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Short communication

Genetic and demographic features of X-linked agammaglobulinemia in Eastern and Central Europe: A cohort study

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ABSTRACT

Primary immunodeficiency disorders are a recognized public health problem worldwide. The prototype of these conditions is X-linked agammaglobulinemia (XLA) or Bruton's disease. XLA is caused by mutations in Bruton's tyrosine kinase gene (*BTK*), preventing B cell development and resulting in the almost total absence of serum immunoglobulins. The genetic profile and prevalence of XLA have not previously been studied in Eastern and Central European (ECE) countries. We studied the genetic and demographic features of XLA in Belarus, Croatia Hungary, Poland, Republic of Macedonia, Romania, Russia, Serbia, Slovenia, and Ukraine. We collected clinical, immunological, and genetic information for 122 patients from 109 families. The *BTK* gene was sequenced from the genomic DNA of patients with a high susceptibility to infection, almost no $CD19^+$ peripheral blood B cells, and low or undetectable levels of serum immunoglobulins M, G, and A, compatible with a clinical and immunological diagnosis of XLA. *BTK* sequence analysis revealed 98 different mutations, 46 of which are reported for the first time here. The mutations included

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single nucleotide changes in the coding exons (35 missense and 17 nonsense), 23 splicing defects, 13 small deletions, 7 large deletions, and 3 insertions. The mutations were scattered throughout the *BTK* gene and most frequently concerned the SH1 domain; no missense mutation was detected in the SH3 domain. The prevalence of XLA in ECE countries (total population 145,530,870) was found to be 1 per 1,399,000 individuals. This report provides the first comprehensive overview of the molecular genetic and demographic features of XLA in Eastern and Central Europe.

1. Introduction

X-linked agammaglobulinemia (XLA; MIM#300300) is a primary immunodeficiency disorder characterized by an early defect in B-lymphocyte differentiation. It is caused by defects in Bruton's tyrosine kinase gene (*BTK*), which encodes a cytoplasmic tyrosine kinase expressed throughout myeloid and B cell differentiation (Tsukada et al., 1993; Vetrie et al., 1993). Affected individuals have almost no peripheral blood B cells and only very small amounts of immunoglobulins of all isotypes. XLA patients are therefore highly susceptible to infections with various types of pathogens, including encapsulated pyogenic bacteria, enteroviruses, and *Giardia lamblia*, against which host defenses are largely based on antibodies (Ochs and Smith, 1996; Plebani et al., 2002). Clinical manifestations of XLA include recurrent infections of the upper and lower respiratory tract and the skin, meningoencephalitis, gastroenteritis, and conjunctivitis (Winkelstein et al., 2006). Infections usually start at four to six months of age, coinciding with the catabolism of IgG of maternal origin. XLA patients may also develop purulent and non-purulent arthritis, hepatitis, osteomyelitis, and protracted enterovirus infection (Winkelstein et al., 2006). Intravenous or subcutaneous immunoglobulin replacement therapy can attenuate, but not completely prevent infectious complications.

The genetic and epidemiological features of primary immunodeficiency disorders (PIDs) have remained largely unexplored in Eastern and Central European (ECE) countries. We provide here the first report of the demographics and mutational spectrum of *BTK* in 122 XLA patients from 10 ECE countries. We report 46 previously unknown mutations in *BTK*. This study was carried out in the framework of the *J Project*, an ECE initiative for increasing awareness and improving the diagnosis of PIDS, including genetic testing for these conditions.

