Am. J. Hum. Genet. 62:1034–1043, 1998

Mutations in Btk in Patients with Presumed X-Linked Agammaglobulinemia

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Summary

In 1993, two groups showed that X-linked agammaglobulinemia (XLA) was due to mutations in a tyrosine kinase now called Btk. Most laboratories have been able to detect mutations in Btk in 80%–90% of males with presumed XLA. The remaining patients may have mutations in Btk that are difficult to identify, or they may have defects that are phenotypically similar to XLA but genotypically different. We analyzed 101 families in which affected males were diagnosed as having XLA. Mutations in Btk were identified in 38 of 40 families with more than one affected family member and in 56 of 61 families with sporadic disease. Excluding the patients in whom the marked decrease in B cell numbers characteristic of XLA could not be confirmed by immunofluorescence studies, mutations in Btk were identified in 43 of 46 patients with presumed sporadic XLA. Two of the three remaining patients had defects in other genes required for normal B cell development, and the third patient was unlikely to have XLA, on the basis of results of extensive Btk analysis. Our techniques were unable to identify a mutation in Btk in one male with both a family history and laboratory findings suggestive of XLA. DNA samples from 41 of 49 of the mothers of males with sporadic disease and proven mutations in Btk were positive for the mutation found in their son. In the other 8 families, the mutation appeared to arise in the maternal germ line. In 20 families, haplotype analysis showed that the new mutation originated in the maternal grandfather or great-grandfather. These studies indicate that 90%–95% of males with presumed XLA have mutations in Btk. The other patients are likely to have defects in other genes.

Received November 10, 1997; accepted for publication March 6, 1998; electronically published April 17, 1998.

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0002-9297/98/6205-0007$02.00

Introduction

X-linked agammaglobulinemia (XLA [MIM 300300]) is a fully penetrant X-linked recessive disorder characterized by the early onset of bacterial infection, profound hypogammaglobulinemia, and a marked decrease in the number of B lymphocytes, the precursors of antibody producing cells (Conley et al. 1994*b*; Ochs and Smith 1996). Although most affected males are recognized to have immunodeficiency in the first few years of life, diagnosis may be delayed, particularly in atypical or sporadic cases (Conley and Puck 1988; Vorechkovsky et al. 1993; Kornfeld et al. 1996). As would be expected for any X-linked disorder that is lethal without medical intervention, 30%–50% of patients with the clinical and laboratory features of XLA have no family history of disease (Haldane 1935; Lederman and Winkelstein 1985). Accurate diagnosis and genetic counseling are further complicated by the fact that there are autosomal recessive disorders that result in a phenotype indistinguishable from XLA (Hoffman et al. 1977; Conley and Sweinberg 1992; Yel et al. 1996; Minegishi et al. 1998).

Carrier testing can confirm the diagnosis of XLA in patients with atypical cases or when there is no family history of disease (Conley et al. 1986; Fearon et al. 1987; Conley and Puck 1988; Allen et al. 1994). Because the defective gene product is intrinsic to the B cell lineage and is not transportable between cells, B cell precursors from obligate carriers that have the mutant X chromosome as the active X do not mature into B cells. As a result, B cells, but not other hematopoietic cell lineages from these women, demonstrate a nonrandom pattern of X chromosome inactivation (Conley et al. 1986; Fearon et al. 1987; Conley and Puck 1988; Allen et al. 1994). However, this approach is labor intensive, and a random pattern of X chromosome inactivation in B cells from the mother of a patient does not rule out the possibility of a new mutation in the maternal germ line.

In 1993, two groups demonstrated that XLA was due to mutations in Btk, a cytoplasmic tyrosine kinase that is expressed throughout B cell and myeloid differentiation (Tsukada et al. 1993; Vetrie et al. 1993). In addition to its catalytic domain, Btk contains several protein-protein interaction domains, including a pleckstrin homology (PH) domain and SH2 and SH3 domains (Tsukada

et al. 1993; Vetrie et al. 1993; Rawlings and Witte 1995). Although the mechanisms by which defects in Btk result in a failure of B cell development have not yet been identified, comparison to other cytoplasmic tyrosine kinases suggests that this enzyme is involved in control of cell proliferation or differentiation (Rawlings and Witte 1995).

The gene encoding Btk consists of 19 exons spread over 37 kb of DNA (Hagemann et al. 1994; Ohta et al. 1994; Rohrer et al. 1994; Sideras et al. 1994). Over 200 mutations have been identified by means of Southern blot analysis, cDNA sequencing, and genomic screening (Vihinen et al. 1996). Gross deletions or insertions detectable by Southern blot analysis constitute ~5% of the mutations; the remaining mutations are scattered throughout the gene and are highly diverse. They include single–base-pair substitutions that cause amino acid substitutions, premature stop codons and splice defects, and small insertions and deletions that result in frameshift mutations. Most laboratories that have screened for mutations in Btk have identified mutations in 80%–90% of the patients evaluated (Bradley et al. 1994; Conley et al. 1994*a*; Hagemann et al. 1994; Hashimoto et al. 1996). It is not clear whether the remaining 10%–20% of patients have mutations in Btk that are not easily detected or whether they have disorders that are phenotypically similar to XLA but are caused by different gene defects.

