Bruton's Tyrosine Kinase Gene Mutations in Turkish Patients with X-Linked Agammaglobulinemia from a Single Center: Novel Mutations in βTK Gene

Tek Merkezden X'e Bağlı Agamaglobulinemi Hastaların Bruton Tirozin Kinaz Gen Mutasyonları: βTK Geninde Yeni Mutasyonlar

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ABSTRACT Objective: X-linked agammaglobulinemia (XLA) is caused by a mutation in the Bruton's tyrosine kinase gene and is characterized by a delay in the maturation and differentiation of B lymphocytes. Patients with XLA have either absent or very low levels of circulating mature B cells (<1%), plasma cells, and immunoglobulins of all isotypes. Recurrent bacterial infections are prevalent in patients with XLA. The absence of B cells, agammaglobulinemia and the clinical features of XLA are similar to autosomal recessive forms such as μ heavy chain, λ5, Igα, Igβ and a BLNK gene defects. The aim of this study was to diagnose Turkish patients with suspected XLA by using BTK mutation analysis. **Material and Methods:** Fourteen male children recruited from 13 different families that were diagnosed with suspected XLA based on recurrent bacterial infections, decreased B cell count in the peripheral blood and hypogammaglobulinemia were included in the study. Clinical and demographic features of patients, serum immunoglobulin levels, peripheral blood B cell counts and BTK gene mutations were reviewed retrospectively. Patients were examined for BTK mutations by polymerase chain reaction. **Results:** Three of 14 patients were the children of consanguineous marriages and 3 families had a history of immune deficiencies. Most of the patients presented with infections, primarily pneumonia. BTK mutation resulting in XLA was detected in 10 patients. Eight patients had previously been diagnosed and 2 patients had novel mutations. **Conclusion:** Mutation analysis must be done to differentiate XLA from other autosomal recessive forms such as μ heavy chain, λ5, Igα, Igβ and a BLNK gene defects and this is necessary to design genetic counseling for newly diagnosed patients.

Key Words: Immunologic deficiency syndromes; diagnosis; child

ÖZET Amaç: X'e bağlı agammaglobulinemi (XLA), Bruton tirozin kinaz (BTK) geninde mutasyon sonucu gelişir ve B lenfositlerinin olgunlaşma ve farklılaşmasındaki gecikme ile karakterizedir. XLA'lı hastaların dolaşan olgun B lenfositleri (<%1), plazma hücreleri ve tüm immunglobulin izotipleri ya yoktur, ya da çok düşüktür. Tekrarlayan bakteriyel enfeksiyonlar sıktır. B hücre yokluğu, agammaglobulinemi ve klinik benzerlikten dolayı, ayırıcı tanıda otozomal resesif geçişli μ ağır zincir, λ5, Igα ve β, BLNK eksikliği göz önünde bulundurulmalıdır. Çalışmada amacımız, XLA düşünülen Türk hastaları BTK mutasyon analizi ile değerlendirmekti. **Gereç ve Yöntemler:** Tekrarlayan bakteriyel enfeksiyonlar, hipogamaglobulinemi, periferik kanda B hücre sayısının düşüklüğü temeline dayanan XLA düşünülen 13 farklı aileden 14 erkek çocuğu çalışmaya alındı. Hastaların klinik ve demografik özellikleri, serum immunglobulin düzeyleri ve periferik kan B hücre oranı ve BTK gen mutasyonları retrospektif olarak irdelendi. BTK gen mutasyon analizi, polimeriz zincir reaksiyonu yöntemi kullanılarak yapıldı. **Bulgular:** On dört hastanın 3'ünde akraba evliliği, 4'ünde ailede immun yetersizlik öyküsü mevcuttu. En sık başvuru sebebi enfeksiyon olup, pnömoni ilk sırada yer alıyordu. XLA'ya sebep olan BTK mutasyonu 10 hastada tesbit edildi. Sekizi daha önce tanımlanmış olup 2'si yeni tanımlanan mutasyondu. **Sonuç:** Klinik ve laboratuar bulgularının benzerliğinden dolayı otozomal resesif geçiş gösteren tablolardan (μ ağır zincir, λ5, Ig α ve β, BLNK eksikliği) ayırmak amacıyla, XLA'nın kesin tanısı için mutasyon analizi yapılmalıdır.Bu genetik danışmanlık açısından da önem arz etmektedir.