2. Methods

2.1. Study population

We analyzed 122 XLA patients from 109 unrelated families from 10 ECE countries. XLA diagnosis in all these patients was based on family history, typical clinical and immunological findings, including recurrent otitis media, sinusitis, bronchitis and pneumonia, an almost total lack of peripheral blood B cells (<2%), and very low levels of serum immunoglobulin isotypes. XLA diagnosis was confirmed genetically by screening for mutations of the *BTK* gene. Blood samples were collected into EDTA, from patients giving informed consent. Fifty-two of the 122 patients studied underwent genetic diagnosis at the Jeffrey Modell Diagnostic Laboratory at the University of Debrecen (Hungary), 29 were underwent genetic diagnosis at the Erasmus University in Rotterdam (The Netherlands), 16 at the Research Center for Medical Genetics in Moscow (Russia), 7 at the Department of Pediatrics in Brescia (Italy), 6 at the Belarusian Research Center for Pediatric Oncology and Hematology in Minsk (Belarus), 6 at St. Jude Children's Research Hospital in Memphis (USA), and 4 at Karolinska University Hospital at Huddinge (Sweden). Samples were transported overnight or on the same day, at room temperature, to one of the seven molecular genetics centers. Two additional patients were analyzed genetically and have been described elsewhere (Jyonouchi et al., 2008). In total, 12 of the patients had been studied before (Jyonouchi et al., 2008; Rohrer et al., 1999; Richter et al., 2001; Noordzij et al., 2002; Fiorini et al., 2004; van Zelm et al., 2008). All investigations were carried out after informed consent had been obtained from the patients or their parents, and were approved by the appropriate institutional review board.

2.2. Immunological assays

B cell counts and serum immunoglobulin determinations were carried out at the various participating ECE immunology centers. Peripheral blood mononuclear cells were isolated from heparin-treated venous blood by density gradient centrifugation. The percentage of $CD19^+$ B-lymphocytes was determined by flow cytometry after incubating the cells with labeled anti-CD19 antibodies or an isotype control. Concentrations of serum IgM, IgG, and IgA isotypes were determined by standard immunochemical assays.

2.3. Mutational analysis of BTK

Genomic DNA was extracted from blood leukocytes according to standard protocols. Mutations were analyzed by amplifying exons 1–19 and the flanking intronic regions of *BTK* by PCR. The primer sequences used at the Debrecen Center were kindly provided by Dr. T. Freiberger. Amplicons were sequenced with the Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA), and analyzed with an ABI 3130 capillary sequencer (Applied Biosystems, Foster City, CA). Sequence variations were described with respect to a reference sequence, GenBank accession no. NM_000061 for *BTK* cDNA, in which the c.1 position corresponds to the A of the ATG translation initiation codon. Mutations were designated as recommended by den Dunnen and Antonarakis (2001).

3. Results

3.1. Types and location of BTK mutations

In our cohort of 122 XLA patients from 15 referral centers, we identified 98 different *BTK* mutations, 46 of which were previously unknown (Table 1 and Fig. 1). Diverse mutations, including missense or nonsense base substitutions, splicing mutations, large and small deletions and insertions, were detected (Fig. 1). Missense mutations were the most frequently identified (35; 36%), followed by splice-site mutations (23; 23.2%), nonsense mutations (17; 17.2%), frameshift due to insertions or deletions (16; 16.2%), and large deletions (7; 7.2%). The mutations were scattered throughout the *BTK* gene, but most frequently affected the SH1 domain of the protein (45; 45.3%), followed by the PH domain (22, 22.3%), the SH2 domain (13; 13.3%), the SH3 domain (7; 7.3%) and the TH domain (6; 6.3%) (Fig. 1). We also identified four large deletions affecting at least two domains of BTK and one large deletion affecting exon 1 (Table 1 and Fig. 1). None of the mutations affected the tyrosine residues in positions 223 and 551, which are phosphorylated during the regulation of the protein. No missense mutation was detected in the SH3 domain.

Table 1
Previously unknown mutations in the *BTK* gene identified in this study.