To facilitate genetic counseling for families in which a male has the clinical features of XLA but no family history of disease, we screened genomic DNA, using SSCP, for mutations in Btk. If a mutation in Btk was not identified, Southern blot analysis was used to identify gross alterations in the gene, cDNA was screened by SSCP, and Btk protein was evaluated by western blot analysis when possible. We also screened for mutations in other genes that might affect B cell development. When a mutation in Btk was identified, genomic DNA from the patient's maternal ancestors was examined to determine the source of new mutations.

Material and Methods

Patients

Twenty-one of the patients included in this study have received care in our clinics. The remaining families were referred for molecular diagnosis by physicians or genetic counselors from 37 different institutions. When possible, a fresh blood sample from an affected patient was analyzed by immunofluorescence staining with antibody to CD19, to confirm the characteristic decrease in B cell numbers (Conley 1985). In some cases, it was not possible to confirm the diagnosis, and only DNA samples from the patient (12 families) or a female relative (eight families) were available. Members of 108 families were screened for mutations in Btk. Six families in whom Btk mutations were not identified were excluded from final analysis in this study because, although the proband had markedly reduced B cell numbers, the patient was not presumed to have XLA. In four of these families, the proband was a female, and in the remaining two, the affected child had intrauterine growth retardation and other morphologic defects atypical of XLA. The DNA sample from one patient was partially degraded and was not further analyzed. Thus, 101 families were included in the final analysis. This study was reviewed by the St. Jude Children's Research Hospital Institutional Review Board, and informed consent was obtained from all subjects.

Mutation Detection

Genomic DNA was obtained from whole blood lysis or from mitogen-stimulated T cells from patients and family members. SSCP analysis was performed as described (Conley et al. 1994*a*) except that the following primer pair (sense: GTGCCTTTAACCTCTGTGCT and antisense: TACCCATGTTTCATACTGTG) was substituted for the exon 16 primer pair. The promoter region (300 bp 5′ to the transcriptional start site) was analyzed with primers (sense: GAGGATAGCTTGATGACC and antisense: GGCCCTGGAGACATATTC) and the polyA tail was analyzed with the primers (sense: CCGAATTTGGCAAGAAT and antisense: TCGAGTTCTCAGTGTAGG). All assays were run at 4°C. Exons demonstrating altered migration were cloned from two separate PCR reactions and sequenced. Family members were then screened, by SSCP, for the same mutation.

Linkage Analysis

Short tandem repeats near the XLA locus at Xq22, DXS178CA (Allen and Belmont 1992), DXS101 (Allen and Belmont 1993), DXS6799, DXS6797, and DXS6804 (Research Genetics) were used for linkage analysis and to assign haplotypes.

Results

We have used SSCP to screen for mutations in Btk in members of 101 families in which an immunodeficient male was thought to have XLA. Thirty of these families included affected cousins or uncles. In 10 families, two or more brothers, but no additional family members, had the clinical and laboratory features of XLA. The patients in the remaining 61 families were presumed to have sporadic XLA. Eighty-three different mutations in Btk were identified in members of 94 families. Seventy-six of the 83 mutations resulted in an abnormal migration of PCR products from a single exon with its flanking

splice sites. Four additional mutations resulted in the consistent failure to amplify one or more exons by PCR. Southern blot analysis demonstrated gross deletions in the Btk gene in the four samples that could not be completely analyzed by SSCP (patients 0703, 0850, 2430, and 2433) and insertions in three additional samples (patients 0060, 0540, and 1107). Of the 83 unique mutations included in this study, 43 have been described elsewhere by our laboratory (Conley et al. 1994*a*; Conley and Rohrer 1995; Farrar et al. 1996; Rohrer and Conley 1998), 5 have been reported by other groups (Bradley et al. 1994; Hagemann et al. 1994; Jin et al. 1995; Hashimoto et al. 1996; Kornfeld et al. 1996), and 35 have not been previously described.

Ten mutations were found in more than one family, 1 of these occurred in three families, and the other 9 were found in two families. To determine whether these 10 mutations were due to independent mutational events or shared ancestry, the haplotype bearing the mutation was analyzed by use of the short tandem repeats linked to Btk at Xq22, or the source of the mutation was identified in one of the families. These studies showed that two of the families, one with a known family history of XLA (patient 0030) and the other thought to have sporadic XLA (patient 0025), were distantly related, as reported elsewhere (Rohrer and Conley 1998). The other nine mutations occurred independently, as shown by haplotype analysis (patients 0200 and 0300, 0770 and 0771, 1030 and 1031, 1110 and 1111, 1500 and 1600, and 2000 and 2017) or identification of the source of the mutation in one of the families (patients 0310, 1426, 1470, and 2273). Four of the sites involved in recurrent mutations were CpG dinucleotides at codons 13, 255, 288, and 520.