Anahtar Kelimeler: İmmünolojik yetersizlik sendromları; tanı; çocuk

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X-Linked agammaglobulinemia (XLA), a hereditary antibody deficiency syndrome, was first described in 1952.[1] XLA is caused by the maturational arrest of B cells early in development, and is characterized clinically by early onset and recurrent bacterial infections.[2,3] Typically, patients with XLA have either absent or very low levels of circulating mature B cells (<1%), plasma cells, and immunoglobulins of all isotypes.[3,4]

The causative gene for XLA, localized to Xq21.3-Xq22, was identified in 1993 and named as Bruton's tyrosine kinase (BTK).[5,6] *BTK* encompasses 37.5 kb containing 19 exons.[2,7] The protein has five distinct structural domains: pleckstrin homology (PH), Tec homology (TH), Src homology 3 (SH3), Src homology 2 (SH2), and catalytic kinase (SH1).[2,7,8] Studies conducted in different countries and ethnic groups have demonstrated that approximately 90% of males with presumed XLA have mutations in *BTK*.[9] In the latest update of the online BTKbase, 1155 entries have been recorded from 974 unrelated families with 602 unique molecular mutations, indicating that most patients have private *BTK* mutations.

The present study aimed to evaluate both *BTK* mutations and clinical features of Turkish patients suspected to have XLA.

MATERIAL AND METHODS

SUBJECTS

A total of 14 Turkish male patients from 13 unrelated families were enrolled in the study conducted in the Istanbul University Cerrahpasa Medical Faculty Children's Hospital (Table 1). Informed consents were taken from the patients. Immunoglobulin levels were evaluated before intravenous gammaglobulin replacement therapy. The percentage of peripheral B cells was assessed by flow cytometry using an anti-CD19 monoclonal antibody (Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

SEQUENCE ANALYSIS OF THE BTK GENE

Molecular analysis of the *BTK* gene was performed in all 14 patients as previously described.[10] All 19 exons of the *BTK* gene were amplified using polymerase chain reaction (PCR) on genomic DNA, followed by direct fluorescent sequencing of PCR

TABLE 1: Clinical data on hypogammaglobulinemic males included in this study.

Patient no	Beginning of symptoms	Age at diagnosis	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	CD19+B cells/&	Family history	Consanguinity	BTK mutation
P 1	3 month	4 years	< 166	0	< 128	3	(-)	(-)	(-)
P 2	6 month	18 month	29	0	< 29	0	(-)	(+)	(+)
P 3	3.5 years	3.5 years	250	0	31	0	(-)	(-)	(+)
P 4a	6 month	3.5 years	No tested	No tested	No tested	0	*S.IgAdef.(2aunts) XLA (Brother)	(-)	(+)
P 4b	3 month	3 month	21	3	< 18	0	S.IgAdef. (2 aunts) XLA (Brother)	(-)	(+)
P 5	4 month	18 month	611	179	55	2	(-)	(+)	(-)
P 6	4.5 month	1.5 years	157	< 25	< 18	0	(-)	(-)	(+)
P 7	3 month	5 years	83	4	12	0	(-)	(-)	(+)
P 8	6 month	2 years	< 140	< 6	< 16	0	(-)	(-)	(+)
P 9	6 month	9 years	< 153	0	< 2	0	(-)	(+)	(-)
P 10	5 years	5 years	< 143	< 23	70	0	**THI (cousin)	(-)	(+)
P 11	5 month	7 years	681	188	25	2	**THI (sister)	(-)	(-)
P 12	3 years	3 years	180	< 28	< 29	0	(-)	(-)	(+)
P 13	8 years	8 years	302	152	8	0	(-)	(-)	(+)

*S.IgA def.: Selective IgA deficiency. **THI: Transient hypogammaglobulinemia of infancy.

products. Sequences of the exons and the splice-sites were compared to the *BTK* reference sequence (NCBI X58957).

Age of onset, age at diagnosis and serum Ig levels were compared in patients with and without BTK mutations by using the Mann-Whitney U test. Median (minimum and maximum) data were used to summarize quantitative data. Statistical significance was set at p-value <0.05.