Pts	Country	Type of mutation	Site of the mutation	Nucleotide change	Protein change	Affected domain
1	Poland	Large deletion	exon 1	–	–	–
2	Romania	Missense	exon 2	c.29T>A	p.F10Y	PH
3	Hungary	Frameshift	exon 2	c.87_99delGTTTCTCTTGACC or c.88_100delTTTCTCTTGACCG or c.89_101delTTCTCTTGACCGT	p.F30fsX52	PH
4	Hungary	Missense	exon 2	c.115T>A	p.Y39N	PH
5	Hungary	Missense	exon 2	c.115T>A	p.Y39N	PH
6	Russia	Splicing	intron 2	c.141+1delG	–	PH
7	Russia	Splicing	intron 3	c.241-1G>T	–	PH
8	Poland	Large deletion	exon 6, 7	c.392_588del197bp	p.V131_Q196del	PH, TH
9	Poland	Large deletion	exon 6, 7	c.392_588del197bp	p.V131_Q196del	PH, TH
10	Poland	Nonsense	exon 6	c.421A>T	p.K141X	TH
11	Poland	Nonsense	exon 6	c.421A>T	p.K141X	TH
12	Ukraine	Splicing	intron 6	c.520+1G>A	–	TH
13	Ukraine	Splicing	intron 6	c.520+1G>A	–	TH
14	Serbia	Splicing	intron 7	c.588+1G>A	–	TH
15	Ukraine	Splicing	intron 7	c.588+2delT	–	TH
16	Slovenia	Frameshift	exon 8	c.685_694delAATGCAAATG or c.686_695delATGCAAATGA	p.N229fsX273	SH3
17	Hungary	Splicing	intron 8	c.777-1G>A	–	SH3
18	Poland	Splicing	intron 9	c.839+2T>C	–	SH3
19	Serbia	Splicing	intron 9	c.839+(4_7)delAGTA	–	SH3
20	Russia	Missense	exon 10	c.881T>C	p.L294P	SH2
21	Belarus	Missense	exon 11	c.950T>G	p.V317G	SH2
22	Belarus	Frameshift	exon 11	c.952_953delTC or c.953_954delCT	p.S318fsX321	SH2
23	Ukraine	Frameshift	exon 12	c.995_1002delGTCATTAT or c.996_1003delTCATTATG or c.997_1004delCATTATGT	p.H333fsX345	SH2
24	Hungary	Missense	exon 12	c.1064T>A	p.I355N	SH2
25	Hungary	Missense	exon 12	c.1064T>A	p.I355N	SH2
26	Hungary	Nonsense	exon 12	c.1087C>T	p.Q363X	SH2
27	Russia	Nonsense	exon 12	c.1087C>T	p.Q363X	SH2
28	Poland	Missense	exon 12	c.1100C>A	p.A367E	SH2
29	Poland	Missense	exon 12	c.1100C>A	p.A367E	SH2
30	Poland	Splicing	intron 12	c.1102+1_11delGTGAGTACCAG	–	SH2
31	Russia	Nonsense	exon 13	c.1171G>T	p.G391X	SH1
32	Russia	Nonsense	exon 15	c.1363G>T	p.E455X	SH1
33	Russia	Frameshift	exon 15	c.1406_delC	p.P469fsX483	SH1
34	Macedonia	Frameshift	exon 15	c.1407_1408insC	p.P469fsX475	SH1
35	Poland	Frameshift	exon 15	c.1457_1458delTG	p.L486QfsX505	SH1
36	Ukraine	Nonsense	exon 15	c.1462G>T	p.E488X	SH1
37	Ukraine	Nonsense	exon 15	c.1462G>T	p.E488X	SH1
38	Serbia	Nonsense	exon 15	c.1480C>T	p.Q494X	SH1
39	Serbia	Nonsense	exon 15	c.1480C>T	p.Q494X	SH1
40	Poland	Missense	exon 15	c.1565T>C	p.L522P	SH1
41	Poland	Missense	exon 15	c.1526T>C	p.M509T	SH1
42	Belarus	Frameshift	exon 16	c.1630delA	p.R544fsX555	SH1
42	Belarus	Frameshift	exon 16	c.1630delA	p.R544fsX555	SH1
44	Hungary	Missense	exon 16	c.1631 G>C	p.R544T	SH1
45	Romania	Splicing	intron 16	c.1631+5G>T	–	SH1
46	Russia	Frameshift	exon 17	c.1697_insT	p.P566fsX572	SH1
47	Russia	Missense	exon 17	c.1730A>T	p.K577I	SH1
48	Macedonia	Missense	exon 17	c.1735G>A	p.D579N	SH1
49	Ukraine	Missense	exon 17	c.1773T>G	p.F583V	SH1
50	Ukraine	Missense	exon 17	c.1773T>G	p.F583V	SH1
51	Poland	Missense	exon 17	c.1750G>C	p.G584R	SH1
52	Poland	Splicing	intron 17	c.1750+3A>C	–	SH1
53	Hungary	Missense	exon 18	c.1846C>G	p.L616V	SH1
54	Macedonia	Nonsense	exon 18	c.1901G>A	p.W634X	SH1
55	Macedonia	Nonsense	exon 18	c.1901G>A	p.W634X	SH1
56	Romania	Missense	exon 19	c.1921C>G	p.R641G	SH1
57	Poland	Missense	exon 19	c.1931T>A	p.F644Y	SH1
58	Hungary	Missense	exon 19	c. 1952 T>G	p.I651S	SH1

