Am. J. Hum. Genet. 54:25-35, 1994

Application of Carrier Testing to Genetic Counseling for X-linked Agammaglobulinemia

R. Cutler Allen,* Ronald G. Nachtman,* Howard M. Rosenblatt,† and John W. Belmont*,†

*Institute for Molecular Genetics and Howard Hughes Medical Institute and †Department of Pediatrics, Baylor College of Medicine, Houston

Summary

Bruton X-linked agammaglobulinemia (XLA) is a phenotypically recessive genetic disorder of B lymphocyte development. Female carriers of XLA, although asymptomatic, have a characteristic B cell lineage-specific skewing of the pattern of X inactivation. Skewing apparently results from defective growth and maturation of B cell precursors bearing a mutant active X chromosome. In this study, carrier status was tested in 58 women from 22 families referred with a history of agammaglobulinemia. Primary carrier analysis to examine patterns of X inactivation in $CD19^+$ peripheral blood cells (B lymphocytes) was conducted using quantitative PCR at the androgen-receptor locus. Obligate carriers of XLA demonstrated >95% skewing of X inactivation in peripheral blood $CD19^+$ cells but not in $CD19^-$ cells. Carrier status for mothers of isolated affected males could be assessed in 10 of 11 families: 7 women showed skewing, and 3 did not. Five carriers were found in six families in which there were no living affected males. Among all those tested, one individual's carrier status was considered to be indeterminate and five women were noninformative for the carrier test. Results obtained by the carrier test were congruent with linkage analysis (where applicable) using the RFLPs DXS178 and DXS94 and two newly developed polymorphic microsatellite markers, DXS178CA and DXS101AAT. Refinements in techniques for primary carrier testing and genetic mapping of XLA now make possible an ordered approach to diagnosis, prenatal diagnosis, and genetic counseling.

Introduction

X-linked agammaglobulinemia (XLA; MIM 300300) is a well-defined genetic disorder characterized by a selective defect in the humoral immune response (Bruton 1952). Affected males have extremely low concentrations of serum immunoglobulins in association with absent or low numbers of B lymphocytes (Rosen et al. 1984; Ochs and Wedgewood 1989). These abnormalities lead to recurrent bacterial or viral infections beginning at 6–18 mo of age. Although rare (estimated birth prevalence $1\text{–}5 \times 10^{-6}$), XLA is an important cause of primary immune deficiency in young children (Conley 1992). XLA results from disturbed growth and differentiation of B lymphocyte precursors in the bone marrow, where reduced numbers and proliferation indices of pre-B cells have been observed (Campana et al. 1990). Females carrying an XLA mutation have normal numbers of peripheral blood B cells, but this population of cells shows profound skewing of X inactivation (Conley et al. 1985, 1988; Fearon et al. 1987; Journet et al. 1992). Such skewing indicates that there is selective growth of B cells having the normal X chromosome active and that the missing gene activity is required by their precursors. The population of mature B cells that exclusively express a normal XLA gene product (because of X inactivation) fully compensates for the defective growth of cells expressing the abnormal gene.

The XLA locus was mapped to Xq22 by linkage analysis (Kwan et al. 1986, 1990; Mensink et al. 1986; Malcolm et al. 1987; Guioli et al. 1989). Additional studies placed the XLA locus within an ~2-million-bp region flanked by two anonymous DNA markers, DXS101 and DXS442 (Lovering et al. 1993; Parolini et al. 1993). No recombination was observed, in any family, between the marker DXS178 and XLA. Screening for B

Received July 15, 1993; revision received September 14, 1993.

Address for correspondence and reprints: John W. Belmont, M.D., Ph.D., Institute for Molecular Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

0002-9297/94/5401-0005$02.00

cell-specific signal transduction proteins, Tsukada et al. (1993) identified a gene encoded in this region, termed it "*BPK*," and showed that its product was abnormally expressed in XLA patient cell lines. The same gene (called "*atk*") was isolated in independent studies using a novel cDNA selection scheme that identified B cell transcripts encoded near DXS178 (Vetrie et al. 1993). *atk* was found to have either intragenic deletion or point mutation cosegregating with XLA in selected families. A missense mutation in the murine gene has been found in the naturally occurring B cell developmental defect *xid,* indicating that it is a murine homologue for the human disease (Rawlings et al. 1993; Thomas et al. 1993). This gene (consensus terminology —*btk*) belongs to a new subfamily of cytoplasmic protein tyrosine kinases. The XLA and *xid* phenotypes strongly suggest that btk, like other known tyrosine kinases, plays a key role in growth-signal transduction or growth-signal amplification in B cells.

Identification of the XLA gene provides important insight into its pathogenesis, but it does not solve the most immediate problems in genetic counseling. In approximately one-half of cases there is no family history of the disease. The possibilities of new mutation in either the patient or his mother or of occurrence of the rare autosomal recessive phenocopy (Conley and Sweinberg 1992) must be considered in isolated cases. In some families, the proband has expired and/or the diagnosis may have been given before clear diagnostic criteria were established. Even in large or well-established XLA pedigrees, carrier and linkage analyses are likely to continue to be useful because of the difficulty of performing routine mutation analysis. We have developed a new highly informative and quantitative method for carrier testing and have also isolated more efficient linkage markers in the region of XLA. Here, we demonstrate the utility of combining direct carrier testing with the new information on gene location, to provide genetic-risk information to XLA family members.

Subjects and Methods

Study Population

Diagnosis of XLA was made on males who had recurrent bacterial infections in the first 2 years of life, low or undetectable serum immunoglobulins (IgG <200 mg/dl; IgA <7.5 mg/dl; IgM <20 mg/dl), absent specific antibody production, low or absent peripheral blood B cells ($CD19^+$ cells and/or $CD20^+$ cells <1% of peripheral blood lymphocytes), and normal T cell numbers and function. Obligate carriers for XLA were women who had an affected son or carrier daughter and at least one affected male relative in the maternal lineage. Normal controls were obtained from an ethnically diverse group of young women (age range 21–36 years). This study was reviewed and approved by the Baylor College of Medicine Institutional Review Board for Human Research.