RESULTS

In the present study, clinical, demographic and mutational characteristics of 14 patients suspected of having XLA were reviewed. The demographic and clinical characteristics of the patients are presented in Table 1. *BTK* gene mutations were identified in 10 (out of 14) patients. *BTK* mutations were found in 1 of the 3 consanguineous families and in 9 of the 11 non-consanguineous families. Three types of mutations were identified: nucleotide substitutions, small nucleotide deletions and splice-site mutations.

NUCLEOTIDE SUBSTITUTIONS IN THE BTK GENE

Four patients had a single nucleotide substitution (Table 2). Three patients (patients 2, 3, and 13) had missense mutations. Patient 2's mutation resulted in an amino acid substitution in the tyrosine kinase domain encoded by exon 18 (c. 1762T>G) (p.W588G). Patient 3 had an amino acid substitution in the PH domain encoded by exon 2 (c.40T>C) (p.S14P). The mutations in patients 2 and 3 had been previously identified. In patient 13, however, a novel amino acid substitution was discovered in the TH domain encoded by exon 6 (c.491G>A) (p.G164D). One patient (patient 6) had a novel point mutation in exon 2 (c.49A>T) (p.K17X) that resulted in a stop codon in the PH domain. The mutations patients 6 and 13 had not been previously reported in the mutation database.

SMALL NUCLEOTIDE DELETIONS IN THE BTK GENE

Small nucleotide deletions leading to a frame shift and a premature stop codon were identified in patients 4a, 4b, 8, and 10 (Table 2). A 5-nucleotide deletion and a premature stop codon mutation were detected in exon 13 (c.1157_1161delCCACT) and affected the TK domain in patients 4a and 4b, who were siblings. In patient 8, a two-nucleotide deletion at nucleotide 115/116 of exon 15 also resulted in a frame shift and a premature stop codon in the TK domain (c.1464_1465delGA) (p.E488fsX 19). A third small nucleotide deletion was a single nucleotide deletion in exon 8 and a premature stop codon in the SH3 domain of patient 10 (c.713delG) (p.G238fsX39). These three mutations have been defined previously.

SPLICE-SITE MUTATIONS IN THE BTK GENE

Two splice-site mutations were identified (Table 2). The first concerned a four-nucleotide deletion

TABLE 2: *Btk* mutations identified in this study.

Patient no	Localization	Nucleotide aberration	Amino acid aberration	Protein Domain
P 2	Exon 18	c.1762T>G	p.W588G	TK
P 3	Exon 2	c.40T>C	p.S14P	PH
P 4a	Exon 13	c.1157_1161delCCACT	p.S386fsX10	TK
P 4b	Exon 13	c.1157_1161delCCACT	p.S386fsX10	TK
P 6	Exon 2	c.49A>T	p.K17X	PH
P 7	Exon 9	c.839+4_+7delAGTA		
P 8	Exon 15	c.1461_1465delGA	p.E488fsX19	TK
P 10	Exon 8	c.713delG	p.G238fsX39	SH3
P 12	Exon 12	c.1102+1G>A		
P 13	Exon 6	c.491G>A	p.G164D	TH

nt. Nucleotide; PH: Pleckstrin homology; TK: Tyrosine kinase; SH3: Src homology 3.

in the donor splice-site of exon 9 in patient 7 (c.839+4_+7delAGTA). The second was a single nucleotide substitution in the donor splice-site of exon 12 in patient 12 (c.1102+1G>A). Both mutations are known XLA disease-causing mutations.

CLINICAL CHARACTERISTICS OF THE PATIENTS

Recruited patients were between 1-18 years. Parental consanguinity was present in 3 of 14 patients. There was a family history of immunodeficiency in 4 of 14 patients (1 XLA, 2 selective IgA deficiency, and 2 transient hypogammaglobulinemia of infancy). Most of the patients were admitted due to infections, and pneumonia was the most common diagnosis (Table 3). One of the patients had permanent hearing loss as a complication of otitis media. Bronchiectasis was detected in 5 patients.