Patients 4 and 5, 8 and 9, 10 and 11, 24 and 25, 28 and 28, 36 and 37, 38 and 39, 42 and 43, 49 and 50, 54 and 55 are siblings. Pts, patients; PH, pleckstrin homology domain; TH, Tec-homology domain; SH3, Src-homology 3 domain; SH2, Src-homology 2 domain; SH1, tyrosine kinase domain. Mutation nomenclature is based on previous recommendations (den Dunnen and Antonarakis, 2001).

3.2. Novel mutations in BTK

We defined 46 new mutations, by comparison with the online *BTK* database www.bioinf.uta.fi/BTKbase. The patients with these new mutations were from nine ECE countries, but mostly from Poland (15; 26%), Hungary (10; 17%), Russia (9; 15%), and Ukraine (8; 14%) (Fig. 2). The new mutations identified included missense (17), splicing (11), frameshift (9) and nonsense (7) mutations, and two large deletions (Table 1, Fig. 1). The missense mutations affected the PH, SH2, and SH1 domains of BTK. Seven new single nucleotide substitutions were identified in the splicing regions of eight patients (Table 1). We also identified four splicing deletions in four patients from four unrelated families. We also found seven small deletions in seven patients from seven unrelated families, and two previously unidentified insertions in two patients from two unrelated families, all of which resulted in a frameshift (Table 1). Large deletions were identified in three patients (Patients 1, 8 and 9; Table 1). Seven new nonsense mutations resulting in premature termination signals and

