two digits joined