In patients with *BTK* mutations, the median age at symptom onset was 6.0 (range 3.0-96.0) months and the median age at diagnosis was 36.0 (range 3.0-96.0) months. The median serum immunoglobulin levels at diagnosis were: IgG: 143 mg/dL (range 21-302), IgM: 18.0 mg/dL (range 8.0-70) and IgA: 6.0 mg/dL (range 0.00-152) in patients with a *BTK* mutation. In patients without a *BTK* mutation, the median age at symptom onset was 4.50 (range 3.0-6.0) months and the median age at diagnosis was 66.0 (range 18.0-108.0) months. The median serum immunoglobulin levels at diagnosis in patients without a *BTK* mutation were as follows: IgG: 388 mg/dL (range 153.0-681.0), IgM: 40.0 mg/dL (range 2.0-128.0) and IgA: 89.50 mg/dL (range 0.0-188.0). The median age at symptom onset and age at diagnosis were not significantly different between the patients with and without a mutation in the *BTK* gene (p= 0.196 and p= 0.255, respectively). In addition, the serum immunoglobulin levels at diagnosis were not significantly different between the two groups (for IgG p= 0.90, for IgA p=0.754, for IgM p= 0.536).

TABLE 3: Types of infection in the patients.

Infections	Mutation (+) n(%)	Mutation (-) n (%)
Pneumonia	8 80%)	3 (75%)
Sinusitis	5 (50%)	3 (75%)
Otitis media	1 (10%)	2 (50%)
Gastroenteritis	1 (10%)	1 (25%)
Menengitis	2 (20%)	
Pyoderma	1 (10%)	
Ecthyma gangrenosum	1 (10%)	
Perianal abscesses		1(25%)
Septic artritis	1(10%)	

DISCUSSION

Since the initial detection of the gene associated with XLA in 1993, the number of genetic studies in XLA patients has increased. The types of mutations causing XLA include missense, nonsense, point, frame-shift, splice-site, deletion, insertion, and premature stop codon mutations, and these mutations in *BTK* may affect any domains of the protein. In general, missense mutations account for 40% of all mutations, whereas nonsense mutations account for 17%, deletions account for 20%, insertions account for 7%, and splice-site mutations account for 16%.[11] This mutational distribution is similar to those listed in the Immunodeficiency Mutation Database.[12] In a study of 56 patients suspected of having XLA, mutations affecting the *BTK* gene were demonstrated in 51 patients. When the types of mutations were examined, it was shown that 22 mutations were missense mutations.[13] Similarly, in another study, the number of missense mutations was found to be higher.[14] In the study by Chan et al., 12 patients were evaluated, and 3 deletion mutations, 8 nucleotide substitution mutations, and 1 insertion-deletion mutation were detected.[15] In a European study, point mutations were more frequent.[16] In a study of Turkish children, novel mutations were identified in 7 of 13 cases and missense mutations were detected in 2 cases, a nonsense mutation was detected in 1 case, and deletion mutations were detected in 4 cases.[17] In the present study, frame-shift and premature stop codon mutations were the most frequent, followed in prevalence by missense mutations.

Although four of the patients presented with clinical and immunologic profiles that were similar to XLA, a *BTK* gene mutation was not identified. However, failure to detect a *BTK* gene mutation in

the coding exons and the splice-sites does not mean that the *BTK* protein will be present in some XLA patients.[18,19] In such cases, other RNA splicing defects or transcriptional defects may be present. Therefore, analysis of *BTK* transcripts should be considered. Alternatively, patients may have mutations in other autosomal recessive agammaglobulinemia genes (μ heavy chain, λ5, Igα, Igβ and a BLNK gene defects). Autosomal recessive forms of agammaglobulinemia cannot be clinically distinguished from XLA, however, the bone marrow precursor B-cell compartment will be affected differently.[20-25] This necessitates flow cytometric analysis of the precursor B-cell compartment in bone marrow for differentiating between the autosomal recessive agammaglobulinemia genes.

In conclusion, in the present study, 10 *BTK* gene mutations were detected in 9 Turkish families suspected of having XLA. Mutation analysis must be done to differentiate XLA from other autosomal recessive forms such as μ heavy chain, λ5, Igα, Igβ and a BLNK gene defects, allowing for improved genetic counseling of newly diagnosed patients.

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