Carrier Analysis

Cell separation and PCR conditions for the carrier assay have been described elsewhere, in detail (Allen et al. 1992). Briefly, B lymphocytes were isolated from the mononuclear cell fraction of 20 cc of blood by using anti-CD19 immunomagnetic beads (Dynal). Purity of the $CD19^+$ cell population was confirmed by flow cytometry using the B cell–specific marker CD20; all cell populations used for DNA analysis were >90% $CD20^+$. To control for skewing arising by stochastic mechanisms, $CD19^-$ cells were analyzed; these $CD19^-$ cells consist predominantly (>90%) of T lymphocytes. A crude DNA extract was prepared from 2.5×10^5 cells and digested with a control enzyme, *Rsa*I, or with the methylation-sensitive enzyme *Hpa*II. The digested DNA was then used as template for PCR (Mullis and Faloona 1987) across the first exon of the androgen receptor. All the control samples and approximately half of the patient samples were analyzed using a PCR in which one primer was labeled with the fluorescent dye JOE (2,7′-dimethoxy-4′,5′-dichloro-6 carboxy-fluorescein; Applied Biosystems, Inc. [ABI]). The products were evaluated using an ABI model 370A automated DNA sequencer; individual allele peak areas were measured using GeneScan software.

Linkage Analysis

DNA was isolated from the peripheral blood (Maniatis et al. 1988) and was analyzed by PCR for RFLPs at DXS178 and DXS94 and for short tandem repeat (STR) polymorphisms DXS178CA and DXS101AAT. The RFLPs at DXS178 and DXS94 were converted to PCR assay by a determination of the sequence around the polymorphic sites. Briefly, plasmid probes p212 and pXG-12 were used to screen a human Charon 4 genomic library. Positive clones were subjected to restriction mapping, and the plasmid subclones containing the putative polymorphic sites were derived. After sequencing of these plasmids, flanking PCR primers were developed. The resulting RFLPs were tested for correlation with samples previously genotyped by Southern blotting. The RFLP at DXS178 was assayed by performing PCR, using primers 5′-GGATTTAGAGACCCATTTGCTGG-3′ and 5′-TACCTACCTGACCATCTTGGTGA-3′. The PCR product was purified,

digested with *Taq*I, and then size fractionated on a 1.5% agarose gel. The RFLP at DXS94 was assayed by PCR, with primers 5′-GAGAATGGTATACCTGAG-AGGATCGA-3′and5′-CAAGTTTGCATTATTGTAT-ATTGAGC-3′. The purified PCR product was digested with *Pst*I. The STRs, DXS178CA and DXS101AAT, were analyzed as described elsewhere (Allen and Belmont 1992, and in press).

Results

The human androgen-receptor (HAR) locus contains a highly polymorphic (90% heterozygosity) trinucleotide repeat in the first exon (Edwards et al. 1992). This repeat is within ~100 bp of two *Hpa*II restriction-enzyme sites that are methylated on the inactive X chromosome but unmethylated on the active X chromosome (Allen et al. 1992). Skewing of X inactivation was easily detected qualitatively by using a radionuclide technique, and detection was further improved to allow quantitation using a fluorescence-tagged primer method (fig. 1). PCR conditions that produce a linear relationship between allele fluorescence peak area and starting cell/DNA template ratio were established in reconstruction experiments using heteromorphic male samples (fig. 2*A*). Normal females were studied as controls for the quantitative X inactivation assay. In contrast to the analysis of the male DNA, quantitation for X inactivation in females requires that the *Hpa*II enzyme digestion be nearly complete. The presence of two *Hpa*II sites within the amplification unit helps in this, since cleavage at only one site disrupts the template. In practice, complete cleavage at the *Hpa*II sites is achieved by 10-fold overdigestion. X inactivation patterns were investigated in both the $CD19^+$ cells (B lymphocytes) and $CD19^-$ cells (>90% T lymphocytes) from 24 subjects who were heterozygous for the trinucleotide repeat polymorphism (fig. 2*B* and 2*C*). Most showed 35%–65% contribution from one X chromosome in both the B and T lymphocytes (mean ± SD = 55% ± 15% for $CD19^-$ cells and 53% ± 16% for $CD19^+$ cells), although two showed >80% activation of one X chromosome. The percentage contribution of one allele in the $CD19^+$ and $CD19^-$ cells from each individual control was highly correlated ($r = .893$, $P < .001$, two-tailed). The number of coherent clones (with respect to X inactivation) contributing to each population can be estimated by applying a simple binomial sampling model (Nesbitt 1972; Fialkow 1973): $\sigma^2 = p(1 - p)/N$, where the variance, σ^2, is related to p, the contribution from one inactive allele, and N, primordial clone pool size. The present data give an estimated pool size of 11 for the T cells and 10 for the B cells. This is identical to recent results that used a somatic cell hybrid technique and maximum-likelihood analysis (Puck et al. 1992). Overwhelming biological evidence indicates that lymphocytes develop from a common pluripotent stem cell and that establishment of X inactivation precedes their lineage allocation in development. Thus, these data support the use of the $CD19^-$ cell population as the most appropriate control for X inactivation pattern in B lymphocytes—that is, under normal circumstances these two cell populations should have very similar allele contributions. The close correlation of the measurement from the two cell populations in an individual also supports the quantitative accuracy of the androgen-receptor-locus method.

Carrier testing was requested for 22 consecutive families referred because of a son with XLA or because of a family history of a closely related affected male (see table 1). These families can be separated into several categories, each presenting unique problems in counseling: (*a*) families with well-established X-linked inheritance, two or more affected individuals available for genotyping, and/or at least one obligate carrier ($n = 5$); (*b*) families with an isolated living XLA proband ($n = 11$); and (*c*) families with no living affected male ($n = 6$). In the first category, 20 individuals from five XLA pedigrees were tested (fig. 3*A*). Six obligate carriers from this group (subjects 04, 05, 12, 13, 15, and 17; subject 14 noninformative) showed the expected skewing of X inactivation in the B cell lineage. Among the remaining at-risk individuals, five showed X inactivation skewing restricted to the B cells, six were not skewed, one was indeterminate, and one was noninformative for the HAR polymorphism. The woman classified as "indeterminate" (subject 20) had >90% skewing, in both her B cells and her T cells, toward the same X chromosome. Her sister has two living sons with typical XLA and has random X inactivation in her T cells but skewed X inactivation in her B cells. This particular individual is interesting in that she demonstrates the necessity for the $CD19^-$ control and the requirement for a reserved interpretation when that cell population is also skewed.