The 93 independently derived mutations included 62 single–base-pair substitutions that resulted in amino acid substitutions (28 mutations), premature stop codons (21 mutations), splice defects (11 mutations), a transcriptional regulatory defect (1 mutation), and a defect in the start codon (1 mutation). An additional 20 mutations were small insertions or deletions. Most of these mutations occurred in the coding regions and caused a frameshift with a secondary premature stop codon; however, three mutations (patients 0620, 1426, and 1427), were 3-bp deletions that would be expected to result in a single amino acid deletion. Four mutations were slightly more complex and resulted in the combination of small insertions with small deletions. DNA from patient 2390 demonstrated a 16-bp deletion of nucleotides 2016–2032 of the coding region, coupled with a 4-bp TTGC insertion. The predicted consequence of this mutation would be an in-frame deletion of amino acids at codon 629–633 with the insertion of a cysteine. Two of the insertion/deletion combinations, in patients 0870 and 1100, would be expected to cause frameshifts. The fourth insertion/deletion, in patient 1120, occurred at the −10 position of the splice-acceptor site in the 10th intron and consisted of a G deletion coupled to an 11-bp insertion (AGGGGAAAACT). Because the insertion occurred immediately 3′ to a cytosine, the new sequence created a splice-acceptor site. Analysis of cDNA from this patient demonstrated the use of cryptic splice sites and exon skipping. The remaining seven mutations were gross deletions or insertions.

As reported elsewhere (Conley et al. 1994*b*; de Weers et al. 1994; Ohta et al. 1994; Jin et al. 1995), there was no clear phenotype/genotype correlation in patients with mutations in Btk, indicating that the specific mutation in Btk is only one of the factors that influences the severity of disease. The age at diagnosis, the severity of the infections prior to diagnosis, and the incidence of complications after diagnosis were highly variable even among patients with premature stop codons and an absence of Btk transcript and protein. The most consistent feature, found in all of the patients analyzed, was a marked reduction in the number of B cells.

Mutations in Btk were identified in 38 of 40 families that included more than one individual with presumed XLA. One of the families in whom we did not find a mutation consisted of two brothers with hypogammaglobulinemia, only one of whom was reported to have decreased numbers of B cells. Fresh blood samples from these individuals were not available to confirm the diagnosis of XLA by immunofluorescence staining of B cells or to evaluate Btk cDNA or protein. These patients may not have XLA. In the other family, the patient had a maternal uncle who died, in early childhood, of infection. This patient had a markedly reduced number of B cells that expressed an immature phenotype, as is characteristic of XLA (Conley 1985). In addition, Btk protein could not be detected in his peripheral blood mononuclear cells by western blot analysis. However, we have not yet been able to identify a mutation in Btk in genomic DNA or cDNA or by Southern blot analysis.

We identified mutations in Btk in 56 of the 61 families with presumed sporadic XLA. In two of the remaining five families, we have shown that the affected males inherited autosomal recessive defects in other genes required for normal B cell differentiation, the μ heavy chain gene (Yel et al. 1996) (MIM 147020) or the $\lambda 5$/14.1 surrogate light chain gene (Minegishi et al. 1998). Both of these patients were compound heterozygotes, and there were no clinical features that would distinguish them from patients with typical XLA. An Epstein-Barr virus–transformed cell line from a third patient had normal Btk cDNA by SSCP, normal Btk message by northern blot, and normal Btk protein by western blot. Therefore, it is unlikely that this patient had X-linked agammaglobulinemia.