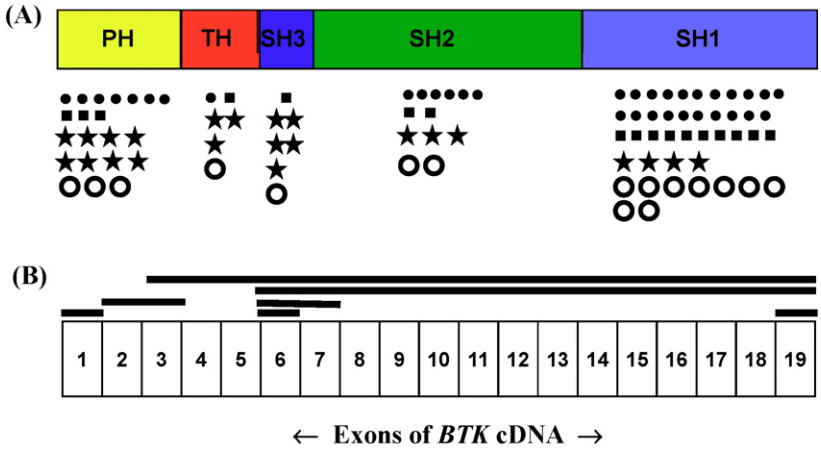

Fig. 1. Domain organization of BTK and localization of novel and recurrent mutations identified in this study. PH, pleckstrin homology domain; TH, Tec-homology domain; SH3, Src-homology 3 domain; SH2, Src-homology 2 domain; SH1, tyrosine kinase domain. Missense (closed circle), nonsense (closed square), splice-site (asterisk), and frameshift (open circle) mutations affecting various domains of the BTK protein are shown in Panel A. Large deletions affecting various exons of the *BTK* cDNA are indicated with horizontal bars in Panel B.

BTK transcripts of abnormal size were detected in 12 patients. One Russian and one Hungarian patient (Patients 26 and 27) carried the same nonsense mutation (Table 1).

3.3. Recurrent mutations in BTK

In addition to the new mutations, we also identified 52 recurrent sequence variants reported in other series (Fig. 1). These mutations were identified mostly in patients from Poland (20; 31%), Ukraine (10; 15%), Russia (9; 14%), Croatia (6; 9%), and Hungary (6; 9%) (Fig. 2).

3.4. Demographics of patients with XLA and BTK mutations in ECE countries

The numbers of XLA patients with new or recurrent *BTK* mutations in the various ECE countries are shown in Fig. 2. Based on the data for nine countries (excluding Russia), we estimate that XLA may affect 1 in 1,399,000 individuals, with significant differences in prevalence between countries (Table 2). These data are likely

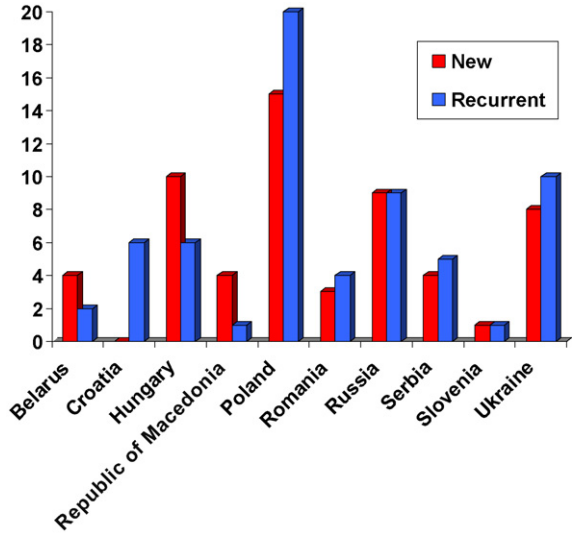

Fig. 2. New and recurrent *BTK* mutations in various Eastern and Central European countries.

Table 2
Prevalence of XLA with *BTK* mutation in European countries and the USA.

Country	Population	Patients	Prevalence	PID centers
Eastern and Central European countries				
Republic of Macedonia	2,022,547	5	1:404,000	1
Hungary	10,050,000	16	1:628,000	4
Croatia	4,491,543	6	1:749,000	1
Slovenia	2,009,000	2	1:1,000,000	1
Poland	38,518,241	35	1:1,100,000	4
Serbia	10,159,056	9	1:1,130,000	1
Belarus	9,689,800	6	1:1,615,000	1
Ukraine	46,287,138	18	1:2,570,000	2
Romania	22,303,552	7	1:3,180,000	3
Total	145,530,870	104	1:1,399,000	15
Western European countries[a]				
Italy	59,337,888	127	1:467,227	NA
France	64,473,140	119	1:541,791	NA
United Kingdom	60,975,000	50	1:1,219,500	NA
Spain	45,116,894	28	1:1,611,317	NA
Germany	82,210,000	26	1:3,161,923	NA
Total	312,112,922	350	1:891,751	NA
USA (2006)[b]	299,398,484	201	1:1,489,544	NA

[a] Based on information from the ESID online database network (http://www.esid.org/esid_registry.php).
[b] Winkelstein et al. (2006); NA, not available; PID, primary immunodeficiency.

to be reliable, as all the PID referral centers from Poland, Ukraine, Hungary, Serbia, Romania, Belarus, Croatia, Republic of Macedonia, and Slovenia participated in this study. The significant variability observed between countries may reflect differences in PID awareness.

