DA5 (5'-GATCCGTATCCCAGGCCTGC-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 *SacI* 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). 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 of Btk and is highly conserved in the SH2 domains of other non-receptor tyrosine kinases.⁷ SH2 domains have been shown to bind tyrosine phosphorylated ligands.⁸ 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 heterogeneous mutations of the btk gene have been found in XLA patients,^{9,10} the three previously reported missense mutations resulting in Arg-288 to Trp, Arg-307 to Gly,¹¹ and Tyr-361 to Cys¹² within the SH2 domain, the mutation in our patient is a new missense mutation within the SH2 domain.

We thank Dr H Kikuchi for helpful advice and discussion. This work was supported in part by grants from the Ministry of Education, Science and Culture, and the Ministry of Health and Welfare, Japan.

YOSHIYUKI OHASHI
SHIGERU TSUCHIYA
TASUKE KONNO

Department of Paediatric Oncology,
Institute of Aging, Development, and Cancer,
Tohoku University,
4-1 Seiryomachi, Aoba-ku, Sendai 980, Japan.

- 1 Bruton OC. Agammaglobulinemia. *Pediatrics* 1952;9:722-7.
- 2 Vetrie D, Vořechovský I, Sideras P, et al. The gene involved in X-linked agammaglobulinemia is a member of the src family of protein-tyrosine kinases. *Nature* 1993;361:226-33.
- 3 Tsukada S, Saffran DC, Rawlings DJ, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* 1993;72:279-90.
- 4 Tsuchiya S, Konno T, Tada K, Ono Y. Epstein-Barr virus-induced lymphoblastoid cell lines from patients with primary immunodeficiency diseases. *Scand J Immunol* 1980;11:155-62.
- 5 Tsuchiya S, Sato T, Nakae S, Katamine S, Konno T, Tada K. Epstein-Barr virus-induced precursor B cell lines from patients with congenital agammaglobulinemia. *Tohoku J Exp Med* 1983;140:133-44.
- 6 Ohashi Y, Yambe T, Tsuchiya S, Kikuchi H, Konno T. Familial genetic defect in a case of leukocyte adhesion deficiency. *Hum Mutat* 1993;2:458-67.
- 7 Overduin M, Mayer B, Rios CB, Baltimore D, Cowburn D. Secondary structure on the Src homology 2 domain of c-abl by heteronuclear NMR spectroscopy in solution. *Proc Natl Acad Sci USA* 1992;89:11673-7.
- 8 Mayer BM, Jackson PK, Baltimore D. The non-catalytic src homology region 2 segment of abl tyrosine kinase binds to tyrosine-phosphorylated cellular proteins with high affinity. *Proc Natl Acad Sci USA* 1991;88:627-31.

- 9 Vořechovský I, Zhou JN, Vetrie D, et al. Molecular diagnosis of X-linked agammaglobulinemia. *Lancet* 1993;341:1153.
- 10 de Weers M, Mensink RGJ, Kraakman, MEM, Schuurman RKB, Hendriks RW. Mutation analysis of the Bruton's tyrosine kinase gene in X-linked agammaglobulinemia: identification of a mutation which affects the same codon as is altered in immunodeficient xid mice. *Hum Mol Genet* 1994;3:161-6.
- 11 Bradley LAD, Sweetman AK, Lovering RC, et al. Mutation detection in the X-linked agammaglobulinemia gene, BTK, using single strand conformation polymorphism analysis. *Hum Mol Genet* 1994;3:79-83.
- 12 Saffran DC, Parolini O, Fitch-Hilgenberg ME, et al. A point mutation in the SH2 domain of Bruton's tyrosine kinase in atypical X-linked agammaglobulinemia. *N Engl J Med* 1994;330:1488-91.