The last two families in whom we did not find mu-

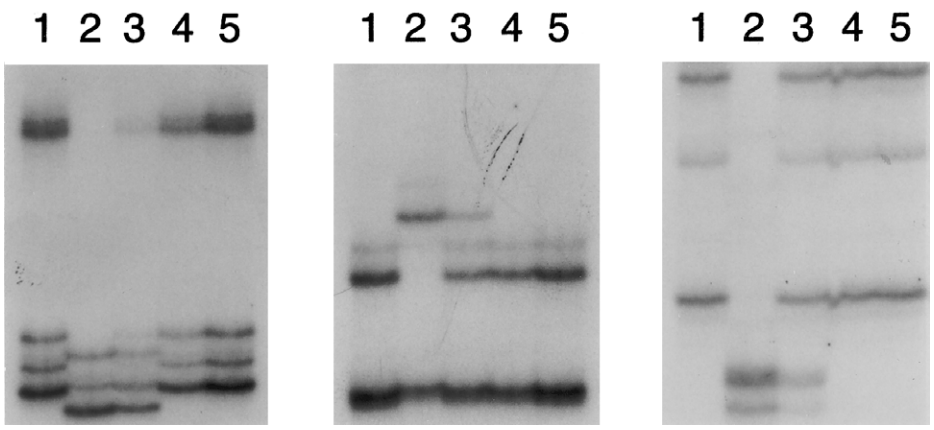

Figure 1 SSCP analysis showing mutations in Btk exon 14 in patient 1426 (*left*), in exon 2 in patient 0310 (*center*), and in exon 3 in patient 0500 (*right*). In each panel DNA from controls (lanes 1 and 5) is compared to the DNA from the patient (lane 2), to a mixture of DNA from the same patient and DNA from a control (lane 3), and to DNA from the patient's mother (lane 4).

tations in Btk were among five families with sporadic disease in whom a proband was not available for analysis; therefore, DNA from the mother (four families) or daughter (one family) of the affected male was analyzed. Mutations in Btk were found in two of the mothers and in the daughter of an affected patient (patient 0420). The mutation in patient 0420, an arginine to cysteine substitution at codon 28, is of particular interest because it is the same mutation found in xid mice, a strain of mice with a relatively mild defect in B cell development (Wicker and Scher 1986). Although patients with mutations in codon 28 have been reported (de Weers et al. 1994; Ohta et al. 1994; Zhu et al. 1994; Hashimoto et al. 1996; Vorechovsky et al. 1997), these mutations have been arginine to histidine or arginine to proline substitutions. It had been speculated that the milder phenotype in xid mice, compared with patients with XLA, might be due to the R28C mutation having less severe consequences than other mutations in Btk (Rawlings and Witte 1994; Smith et al. 1994); however, more recent studies indicate that mice that express no Btk have a phenotype that is similar to that of xid mice (Kerner et al. 1995; Khan et al. 1995). The patient with the R28C mutation died at 45 years of age, of chronic pulmonary disease. He was presumed to have XLA, because of very low concentrations of serum immunoglobulins and markedly reduced numbers of B cells. However, it is notable that he survived until middle age during the period when most patients with XLA did not reach adulthood.

The absence of a Btk mutation in the two other mothers could be attributed to a new mutation in the maternal gamete, failure to detect the mutation by SSCP screening, or mutations in other genes. If we exclude the patients in whom we were not able to confirm the marked decrease in B cell numbers that is characteristic of XLA, mutations in Btk were identified in 43 of 46 patients with presumed sporadic XLA.

DNA was available for study from 49 of the mothers of patients with sporadic XLA and documented mutations in Btk, including the mothers of the three patients who were deceased. Southern blot analysis (5 families) or SSCP (44 families) was used to determine whether the mother was a carrier of the mutation. In 41 of the families, DNA from the mother showed the abnormal allele found in her son and the normal allele, which indicated that she was a carrier of XLA. In the remaining eight families, the mother's DNA was normal by SSCP analysis. To rule out the possibility that the abnormal allele was not detected because of preferential amplification of the normal versus the mutant allele, equal mixtures of DNA from the affected son and a normal control were analyzed by SSCP in seven of the eight families. In each of the seven families, the mixture of the normal and abnormal allele permitted the identification of the abnormal allele (fig. 1). DNA was not available from the deceased father of patient 0410; however, the fact that we were able to identify the mutant allele in the heterozygous daughter indicated that the failure to identify the mutant allele in the mother of the patient could not be attributed to selective amplification of the normal allele. These findings indicate that in 8 of 49 families, the mother of the patient was a somatic or gonadal chimera for the new mutation, or, more likely, the new mutation arose in the maternally derived gamete. In seven of the families that included affected brothers but no affected maternal cousins or uncles, DNA samples from the mothers of the patients were studied. In all seven families, the mother was heterozygous for the mutation seen in her sons.

To identify the source of mutation, we evaluated all available family members, including maternal aunts, grandparents, and/or great-grandparents, in 20 families in which the mother of a patient with sporadic XLA was a carrier of the mutation found in her son. Genomic DNA was screened by SSCP for the mutation found in the proband, and linkage analysis, using the highly polymorphic short tandem repeats at DXS6799, DXS6797, DXS178, DXS101, and DXS6804, was performed, to determine the haplotype bearing the mutation. In five of these families, the maternal grandmother was a carrier of the mutation found in her grandson. In 15 families, neither of the maternal grandparents was a carrier of the mutation, and the mutation occurred on the grandpaternal haplotype (fig. 2). In one of the families in which the maternal grandmother was a carrier of XLA and in four additional families that included more than one affected male, we were able to demonstrate that the mutation occurred on the maternal grandpaternal or great-grandpaternal allele.