4. Discussion

PIDs have recently been recognized as a global public health problem affecting at least 1 in every 250 individuals (Casanova and Abel, 2007). However, patients with these conditions remain largely neglected, particularly in countries with poor socioeconomic conditions. Five years ago, we launched a program focusing on PIDs, the *J Project*, to increase awareness and promote early diagnosis of PIDs, through methods including genetic testing, in the countries of Eastern and Central Europe (Maródi and Notarangelo, 2007). We have focused in particular on XLA, the prototype immunodeficiency disorder, because this condition is reasonably easy to diagnose and to treat with regular immunoglobulin infusions. We provide here

Table 3
Demographic and immunological data for patients with novel *BTK* mutations.

Patient	Country	Age (years)	Age at diagnosis (years)	B cells[a]	sIgA (g/l)	sIgG (g/l)	sIgM (g/l)
1	Poland	11	8 6/12	0.30	0.07	0.30	0.07
2	Romania	10	2 6/12	0.10	0.15	0.53	0.20
3	Hungary	11	7	0.04	0.06	7.00[b]	0.05
4	Hungary	31	3/12	0.20	0.02	3.66[b]	0.18
5	Hungary	34	1 6/12	0.30	0.02	6.32[b]	0.18
6	Russia	23	3	0.10	UD	1.50	UD
7	Russia	6	4	0.01	0.06	0.83	0.04
8	Poland	8	1	UD	0.06	0.10	0.15
9	Poland	5	2/12	UD	0.08	3.73[b]	0.26
10	Poland	26	7	UD	UD	0.13	UD
11	Poland	24	5	UD	0.19	0.42	0.23
12	Ukraine	11	5	UD	UD	0.08	0.01
13	Ukraine	11	11	UD	UD	UD	UD
14	Serbia	9	3	0.40	0.22	0.30	0.12
15	Ukraine	17	6	UD	UD	UD	UD
16	Slovenia	2 6/12	7/12	0.04	0.06	0.10	0.11
17	Hungary	13	5	UD	0.45	0.08	0.06
18	Poland	9	4	UD	UD	0.32	0.17
19	Serbia	5	1	0.20	0.08	0.60	0.05
20	Russia	6 6/12	3	UD	0,06	0,11	0,18
21	Belarus	5	4	0.10	0.02	0.77	UD
22	Belarus	7	5	0.10	0.07	4.20[b]	0.03
23	Ukraine	11	7	UD	0.02	0.30	0.03
24	Hungary	27	16	0.02	0.06	5.05[b]	0.05
25	Hungary	27	16	0.04	0.06	5.45[b]	0.08
26	Hungary	†22	3	0.20	0.08	1.20	0.05
27	Russia	23	8	0.02	0.06	1.21	0.14
28	Poland	17	7	0.20	0.12	2.89	0.14
29	Poland	34	12	2.00	UD	0.15	0.45
30	Poland	†(age unknown)	1	UD	UD	0.17	UD
31	Russia	8	3	UD	0.06	0.31	0.06
32	Russia	2	1	UD	0.06	0.33	0.04
33	Russia	9	4	0.01	0.06	1.12	0.26
34	Macedonia	8	1	0.30	0.28	1.61	0.03
35	Poland	18	5	UD	UD	0.05	0.08
36	Ukraine	9	4	UD	UD	0.05	0.02
37	Ukraine	1	6/12	UD	UD	0.02	UD
38	Serbia	†17	4	UD	UD	0.50	0.20
39	Serbia	23	8/12	UD	UD	1.40	UD
40	Poland	10 6/12	2 6/12	0.10	0.06	0.15	0.19
41	Poland	9	6	0.20	UD	0.15	0.19
42	Belarus	21	6	0.10	0.04	3.67[b]	UD
43	Belarus	7	3	0.30	0.03	0.08	0.06
44	Hungary	14	2	0.10	0.02	3.28[b]	0.18
45	Romania	3	3	UD	0.16	3.15[b]	0.13
46	Russia	14	2	UD	0.06	0.33	0.04
47	Russia	5	2	UD	0.06	0.33	0.41
48	Macedonia	25	8	0.80	0.08	1.50	0.10
49	Ukraine	13	9	UD	0.01	0.06	0.01
50	Ukraine	16	12	UD	UD	0.08	0.01
51	Poland	12	2	0.20	UD	0.30	0.52
52	Poland	5	1	0.30	0.07	0.24	0.19
53	Hungary	35	23	0.10	0.05	4.06[b]	0.05
54	Macedonia	20	7	UD	UD	3.50[b]	UD
55	Macedonia	16	3	UD	UD	1.20	0.86
56	Romania	12	7	UD	0.16	2.54[b]	0.05
57	Poland	4	1 6/12	0.50	0.22	1.20	0.44
58	Hungary	17	3	UD	0.05	3.88[b]	0.04