The second category of families (fig. 3*B*)—in which there is an isolated case of XLA—accounted for nearly half the families. In this group, 11 mothers of affected males were tested: 7 (subjects 21, 24, 31, 33, 35, 37, and 39) showed skewing, 3 (subjects 27, 30, and 36) were not skewed, and 1 (subject 34) was noninformative. These data, although limited, fit well with expectations for occurrence of new mutation in an X-linked lethal

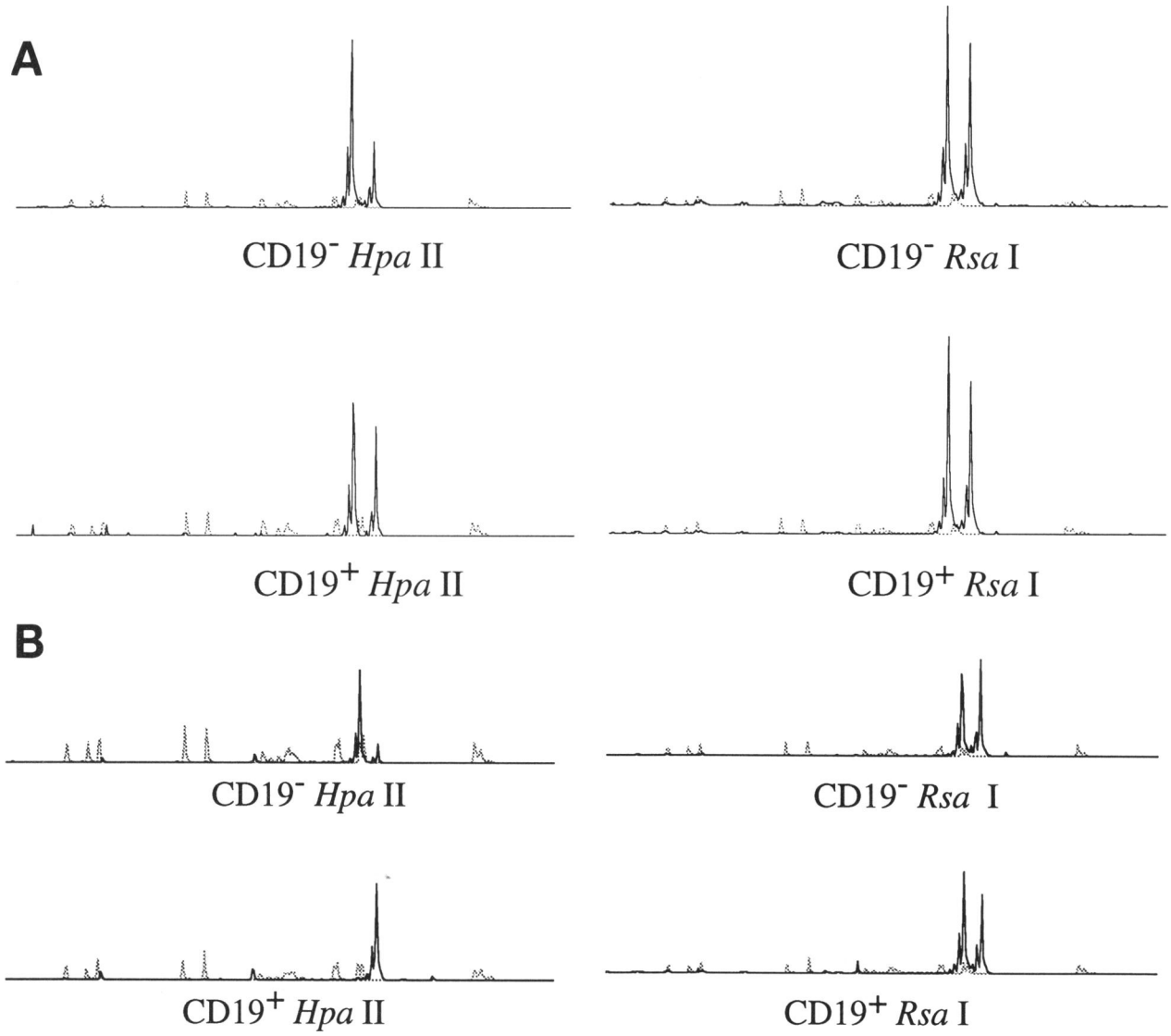

Figure 1 Allele profiles at the androgen-receptor locus analyzed by fluorescence PCR. *A*, Normal female. PCR was performed after *Hpa*II or *Rsa*I digestion of DNA from either $CD19^+$ (B lymphocytes) or $CD19^-$ cells (solid line). Each sample was loaded with an internal molecular-weight marker standard (dotted line). *B*, XLA carrier. Note that in this particular individual the $CD19^-$ cells are partially skewed, but to the allele other than the one to which the $CD19^+$ cells are skewed. The mean allele contribution in the $CD19^-$ cells from the XLA carrier group was 50.2% (range 7%–85%).

disorder (Chase and Murphy 1973). An additional nine individuals (grandmothers, aunts, and sisters) from the families with a single affected male were tested. Carrier state was detected in only two (subjects 38 and 40). The nonskewed individuals, along with the normal controls, reinforce the observation that lineage-restricted skewing is a pathognomonic abnormality in XLA carriers. Taken together with the results from the obligate carriers, these data also indicate that the carrier state is fully penetrant—that is, there were no "skipped generations." It is important to note that when the mother of an XLA patient does not have skewing of X inactivation in her B cells, the possibility of gonadal mosaicism (Parolini et al. 1993) or autosomal recessive agammaglobulinemia should be considered in counseling. At this time, prenatal diagnosis using genetic methods is practically precluded in this group.