In previous studies, we identified a family in which a healthy father transmitted the XLA defect to two of his daughters, indicating gonadal or somatic chimerism (patient 2500) (Parolini et al. 1993). To assess the frequency of this event, we evaluated 11 sisters of seven women who were carriers of XLA and whose mutation occurred on the paternal haplotype. None of the 11 sisters were carriers of the mutations seen in their nephews.

Studies evaluating the source of a new mutation in other genetic disorders have suggested that certain types of mutations may be more likely to occur in male gametes than in female gametes; therefore, we examined the specific mutations in Btk that originated in paternal versus maternal gametes. As shown in table 1, 13 of 20 mutations derived from paternal gametes were single–base-pair substitutions, 5 were small deletions, 1 was a 3-bp deletion accompanied by a 5-bp insertion, and the last was a large insertion. By contrast, three of eight mutationsderived from maternal gametes were single–base-pair substitutions, three were small deletions, one was a 16-bp deletion accompanied by a 4-bp insertion, and one was a single–base-pair insertion (table 1).

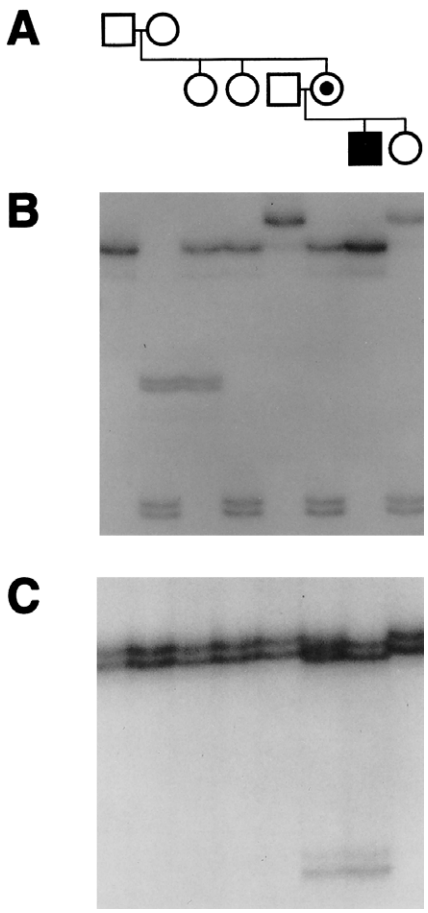

Figure 2 Pedigree for patient 1000 is shown in *A*. DNA from each individual was analyzed by PCR for the polymorphism at DSX101 (*B*) and by SSCP for the mutation in exon 8 (*C*).

Discussion

This study indicates that mutations in Btk can be found in ~90%–95% of males with sporadic immunodeficiency characterized by early onset of infections, hypogammaglobulinemia, and <1% B cells. The vast majority of the mutations in these patients and in patients with a family history of antibody deficiency could be detected by SSCP screening of genomic DNA. Mutations that could be identified only by cDNA analysis or Southern blotting did occur and have been reported in other series (Jin et al. 1995), but in our study, these constituted only 3% of the total mutations.

In 7 of 101 families, a mutation in Btk was not identified. Mutations in other genes required for B cell development were identified in two patients who were clinically indistinguishable from patients with typical XLA. Fresh blood samples that could be used for lymphocyte analysis and to provide cDNA or protein were not available from affected males in four families in whom we did not identify mutations in Btk, which made it difficult to determine whether these patients had findings typical of XLA. Strong evidence for the diagnosis of XLA was found in only one patient in whom a mutation in Btk was not detected. This patient may have a mutation that affects either Btk transcription or the splicing of the Btk message in a region of the gene that was not examined.

Past studies have indicated that ~10% of patients with the clinical and laboratory characteristics of XLA are females (McKinney et al. 1987; Conley and Sweinberg

1992) and have suggested that an equal proportion of males with the same phenotype might have disorders that were etiologically unrelated to XLA (Conley and Sweinberg 1992). The percentage of males in this study without mutations in Btk fits well with this estimate. We continue to analyze DNA from several females with a disease phenotypically similar to XLA, in whom the genetic etiology of the disease remains unclear, suggesting that there are additional genes, combinations of genes, or environmental factors that can result in an XLA-like phenotype.

The distribution of mutations in Btk reported in this study is similar to that recorded in a database of Btk mutations contributed by many laboratories (Vihinen et al. 1996). However, it has not been possible to determine whether all of the mutations in that registry were independently derived, as some families may have been studied by more than one institution. All but 1 of the 94 mutations identified in this study represented independent mutational events. The high incidence of independently derived mutations in our series contrasts with the findings in another potentially lethal X-linked disorder, hemophilia B, in which 25% of patients in the United States have mutations that can be attributed to three founders (Ketterling et al. 1991). It has been postulated that these three mutations result in a milder form of hemophilia and are thus maintained in the population (Ketterling et al. 1991). The absence of similar, shared mutations in Btk suggests that a mild form of XLA, consistent with survival in a preantibiotic era, is uncommon or nonexistent.