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 al¹ excluding linkage to RARA (17q21), F13A1 (6p24-25), and CRTL1 (5q15) 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 translocations with associated syndromes which include CL(P), proximity to loci known to cause syndromes which include CL(P), and from previously published association and linkage studies.¹

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.¹ The *Pst* RFLP in RARA was tested using Southern gel and Genius non-radioactive techniques.² Tightly linked flanking short tandem repeat PCR markers at the RARA locus, Thr1, Mfd188 (D17S579), D17S800, and Hox2B and CRTL were amplified and separated on 8% sequencing gels,² and visualised by silver staining using the GELCODE® system.³ 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 0.001 allele frequency.⁴

The lod scores at $\theta=0$ were 1.14, -9.57, -9.72, -8.87, and -2.49 for RARA, Thr1, D17S579, D17S800, and Hox2B, respectively. The summed lod scores are shown in the table. Although RARA showed a small positive lod score, the families were generally uninformative. However, haplotype analysis of flanking markers (Thr1, 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 $\theta=0.1$ also excluded this gene. We have previously reported exclusion of the F13A1 locus and the entire region spanning from F13A1 to

TCTE, which included the HLA region.³

Our findings and those of Vintiner et al¹ 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).⁵

JONATHAN D STEIN
JACQUELINE T HECHT
Department of Pediatrics,
University of Texas Medical School
PO Box 20708,
Houston, TX 77225, USA
SUSAN H BLANTON
Department of Pediatrics,
University of Virginia - Charlottesville,
Charlottesville, VA, USA

- 1 Vintiner GM, Lo KK, Holder SE, Winter RM, Malcolm S. Exclusion of candidate genes from a role in cleft lip with or without cleft palate: linkage and association studies. *J Med Genet* 1993;30:773-8.
- 2 Sambrook J, Fritsch F, Maniatis T. *Molecular cloning. A laboratory manual*. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
- 3 Hecht JT, Wang Y, Conner B, Blanton SH, Daiger SP. Nonsyndromic cleft lip and palate: no evidence of linkage to HLA or factor 13A. *Am J Hum Genet* 1993;52:1230-3.
- 4 Hecht JT, Yang P, Michels V, Buetow K. Complex segregation analysis of nonsyndromic cleft lip and palate. *Am J Hum Genet* 1991;49:674-81.
- 5 Chenivix-Trench G, Jones K, Green AC, Duffy DL, Martin NG. Cleft lip with or without cleft palate: associations with the transforming growth factor alpha and retinoic acid receptor loci. *Am J Med Genet* 1991;41:44-8.

Further report of a patient with humeroradioulnar synostosis and hydronephrosis

A case of humeroradioulnar synostosis with lambdoid 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.¹⁻⁴

The proband was the first child of young and healthy non-consanguineous parents. He was born at term following caesarean section because of cephalopelvic disproportion. Birth weight and length were 3490 g and 51 cm, respectively. Apart from the upper limb defects 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 malformed 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

Lod scores for CLP v chromosome 17 markers and in CL(P) families

Marker	Recombination fraction (θ)						
	0.00	0.001	0.01	0.05	0.10	0.20	0.30
Thr1	-9.57	-9.12	-7.47	-4.61	-2.80	-1.33	-0.63
RARA	1.14	1.14	1.09	0.90	0.69	0.37	0.16
D17S579	-16.35	-14.11	-9.38	-4.80	-2.85	-1.25	-0.57
D17S800	-8.87	-7.01	-3.76	-1.25	-0.33	0.31	0.43
Hox2b	-2.49	-2.27	-1.29	-0.06	0.38	0.53	0.39

X rays of the upper limbs showing (top) humeroradioulnar synostosis and (below) humeroradial synostosis and absent thumb ossification.

along their axis. On the right side only absence of the thumb was observed.

Radiographs obtained at 4 months showed left humeroradioulnar synostosis, two metacarpophalangeal bones (probable fusion of a third metacarpal bone), and the middle phalanx of the 5th finger was absent. On the right, there was humeroradial synostosis and thumb ossification was absent (figure). Lower limb radiographs disclosed no abnormality. Skull x ray failed to show craniosynostosis as reported by Edwards *et al.*¹

An abdominal ultrasound scan showed hydronephrosis of the left kidney, with a pyelogram suggestive of left pyeloureteral stenosis. This finding, however, could be coincidental, but should be searched for in similar cases.

This case represents an example of the possible variability of this condition, the pathogenesis of which still remains obscure.²

R BOY
D HOROVITZ
F R VARGAS
J G BARBOSA NETO
J C LLERENA JR
J C C ALMEIDA
Centro de Genética Médica,
Instituto Fernandes Figueira,
Fundação Oswaldo Cruz,
Av Ruy Barbosa 716,
Flamengo, CEP 22250-020,
Rio de Janeiro, Brazil.
J C LLERENA JR
J C C ALMEIDA
Unidade de Citogenética Humana,
IBCCF, UFRJ.