In the remaining six families referred for testing,

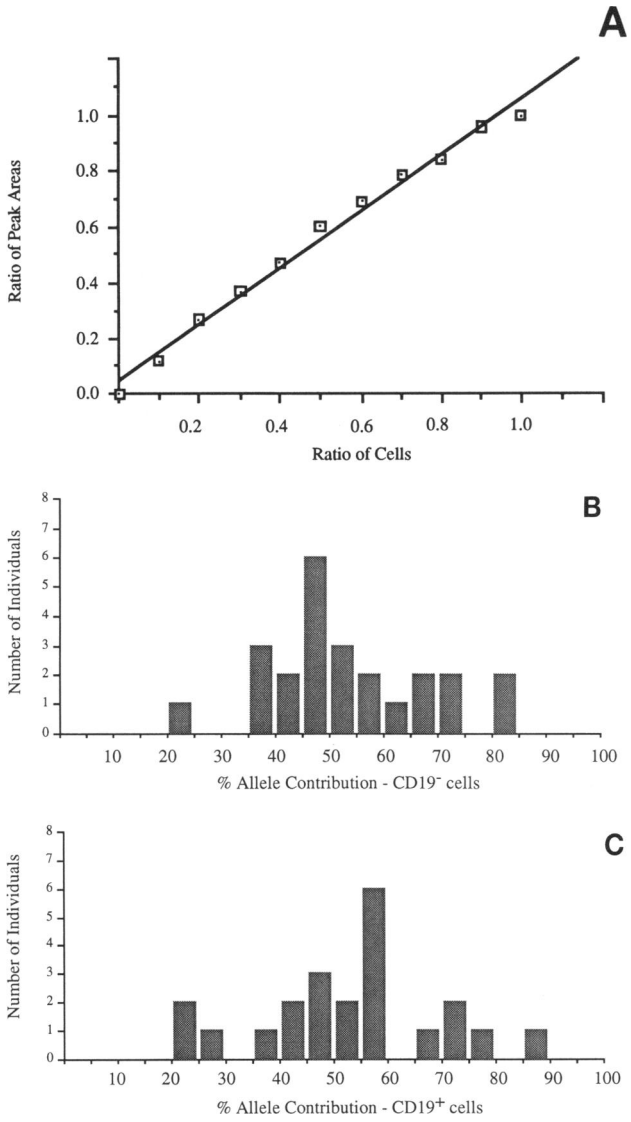

Figure 2 *A*, Quantitation of the androgen-receptor PCR reaction. $CD19^+$ cells from two males who have different androgen-receptor alleles were mixed, DNA extracted, and PCR performed. The products were analyzed on an automated sequencer as described in the Carrier Analysis section ($r = .98$, $P < .005$). *B*, Distribution of X inactivation patterns observed in the $CD19^-$ cells in normal females. Percentage allele contribution was calculated as [peak area p/ (peak area p + peak area q)] × 100, where the lower-molecular-weight allele was arbitrarily designated as p. The data are plotted in intervals of 5%. *C*, Distribution of X inactivation patterns observed in the $CD19^+$ cells in normal females.

there were no living affected males (figs. 3C and 4). These cases are particularly troublesome if the proband(s) expired before definitive immunologic evaluation was available. The mother of the affected male was tested in five of these families: in three cases (subjects 41 and 47 and fig. 4) she was found to be a carrier, in another case (subject 50) no skewed X inactivation was found, and in the last case (described below) skewing was detected in both the B cells and T cells in the mothers and the sisters of the expired probands. This last result suggested a reevaluation of the primary diagnosis. In the aunts and sisters of the expired agammaglobulinemia patients, two of eight were found to be carriers and one was noninformative. As a whole, this group of subjects demonstrates the way in which carrier testing can be used to clarify genetic risk in situations in which linkage analysis is impossible. Carrier testing also serves to reinforce or weaken the putative historical diagnosis of XLA.

For nine individuals in six families, it was possible to obtain corroborative predictive data on carrier status by using linkage analysis with three or four probes that are closely linked to the XLA locus (10–16). Two of these probes, DXS94 and DXS178, detect RFLPs. We have simplified detection of these RFLPs by determining the flanking sequence and developing appropriate PCR primers. Two other markers have been developed that are associated with STRs. We developed both a polymorphic CA repeat marker (11 alleles, 75% heterozygosity) from a YAC clone positive for DXS178 and a polymorphic ATT repeat marker (15 alleles, 80% heterozygosity) from a YAC positive for DXS101A. DXS178CA and DXS101AAT are 165 kb and ~850 kb, respectively, telomeric to *btk*. No discordance between Xq22 haplotype and X inactivation studies was found in either noncarriers or carriers. We conclude that the combination of quantitative X inactivation analysis with linkage provides a highly reliable assessment of XLA carrier status. This unselected clinical experience also emphasizes the fact that only a subset of consultands will come from families with a pedigree structure that would allow use of linkage analysis alone to assign carrier state. Thus, X inactivation testing can be a critical tool in evaluating mothers, aunts, and sisters of XLA patients for whom there is no extended family history.

In 10 families, the consultand was pregnant at the time of case ascertainment; three of these elected to proceed with genotyping using DNA markers in Xq22. In one of the cases (fig. 4), the mother was determined to be a carrier by X inactivation analysis, and the fetus was subsequently predicted to be affected using linkage. Individual IV-1 had expired, but recent diagnostic testing had shown the definitive characteristics of XLA. A DNA sample from this patient had been banked. The

Table 1

Summary of Carrier Analysis

Subgroup	Families	Mothers				Other Relatives			
		Skewed	Nonskewed	Noninformative	Indeterminate	Skewed	Nonskewed	Noninformative	Indeterminate
Multiple affected	5	8[a]	...	...	...	3	6	2	1
Isolated proband	11	7	3	1	...	2	6	1	...
No living affected	6	3	1	–	1[b]	2	9	1	1[b]
Total	22	18	4	1	1	7	21	4	2

[a] Includes six obligate carriers.
[b] Suspected carrier of XLSCID.

consultand, her mother, and her first cousin had B cell–restricted skewing of X inactivation, findings that then allowed more certain application of linkage to the prenatal testing. The diagnosis of XLA was confirmed postnatally. Of the two other prenatal cases, both were predicted to be unaffected; one of these cases has been confirmed postnatally, the other is pending. In a fourth case (last pedigree in fig. 3C), a 23-year-old woman (subject 53) sought prenatal counseling at 18 wk gestation with a male fetus. Her two male sibs were described as having had Bruton disease but as having expired in infancy. Quantitative immunoglobulins, immunophenotyping, and in vitro immune responses were unavailable at the time these infants died, and no pathologic material was available. Skewing of X inactivation in both $CD19^+$ and $CD19^-$ cell populations (but not in granulocytes) was detected in the consultand and her mother (subject 52). Since carriers of X-linked severe combined immune deficiency (XLSCID) show skewing of X inactivation in both the T lymphocytes and B lymphocytes (Puck et al. 1987, 1992; Conley et al. 1988; Hendriks et al. 1992; Wengler et al. 1993), the carrier testing result in this family strongly suggests revision of the diagnosis from XLA to XLSCID (fig. 5). This case illustrates the possible contribution of primary carrier testing to disease identification, when adequate diagnostic information on the proband is not available.