In a family with a sporadic case of an X-linked disease, the probability that the mother of the patient is a carrier of that disease is influenced by the source of new mutations. If most new mutations occur in male gametes, as is seen in several autosomal dominant as well as X-linked disorders (Stephens et al. 1992; Goldberg et al. 1993; Palau et al. 1993; Carlson et al. 1994; Rossiter et al. 1994; Tuchman et al. 1995; Becker et al. 1996; Lazaro et al. 1996), the mother would almost always be a carrier. The observation that most new mutations are of paternal origin has in some cases been attributed to the fact that spermatogenesis is associated with a 10-fold higher number of cell divisions compared with oogenesis (Carlson et al. 1994); however, the source of new mutations may be affected by the type of mutation. Duchenne muscular dystrophy is caused by deletions in the dystrophin gene in 60%–70% of patients; 60% of these deletions originate in female gemetes (van Essen et al. 1992). In hemophilia A, 35%–50% of mutations are due to large intragenetic inversions that disrupt the sequence between exon 22 and exon 23. These inversions are derived almost exclusively from male gametes (Rossiter et al. 1994; Becker et al. 1996). Charcot-Marie-Tooth disease is usually caused by a 1.5-Mb duplication on chromosome 17p11.2 (Lupski et al. 1991). This mutation also almost always originates in a male gamete (Palau et al. 1993).

Of the 49 mothers of patients with sporadic XLA and proven mutations in Btk in our series, 84% were carriers of the mutation found in their sons. This is similar to the percentage of mothers of patients with sporadic hemophilia A, hemophilia B, or X-linked severe combined immunodeficiency, who are carriers of the mutations found in their sons (Knobloch et al. 1993; Becker et al. 1996; Puck et al. 1997). In the series reported here, the proportion of mutations originating in male gametes that were point mutations (13 [66%] of 20) was similar to the proportion of point mutations in the entire group of independently occurring mutations (62 [69%] of 93). By contrast, three of eight of the mutations occurring in female germ cells were point mutations, and there was a slight enrichment for small deletions and/or insertions in this group of mutations. In three of the eight cases in which the maternal gamete was the source of the mutation, linkage analysis demonstrated that the mutant X chromosome was the paternally derived X. The source of the mutant X chromosome could not be determined in the other families.

In the absence of mutation detection, this study allows a physician or genetic counselor to predict the reproductive risk of the parents, sisters, and maternal aunts of males with sporadic defects in B cell development. Both parents have a small but appreciable (3%–5%) risk of being carriers of rare autosomal recessive defects in B cell development. The risk that the mother of the patient is a carrier of XLA can be calculated on the basis of the percentage of males with mutations in Btk (92%) times the percentage of mothers who carry the mutation found in their son (84%), or ~77%. This is an approximation because, although this study did not identify any women who were gonadal chimeras, in other similar disorders—for example, X-linked severe combined immunodeficiency—maternal gonadal chimeras have been reported (Puck et al. 1995). This rare event would slightly increase the predicted risk to the mother. The risk to the sister of the affected male is half the risk to the mother (38%). The risk that a maternal aunt is a carrier of XLA can be estimated on the basis of the observation that the maternal grandmother was a carrier of the mutation found in her grandson in 5 (16%) of 30 families available for analysis. Even when coupled with the small risk that either of the patient's maternal grandparents might be a gonadal chimera, the probability that the maternal aunt is a carrier of XLA is <10%. These figures, based on a relatively large number of families presumed to have XLA, should facilitate genetic counseling in families with sporadic disease.