- 1 Edwards TJC, Haan EA, Humphrey IJ. Humeroradioulnar synostosis in a patient with lambdoid synostosis. *J Med Genet* 1993;30: 81-2.
- 2 Gollop TR, Coates V. Apparent bifurcation of distal humerus with oligoectrosyndactyly. *Am J Med Genet* 1983;14:591-3.
- 3 Hersh JH, Joyce MR, Profumo LE. Humero-radio-ulnar synostosis: a new case and review.

Am J Med Genet 1989;33:170-1.

- 4 Leroy JG, Speeckaert MTC. Humeroradioulnar synostosis appearing as distal humeral bifurcation in a patient with distal phocomelia of the upper limbs and radial ectrodactyly. *Am J Med Genet* 1984;18:365-8.

BOOK REVIEW

If you wish to order or require further information regarding the titles reviewed here, please write to or telephone the BMJ Bookshop, PO Box 295, London WC1H 9JR. Tel 071 383 6244. Fax 071 383 6662. Books are supplied post free in the UK and for BFPO addresses. Overseas customers should add 15% for postage and packing. Payment can be made by cheque in sterling drawn on a UK bank or by credit card (Mastercard, Visa, or American Express) stating card number, expiry date, and full name. (The price and availability are occasionally subject to revision by the Publishers.)

Human Population Genetics: A Centennial Tribute to J B S Haldane. Editor Partha P Majumder. (Pp 348; \$85.00.) New York: Plenum Press. 1994.

In the excitement of the rapid developments of today, it is easy for readers of this journal to be unaware of the debt that they owe to J B S Haldane, for much of the theory on which clinical genetics is founded originated with his work. His influence, great in all fields of genetics human and non-human, was equally

great in physiology and biochemistry. He wrote some 400 scientific articles, 20 books, and numerous essays, and at least 20 of his pupils were elected Fellows of the Royal Society. It was therefore fitting to commemorate the centenary of his birth, and the same idea occurred to several. Thus a one day symposium was organised at University College London on behalf of the Biometric Society, by Professor C A B Smith, and there seven of Haldane's friends and colleagues spoke on aspects of his work and ideas, essentially a personal tribute. A much more ambitious celebration, a full conference, was organised in December 1992 at the Indian Statistical Institute in Calcutta, to which Haldane retired. The proceedings of that conference form the subject of this book. Its object was to evaluate the effect of Haldane's contributions in various areas of human genetics, in the light of the more recent developments.

C R Rao, a former Director of the Research and Training School of the Indian Statistical Institute, and jointly responsible (with Mahalanobis) for attracting Haldane there, gave the opening address of which a condensed version opens this book, illustrating with examples the scientific method followed and advocated by Haldane. The technical papers are then grouped in five sections.

The first, devoted to population genetics and evolution, opens with a highly provocative paper by W J Ewens, criticising several aspects of Haldane's thought and work. The very readable style of this criticism continues in Ewens's discussion of key developments in population genetics post 1955, in which flesh has been added to the Fisher-Haldane-Wright skeleton, for example, the incorporation of multilocus systems and the development of stochastic theory appropriate for the situation of infinitely many alleles that is emerging from recent molecular work. Other noteworthy contributions in this section are those by N Takahata on the evolution of the immune system, and by T Gojobori and T Imanishi which draws together a great deal of MHC gene frequency data, and the phylogenetic trees based on them show the importance of the major racial groupings in contributing to variation at these loci.

The second part concerns the formal genetics of man, and the papers here are outstanding, though not easy reading for the mathematically fearful. C C Li reviews the several methods of segregation analysis that have been developed in the post Haldane period. They are essentially applicable for cases of complete ascertainment, which is much more attainable today with modern computerisation of records than at the time of Haldane's pioneer work in the early thirties. Li feels that the segregation models involving varying values of ascertainment probabilities are somewhat arbitrary and inadequate, largely on account of complex social factors that contribute to the completeness or incompleteness of ascertainment. To overcome these difficulties he recommends striving for complete ascertainment by establishing adequate systems of registration and reporting by health agencies. R Elston re-examines recent developments in the theory of segregation analysis. He describes the two well established multiparameter models available for performing likelihood based analyses (the transmission probability and the mixed model). P P Majumder's chapter, also on segregation analysis is complementary, for he concentrates on a multilocus epistatic model with or without variable age of onset, but finally provides a reminder of the value of a