Discussion

An ordered approach to genetic analysis of XLA families should include sequential application of carrier testing, linkage (where feasible), and mutation detection. Previously, carrier analysis has relied on either production of somatic cell hybrids (Conley and Puck 1988) or methylation analysis using Southern blotting with either PGK, HPRT, or M27β probes (Journet et al. 1992). In the hybrid method, X inactivation patterns were assessed by fusing an individual's B cells with an HPRT-deficient rodent cell line, selecting for HPRT-expressing clones, and determining whether the maternal or paternal X chromosome from the subject was selectively retained. In the largest published compilation (Conley and Puck 1988), 15 women from typical, atypical, or sporadic XLA pedigrees were analyzed with this method. Although analysis using hybrids is accurate and quantitative, hybrids are technically cumbersome and time-consuming. Controls for constitutional skewing are not easily performed. Detection of hypermethylation at the PGK or HPRT loci on the inactive X chromosome is limited by their relatively low heterozygosity (Fearon et al. 1987). Methylation at the M27β locus appears to be influenced by factors other than X inactivation, making that analysis unreliable in an unknown subset of patients (Cachia et al. 1992). As demonstrated here, the androgen-receptor locus is highly polymorphic and the assay for methylation can be made quantitative. This single test overcomes most of the technical obstacles to routine XLA carrier testing: (1) it requires only a small sample of peripheral blood, from which highly purified B lymphocytes can be isolated; (2) the maternal and paternal alleles can be distinguished in >90% of women; (3) methylation of the CpG island adjacent to the polymorphic trinucleotide repeat is highly correlated with X inactivation; (4) quantitative estimatation of allele contribution can be obtained; and (5) the non–B cell mononuclear cells provide a reliable control for constitutional skewing of X inactivation. Primary carrier testing for XLA is especially useful in the counseling of women with an affected brother or for those who have had one affected son, since it can, in principle, identify those cases in which the recurrence risk is high. The youngest subject studied by us is 9

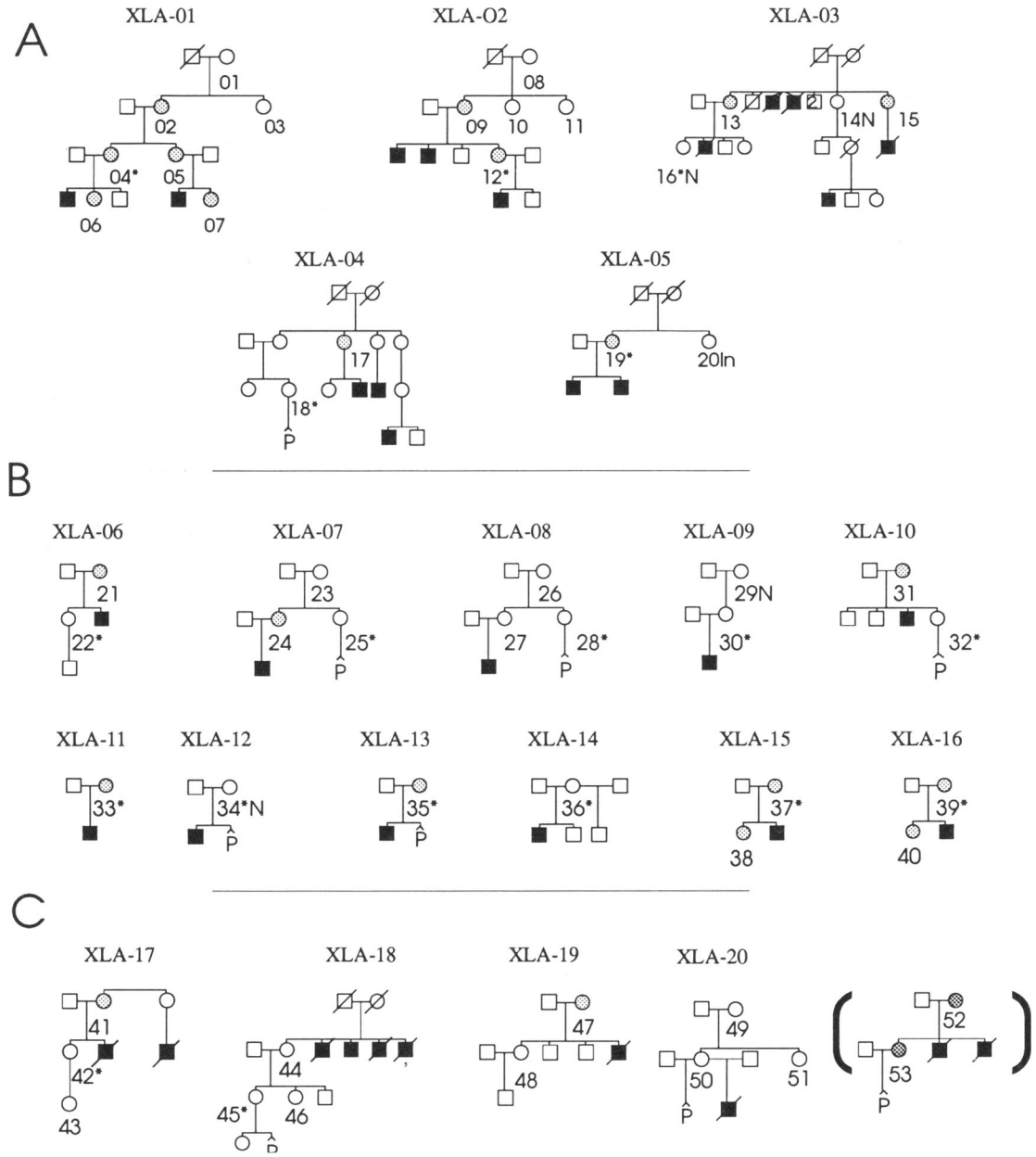

Figure 3 Carrier detection in XLA families. Subjects are numbered for reference in the text; * = consultands; N = noninformative for the androgen-receptor polymorphism; and In = indeterminate (for explanation, see text). Blackened circles indicate women with skewed X inactivation. *A*, Multiple living affected and/or obligate carriers. *B*, Isolated living affected males. *C*, No living affected.