Table 1

Mutations in Btk in Patients with X-Linked Agammaglobulinemia

Subject	Codon[a]	Intron/Exon	Domain	Change[b]	Effect[c]	Family History[d]	Source of Mutation[e]
0015*[f]	…	Intron 1	5′ untranslated	G → A	Splice-donor defect (−1)	S	…
0025	…	Intron 1	5′ untranslated	T → G	Regulatory defect (+6)	S	…
0030	…	Intron 1	5′ untranslated	T → G	Regulatory defect (+6)	C, U, EG	…
0060*	…	…	…	Insertion (13 kb) within intron 1	Frameshift	B	…
0100	1	Exon 2	PH	ATG → GTG	No start	S	…
0200	13	Exon 2	PH	CGA → TGA	Arg → Stop	U	…
0300	13	Exon 2	PH	CGA → TGA	Arg → Stop	B	…
0310	13	Exon 2	PH	CGA → TGA	Arg → Stop	S	Mother (M)
0400	15	Exon 2	PH	CAA → TAA	Gln → Stop	U	…
0410*	21	Exon 2	PH	TCA → TAA	Ser → Stop	S	…
0420*	28	Exon 2	PH	CGC → TGC	Arg → Cys	S	Mother (M)
0450	40	Exon 2	PH	TAT → TGT	Tyr → Cys	S	MGF (P)
0470	62	Exon 3	PH	A del	Frameshift	B, U, EG	…
0480	71	Exon 3	PH	A del (7 As)	Frameshift	B	…
0500	76, 77	Exon 3	PH	GAAA del	Frameshift	S	Mother (M)
0507*	77, 78	Exon 3	PH	GACA del	Frameshift	S	…
0520*	80	Exon 3	PH	CCG → CCA	Splice-donor defect (−1)	S	MGGF (P)
0540*	…	…	…	Insertion (>30 kb) within intron 4	No transcript	S	MGF (P)
0590*	112	Exon 5	PH	TAC → TAA	Tyr → Stop	S	…
0600	113	Exon 5	PH	GTC → GAC	Val → Asp	S	…
0620	114, 115	Exon 5	PH	TCT del	Phe deletion	S	…
0621	115	Exon 5	PH	TCC → TTC	Ser → Phe	S	MGF (P)
0630*	117	Exon 5	PH	ACT → CCT	Thr → Pro	S	…
0700	130	Exon 5	PH	A del (5 As)	Frameshift	B	…
0703*	…	…	…	Deletion (>30 kb) of exons 6–19	…	S	…
0710	(131)	Intron 5	PH	A → G	Splice acceptor (−2)	S	…
0730	145	Exon 6	TH	TGC → TGA	Cys → Stop	C, EG	…
0770	166	Exon 6	TH	CAA → TAA	Gln → Stop	U	…
0771	166	Exon 6	TH	CAA → TAA	Gln → Stop	U, C	…
0780*	(174)	Intron 6	TH	G → A	Splice acceptor (−1)	S	…
0800	186	Exon 7	Proline rich	A ins (6 As)	Frameshift	S	Mother (P)
0810	187, 188	Exon 7	Proline rich	TCTT del	Frameshift	U	MGGF (P)
0820*	189	Exon 7	Proline rich	C del (5 Cs)	Frameshift	S	Mother (M)
0850*	…	…	…	Deletion (2.6 kb) of exons 8–10	Frameshift	B, EG	…
0870*	203, 204	Exon 8	Proline rich	CGC del and TGGTG ins	Frameshift	S	MGF (P)
0900	218	Exon 8	Proline rich	A del (5 As)	Frameshift	B	…
0940*	239	Exon 8	SH3	G del	Frameshift	S	…
0980	244	Exon 8	SH3	TTG → TAG	Leu → Stop	B	MGF (P)
1000	252	Exon 8	SH3	TGG → TAG	Trp → Stop	S	MGF (P)
1030	255	Exon 8	SH3	CGA → TGA	Arg → Stop	S	…
1031	255	Exon 8	SH3	CGA → TGA	Arg → Stop	U	…
1070*	(259)	Intron 8	SH3	T → C	Splice-donor defect (+2)	C, EG	…
1100	261	Exon 9	SH3	G del TTA ins	Frameshift	S	…
1107*	(280)	…	…	500-bp ins starting at +5 position of splice-donor site of intron 9	…	S	…
1110*	288	Exon 10	SH2	CGG → CAG	Arg → Gln	S	…
1111†	288	Exon 10	SH2	CGG → CAG	Arg → Gln	S	…
1120	(298)	Intron 10	SH2	G del, 11-bp ins	Splice-acceptor defect (−9)	C, EG	…
1130	(298)	Intron 10	SH2	A deletion	Splice-acceptor defect (−2)	U	…
1131	(298)	Intron 10	SH2	A → C	Splice-acceptor defect (−2)	B	…
1133	(298)	Intron 10	SH2	G → A	Splice-acceptor defect (−1)	U	…
1150	302	Exon 11	SH2	GGA → GAA	Gly → Glu	S	MGF (P)
1160*	307	Exon 11	SH2	AGA → ACA	Arg → Thr	S	MGF (P)

(*continued*)

Table 1 (continued)