Patients 5 and 6, 7 and 8, 20 and 21, 24 and 25, 32 and 33, 34 and 35, 37 and 38, 43 and 44, 48 and 49 are siblings. UD, undetectable; †, patient died.

[a] Percentage of blood lymphocytes.

[b] IgG trough levels in patients on regular intravenous immunoglobulin substitution treatment.

the first description of the demographic and genetic features of XLA in 10 ECE countries and describe 46 previously unknown mutations of the *BTK* gene in 58 patients.

We identified 17 new missense mutations resulting in single amino acid substitutions in the PH, SH2, and SH1 domains of the BTK protein. Evidence for the involvement of these sequence variants in causing disease was provided by the typical clinical phenotypes of XLA and the absence or very small numbers of circulating $CD19^+$ B cells and low or undetectable concentrations of serum immunoglobulin isotypes (Table 3). In addition, all but the p.F10Y and p.A367E missense mutations described here affected conserved amino acids (Lindvall et al., 2005). The cosegregation of missense mutations in 7 of the 17 families provided further confirmation that these missense mutations were involved in disease. We also sequenced the gene in six women, the mothers of nine of the patients studied. These women were found to carry heterozygous missense mutations. We detected previously unknown missense mutations in at least two members of seven families, as follows: mother and son in the families of patients 2, 56 and 58; mother and two heterozygous twins (Patients 24 and 25); two Polish brothers

(Patients 28 and 29); a mother and her two sons (Patients 4 and 5, and Patients 49 and 50).

Two previously unknown missense mutations affecting the PH domain were identified in Patients 2, 4, and 5 (Table 1). BTK binds phosphatidylinositol-3,4,5-triphosphate through its PH or membrane localization domain. Replacement of the non-polar phenylalanine residue in position 10 with a hydrophilic tyrosine residue may be deleterious for cell membrane binding (Conley et al., 1998; Holinski-Feder et al., 1998).