years of age. It is unknown at what age the skewing of X inactivation would be detected, but on the basis of the natural history of the disease in males, it is highly likely that female carriers would have B lineage skewing in early infancy or even in fetal development. Likewise, we have not made a systematic survey of different age ranges of women in the control group. On the basis of clone selection in the hematopoietic system, it is conceivable that X allele usage and skewing might be affected by age. At the present time there is no evidence for this. The assay, itself, should have wide application in the X-linked immune deficiencies and in other constitutional and somatic disorders in which there is skewing of X inactivation.

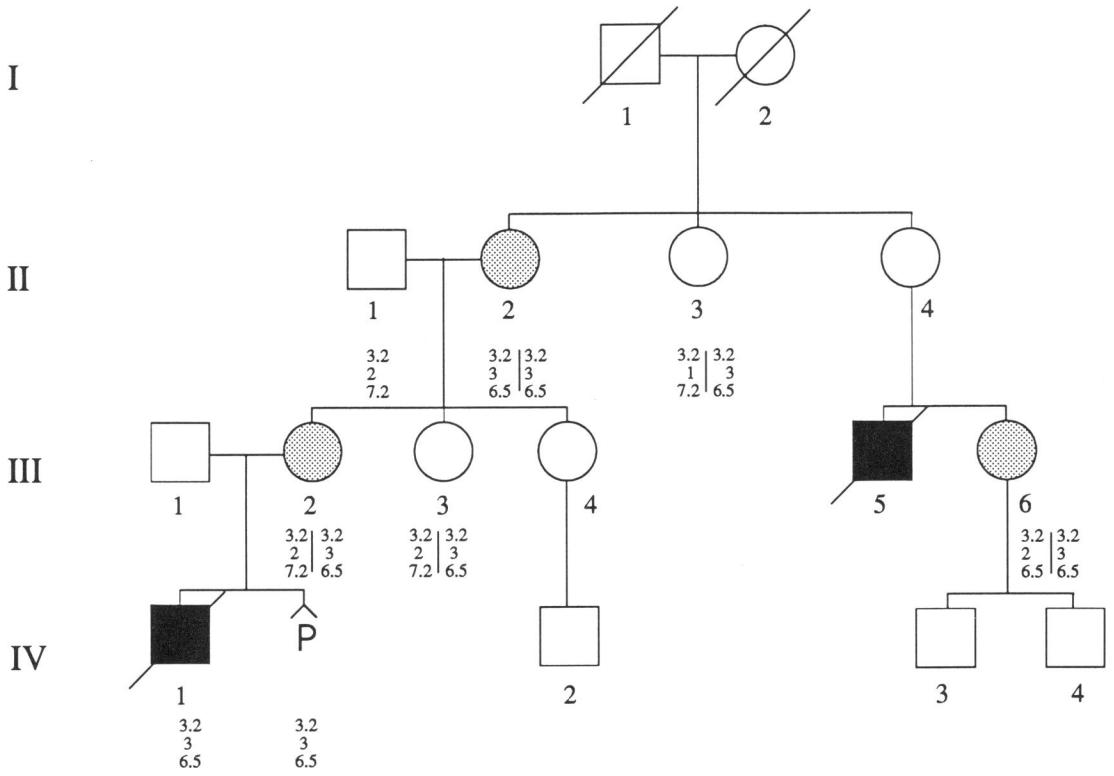

Figure 4 Prenatal diagnosis in a family with a history of XLA. The shaded females (II-2, III-2, and III-6) exhibit >95% skewing of X inactivation when the androgen-receptor assay is used. Individuals II-3 and III-3 did not show skewing. II-4 and III-4 were unavailable for study. A genomic DNA sample was available from the affected male IV-1. Karyotyping of the pregnancy confirmed 46,XY chromosomes. The numbers below each individual indicate the haplotype generated by the three markers DXS178, DXS178CA, and DXS94. This family was studied before the development of DXS101AAT.

Development of new highly polymorphic microsatellite repeat markers has also facilitated linkage analysis of families affected by XLA. Informative haplotypes can be generated for >95% of at-risk families. No recombination has been observed between DXS178 and *btk*, and only a single recombinant with DXS101 has been reported. When several more linked markers are combined, essentially all subjects should have informative haplotypes (de Weers et al. 1992). Identification of the gene responsible for XLA raises the possibility of directly detecting mutations. Mutation scanning methods (e.g., see Orita et al. 1989) should now be applied to XLA families, both for diagnostic purposes and to further clarify the molecular basis of the disease. Until a greater range of mutations are described, however, caution is warranted, since at some loci even extensive scanning of the coding sequence may fail to detect a substantial percentage of mutations (Higuchi et al. 1991, 1992).

Recently, the genes for two other X-linked immunodeficiencies have been identified. X-linked hyper-IgM syndrome is caused by defects in the CD40 ligand (Allen et al. 1993*a*; Aruffo et al. 1993; Disanto et al. 1993, Fuleihan et al. 1993; Korthauer et al. 1993). In this condition female carriers do not have skewing of X inactivation in either the B cells or T cells, presumably because the CD40 ligand is expressed exclusively by activated T cells but is not required for T cell growth. There is no intrinsic defect of the B cells in hyper-IgM syndrome, and carriers have normal immune responsiveness, although CD40 ligand expression may be diminished in the T cell population as a whole. Linkage analysis using a polymorphism in the CD40 ligand gene (Allen et al. 1993*b*) and direct mutation detection are clinically feasible because of the small size of the coding sequence. XLSCID results from defects in the γ chain of the IL-2 receptor (Noguchi et al. 1993). This causes an intrinsic defect in both B cell growth and T cell growth, resulting in skewing of X inactivation in lymphoid cells but not in the myeloid lineages. By including