Subject	Codon[a]	Intron/Exon	Domain	Change[b]	Effect[c]	Family History[d]	Source of Mutation[e]
1180	315	Exon 11	SH2	TAT → TAG	Tyr → Stop	S	…
1200	325	Exon 11	SH2	G del (4 Gs)	Splice defect	S	MGF (P)
1210*	326–330	Exon 12	SH2	ACCCTCAAGGGGT del	Frameshift	S	MGF (P)
1250*	348	Exon 12	SH2	GAG → TAG	Glu → Stop	U	…
1300	361	Exon 12	SH2	TAC → TGC	Tyr → Cys	B, EG	…
1400	375	Exon 13	SH2	TAT → TAG	Tyr → Stop	S	MGF (P)
1420*	391	Exon 13	Kinase	A → G	Creates cryptic splice site	C	…
1426*	404	Exon 14	Kinase	TTC del	Phe del	S	Mother (M)
1427†	404	Exon 14	Kinase	TTC del	Phe del	S	…
1440	430	Exon 14	Kinase	AAG → AGG	Lys → Arg	S	…
1445*	445	Exon 14	Kinase	GAA → GAC	Glu → Asp	B	…
1450*	460	Exon 15	Kinase	TTG → TAG	Leu → Stop	S	…
1470	509	Exon 15	Kinase	ATG → ATA	Met → Ile	S	MGF (P)
1471	509	Exon 15	Kinase	ATG → ATA	Met → Ile	S	…
1500	520	Exon 15	Kinase	CGA → CAA	Arg → Gln	B	MGF (P)
1600	520	Exon 15	Kinase	CGA → CAA	Arg → Gln	U, C, EG	…
1700	520	Exon 15	Kinase	CGA → TGA	Arg → Stop	S	MGF (P)
1720	(522)	Intron 15	Kinase	TTTG del	Splice-acceptor defect (−9–12)	B	…
1750*	525	Exon 16	Kinase	CGA → GGA	Arg → Gly	S	Mother (M)
1800	527, 528	Exon 16	Kinase	TTTG del	Frameshift	S	MGF (P)
1830*	533	Exon 16	Kinase	G del (2 Gs)	Frameshift	S	…
1850*	535	Exon 16	Kinase	GTT → TTT	Val → Phe;	C	…
1900	542	Exon 16	Kinase	CTG → CCG	Leu → Pro	U	…
2000	(544)	Intron 16	Kinase	G → T	Splice-donor defect (+1)	B	…
2017	(544)	Intron 16	Kinase	G → T	Splice-donor defect (+1)	S	…
2100	562	Exon 17	Kinase	CGG → TGG	Arg → Trp	C	…
2110*	563	Exon 17	Kinase	TGG → TTG	Trp → Leu	C, U	…
2116*	566	Exon 17	Kinase	G del (2Gs)	Frameshift	S	MGF (P)
2200	581	Exon 17	Kinase	TGG → CGG	Trp → Arg	S	…
2210	582	Exon 17	Kinase	GCT → GTT	Ala → Val	U	…
2220	584	Exon 17	Kinase	G → A	Splice-donor defect (−1)	C	…
2270*	588	Exon 18	Kinase	TGG → TAG	Trp → Stop	S	…
2273†	588	Exon 18	Kinase	TGG → TAG	Trp → Stop	U	MGGF (P)
2280	589	Exon 18	Kinase	GAA → GAT	Glu → Asp	S	…
2300	591	Exon 18	Kinase	TAC → TAA	Tyr → Stop	S	…
2320*	594	Exon 18	Kinase	GGG → GAG	Gly → Glu	S	…
2370	622	Exon 18	Kinase	GCT → CCT	Ala → Pro,	S	…
2390*	629–634	Exon 18	Kinase	16-bp del and 4-bp ins	…	S	Mother (M)
2400	630	Exon 18	Kinase	ATG → AAG	Met → Lys	C	…
2430*	…	…	…	Deletion (7.5 kb) of exon 19	Incomplete protein	S	…
2433*	…	…	…	Deletion (12 kb) of exon 19	Incomplete protein	S	…
2500	652	Exon 19	Kinase	CTA → CCA	Leu → Pro	B, C	MGGF (P)

[a] For mutations that occur in introns, the codon at the splice site is noted in parentheses.

[b] If an insertion (ins) or deletion (del) occurs in a run of repeated base pairs, the number of base pairs is indicated in parentheses.

[c] The position of splice defects is indicated as a minus sign (−) if the defect is 5′ to the splice site and as a plus sign (+) if the defect is 3′ to the splice site.

[d] S = sporadic; B = brothers; U = uncles; C = cousins; and EG = earlier generations.

[e] MGF = maternal grandfather; MGGF = maternal great-grandfather; M = mutations that are assumed to have originated in maternal germ cells; and P = mutations that are assumed to have originated from paternal germ cells.

[f] An asterisk (*) indicates that the mutation found in this patient has not been previously described. If a new mutation occurred in more than one patient, the second patient is indicated by a dagger (†).

Acknowledgments

We are grateful to the families for their participation in this study and to the physicians and genetic counselors who referred the families for analysis. These studies were supported in part by National Institutes of Health grant AI25129, March of Dimes grant FY97-0384, and NCI CORE grant P30 CA21765; by American Lebanese Syrian Associated Charities; and by funds from the Federal Express Chair of Excellence.

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