For the SH2 domain, we identified a p.V317G mutation in a Belarusian patient (Patient 21), a p.I355N substitution in Hungarian heterozygous twins (Patients 24 and 25), and a p.A367E substitution in two Polish brothers (Patients 28 and 29). During BTK activation by Src family kinases, the Y551 tyrosine residue in the SH1 domain is phosphorylated, leading to autophosphorylation of the other regulatory phosphorylation target, Y223 (Mohamed et al., 2009). The SH2 domain then recruits the B cell linker adapter protein to the plasma membrane, together with phospholipase C-γ2. Several residues are involved in phosphotyrosyl protein binding to SH2, and residues outside the strict pY binding region may be essential for affinity and specificity. All three of the mutations identified in this study are within six residues, upstream or downstream, of Y361, providing strong support for the hypothesis of a disease-causing effect (Vihinen et al., 2000).

The remaining 11 missense mutations identified concern the catalytic domain of BTK. In particular, the previously unidentified p.R641G mutation found in a Romanian patient (Patient 56) affects the lower lobe of the SH1 domain. Although this *BTK* mutation was considered to be a new mutation, the replacement of Arg 641 by a His or Cys residue has been reported before (Gaspar et al., 1995). This position is of key importance and this substitution may damage the ionic bond between E567 and R641, thereby destabilizing the kinase structure (Jin et al., 1995; Saha et al., 1997; Speletas et al., 2001).

In this study, we aimed to estimate the prevalence of XLA in ECE countries despite the fact that there is unevenness in the data capture due to large underserviced areas. We compared the current prevalence with previous reports from other parts of the world. Winkelstein et al. (2006) recently reported on 201 XLA patients included in the United States XLA registry. Based on the population of USA in 2006 (299,398,484) the number of patients studied by Winkelstein suggests a prevalence of XLA in the United States of 1 in 1,489,544 individuals, consistent with our findings for nine ECE countries (Table 2). The data published by Winkelstein et al. can be considered reliable as they were collected between 1999 and 2006 from academic societies and appropriate academic departments with residency training. Our data, suggesting a prevalence of 1 in 1,399,000 individuals for XLA, are validated by the involvement in this study of all recognized PID centers in nine ECE countries (data from Russia were not included because some of the major centers in this country chose not to collaborate on this project). Data available from the ESID Registry showed a prevalence of 1 in 1,891,781 for XLA with *BTK* mutations in five Western European countries with a population of 312,112,922. These data clearly indicate that awareness of XLA is greater in these countries than in Eastern Europe and the USA (Table 2).

We believe that XLA is underdiagnosed in both Europe and the USA. The average age at diagnosis of XLA for the 58 patients carrying previously unknown *BTK* mutations was five years and one month, and the diagnosis was established beyond the age of 10 years in six patients (Table 3). There is only one PID center per 7 million people in Romania and one PID center per 23 million people in Ukraine. The reported prevalence of XLA was lowest for these two countries, whereas the highest prevalences were reported for Macedonia, Hungary, and Croatia, which have one PID center per 2–4.5 million inhabitants (Table 2). These data clearly suggest that a lack of access to diagnostic facilities, a lack of awareness, or both may be the principal reason for the underdiagnosis of XLA, and probably of other PIDs, in Eastern and Central Europe. There is therefore a clear need to ensure that diagnostic facilities are made available in these countries.

In conclusion, we collected information about mutations in *BTK* over a period of five years, in 10 ECE countries. We present here 98 different mutations, including 46 previously unknown sequence variants. The data obtained in this study indicate that the spectrum of *BTK* mutations in ECE countries is similar to that in other countries and continents, and do not show a distinct pattern which is different then the rest of the world. An analysis of *BTK* mutations provides valuable information for the counseling of female family members living in parts of ECE countries in which treatment for XLA is not available. This joint effort in Eastern and Central Europe could serve as the basis for ongoing education and research collaboration between PID centers in ECE countries and advanced, cutting-edge centers in Western Europe and elsewhere. Collaborative studies are of particular importance for the evaluation of secondary prevention strategies, such as the use of long-term prophylactic antibiotics, which could potentially decrease the risk of infection in ECE countries in which immunoglobulins are difficult to obtain.

Conflict of interest

L. Marodi declares that he is on the Board of ESID.

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