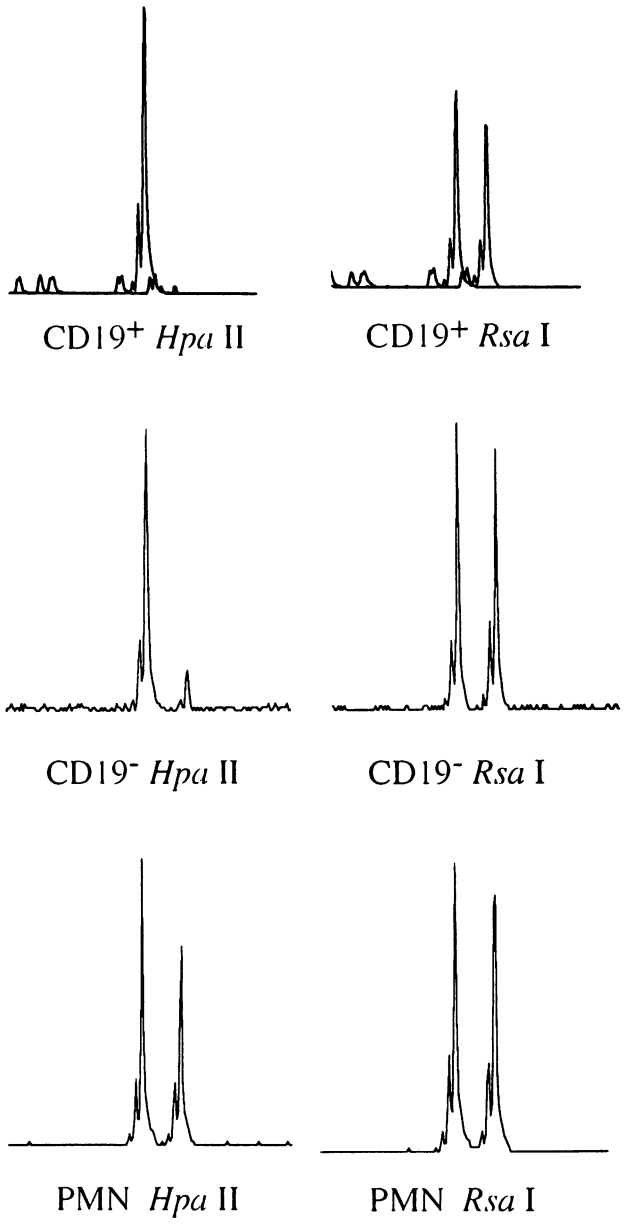

Figure 5 Skewing of X inactivation in a suspected carrier of XLSCID. Peripheral blood lymphocytes from subject 53 (fig. 3) were separated into $CD19^+$ and $CD19^-$ populations. A fraction of peripheral blood leukocytes enriched for granulocytes was prepared on a dextran gradient. The pattern of X inactivation was assayed as described in the legend to fig. 1.

the additional myeloid cell controls, the androgen-receptor assay may be used for direct carrier testing in this disorder as well (authors' unpublished observations). More precise genetic counseling for these conditions can be expected to have an important influence on pregnancy planning, utilization of prenatal diagnosis, and decisions on treatment options.

Acknowledgments

This work was supported by the Howard Hughes Medical Institute. The authors wish to thank Dr. Mary Ellen Conley for critical reading of the manuscript.

References

Allen RC, Armitage RJ, Conley ME, Rosenblatt H, Jenkins NA, Copeland NG, Bedell MA, et al (1993*a*) CD40 ligand defects responsible for X-linked hyper-IgM syndrome. Science 259:990–993

Allen RC, Belmont JW (1992) Dinucleotide repeat polymorphism at the DXS178 locus. Hum Mol Genet 1:216

———. Trinucleotide repeat at the DXS101 locus. Hum Mol Genet (in press)

Allen RC, Spriggs MK, Belmont JW (1993*b*) Dinucleotide repeat polymorphism in hte human CD40 ligand gene. Hum Mol Genet 2:828

Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW (1992) Methylation of *Hpa*II and *Hha*I sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am J Hum Genet 51:1229–1239

Aruffo A, Farrington M, Hollenbaugh D, Li X, Milatovich A, Nonoyama S, Bajorath J, et al (1993) The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. Cell 72:291–300

Bruton OC (1952) Agammaglobulinemia. Pediatrics 9:722–727

Cachia PG, Culligan DJ, Thomas ED, Whittlaker J, Jacobs A, Padua R (1992) Methylation of the DXS255 hypervariable locus 5′ CCGG site may be affected by factors other than X-chromosome activation status. Genomics 14:70–74

Campana D, Farrant J, Inamdar N, Webster AD, Janossy G (1990) Phenotypic features and proliferative activity of B cell progenitors in X-linked agammaglobulinemia. J Immunol 145:1675–1680

Chase GA, Murphy EA (1973) Risk of recurrence and carrier frequency for X-linked lethal recessives. Hum Hered 23:19–26

Conley ME (1992) Molecular approaches to analysis of X linked immune deficiencies. Annu Rev Immunol 10:215–238

Conley ME, Brown P, Packard AR, Buckley RH, Miller DS, Raskind WH, Singer JW, et al (1985) Expression of the gene defect in X-linked agammaglobulinemia. N Engl J Med 315:564–567

Conley ME, Lavoie A, Briggs C, Brown P, Guerra C, Puck JM (1988) Nonrandom X chromosome inactivation in B cells

from carriers of X chromosome-linked severe combined immunodeficiency. Proc Natl Acad Sci USA 85:3090–3094

Conley ME, Puck JM (1988) Carrier detection in typical and atypical X-linked agammaglobulinemia. J Pediatr 112:688–694

Conley ME, Sweinberg SK (1992) Females with a disorder phenotypically identical to X-linked agammaglobulinemia. J Clin Immunol 12:139–143

de Weers M, Mensink RGJ, Kenter M, Schuurman RKB (1992) Three dinucleotide repeat polymorphisms at the DXS178 locus. Hum Mol Genet 1:653

Disanto JP, Bonnefoy JY, Gauchat JF, Fischer A, de Saint Basile G (1993) CD40 ligand mutations in X-linked immunodeficiency with hyper-IgM. Nature 361:541–543

Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R (1992) Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 12:241–253

Fearon ER, Winkelstein JA, Civin CI, Pardoll DM, Vogelstein B (1987) Carrier detection in X-linked agammaglobulinemia by analysis of X chromosome inactivation. N Engl J Med 316:427–431

Fialkow PJ (1973) Primordial cell pool size and lineage relationships of five human cell types. Ann Hum Genet 37:39–48

Fuleihan R, Ramesh N, Loh R, Jabara H, Rosen FS, Chatila T, Fu SM, et al (1993) Defective expression of the CD40 ligand in X chromosome-linked immunoglobulin deficiency with normal or elevated IgM. Proc Natl Acad Sci USA 90:2170–2173

Guioli S, Arveiler B, Bardoni B, Notarangelo LD, Panina P, Duse M, Ugazio A, et al (1989) Close linkage of probe p212 (DXS178) to X-linked agammaglobulinemia. Hum Genet 84:12–21

Hendriks RW, Kraakman ME, Schuurman RK (1992) X chromosome inactivation patterns in haematopoietic cells of female carriers of X-linked severe combined immunodeficiency determined by methylation analysis at the hypervariable DXS255 locus. Clin Genet 42:114–121

Higuchi M, Antonarakis SE, Kasch L, Oldenburg J, Economou-Petersen E, Olek K, Arai M, et al (1991) Molecular characterization of mild to moderate hemophilia A: detection of the mutation in 25 of 29 patients by denaturing gradient gel electrophoresis. Proc Natl Acad Sci USA 88:8307–8311

Higuchi M, Kazazian HH, Kasch L, Warren TC, McGinniss MJ, Phillips JA, Kasper C, et al (1992) Molecular characterization of severe hemophilia A suggests that about half the mutations are not within the coding regions and splice junctions of the factor VIII gene. Proc Natl Acad Sci USA 88:7405–7409

Journet O, Durandy A, Doussau M, Le Deist F, Couvreur J, Griscelli C, Fischer A, et al (1992) Carrier detection and prenatal diagnosis of X-linked agammaglobulinemia. Am J Med Genet 43:885–887

Korthauer U, Graf D, Mages HW, Briere F, Padayachee M, Malcolm S, Ugazio AG, et al (1993) Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. Nature 361:539–541

Kwan S-P, Kunkel L, Bruns G, Wedgwood RJ, Latt S, Rosen FS (1986) Mapping of the X-linked agammaglobulinemia locus by use of restriction fragment length polymorphism. J Clin Invest 77:649–652

Kwan S-P, Terwilliger J, Parmley R, Raghy G, Sandkuyl LA, Ott J, Ochs H, et al (1990) Identification of a closely linked DNA marker, DXS178, to further refine the X-linked agammaglobulinemia locus. Genomics 6:238–242

Lovering R, Middleton-Price HR, O'Reilly M-A, Genet SA, Parkar M, Sweatman AK, Bradley LD, et al (1993) Genetic linkage analysis identifies new proximal and distal flanking markers for the X-linked agammaglobulinemia gene locus, refining its localization in Xq22. Hum Mol Genet 2:139–141

Malcolm S, de Saint Basile G, Arveiler B, Lau YL, Szabo P, Fischer A, Griscelli IC, et al (1987) Close linkage of random DNA fragments from Xq21.3-22 to X-linked agammaglobulinemia (XLA). Hum Genet 77:172–174

Maniatis T, Fritsch EF, Sambrook J (1988) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Mensink EJBM, Thompson A, Schot JDL, van der Greef WMM, Sandkuyl LA, Schuurman RKB (1986) Mapping of a gene for X-linked agammaglobulinemia and evidence for genetic heterogeneity. Hum Genet 73:327–332

Mullis KB, Faloona FA (1987) Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol 155:335–350

Nesbitt MN (1972) X chromosome inactivation mosaicism in the mouse. Dev Biol 26:252–263

Noguchi M, Yi H, Rosenblatt HM, Filipovich AH, Adelstein S, Modi WS, McBride OW, et al (1993) Interleukin 2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. Cell 73:147–157

Ochs H, Wedgwood RD (1989) Disorders of the B cell system. In: Steihm ER (ed) Immunologic disorders of infants and children. WB Saunders, Philadelphia, pp 226–256

Orita M, Suzuki Y, Sekiya T, Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874–879

Parolini O, Hejtmancik JF, Allen RC, Belmont JW, Lassiter GL, Henry MJ, Barker DF, et al (1993) Linkage analysis and physical mapping near the gene for X-linked agammaglobulinemia at Xq22. Genomics 15:342–349

Puck JM, Nussbaum RL, Conley ME (1987) Carrier detection in X-linked severe combined immunodeficiency based on patterns of X chromosome inactivation. J Clin Invest 79:1395–1400

Puck JM, Stewart CC, Nussbaum RL (1992) Maximum-likelihood analysis of human T-cell X chromosome inactivation patterns: normal women versus carriers of X-linked severe

combined immunodeficiency. Am J Hum Genet 50:742–748

Rawlings DJ, Saffran DC, Tsukada S, Largaespada DA, Grimaldi JC, Cohen L, Mohr RN, et al (1993) Mutation of unique region of Bruton's tyrosine kinase in immunodeficient XID mice. Science 261:358–361

Rosen FS, Cooper MD, Wedgwood RJP (1984) The primary immunodeficiencies. I. N Engl J Med 311:235–242

Thomas JD, Sideras P, Smith CL, Vorechovsky I, Chapman V, Paul WE (1993) Colocalization of X-linked agammaglobulinemia and X-linked immunodeficiency genes. Science 261:355–358

Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I, Sparkes RS, et al (1993) Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. Cell 72:279–290

Vetrie D, Vorechovsky I, Sideras P, Holland J, Davies A, Flinter F, Hammarstrom L, et al (1993) The gene involved in X-linked agammaglobulinemia is a member of the src family of protein tyrosine kinases. Nature 361:226–233

Wengler GS, Allen RC, Parolini O, Smith H, Conley ME (1993) Nonrandom X chromosome inactivation in natural killer cells from obligate carriers of X-linked severe combined immunodeficiency. J Immunol 150:700–704