SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

DISEASE-CAUSING MUTATIONS
IN SELECTED PRIMARY IMMUNODEFICIENCIES

BEÁTA TŐTH
Supervisor: Prof. Dr. László Maródi

UNIVERSITY OF DEBRECEN
GYULA PETRÁNYI DOCTORAL SCHOOL

DEBRECEN, 2011
INTRODUCTION

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 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, 1993; Vetrie, 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, 1996; Plebani, 2002). Clinical manifestations of XLA include recurrent infections of the upper and lower respiratory tract and the skin, meningoencephalitis, gastroenteritis, and conjunctivitis (Winkelstein, 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, 2006).

The hyper-IgE syndrome (HIES) is a multisystem primary immunodeficiency disorder characterized by pyogenic skin and lung infections, pneumatocele formation, severe eczema, and extreme elevation of serum IgE (Davis, 1966; Grimbacher, 2005; Buckley, 2001). Additional manifestations include distinctive facial features, hyperextensibility of the joints, cranial synostosis, scoliosis, abnormal dentition, and pathological fractures (Grimbacher, 2005; Buckley, 2001). Central nervous system lesions, coronary artery aneurysms, and Chiari’s malformation may also be part of the complex clinical pathology (Ling, 2007; Freeman, 2007). The most common pathogens of infections in HIES patients include *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and Candida. Staphylococcal skin abscesses typically fail to demonstrate erythema and warmth, and hence are called “cold abscesses” (Davis, 1966). Both autosomal recessive (AR) and autosomal dominant (AD) inheritance has been described, but most HIES cases are sporadic (Renner, 2004; Grimbacher, 2005). Recent studies have showed mutations in the signal transducer and activator of transcription-3 gene (STAT3) in chromosome 17q21 as major causes of AD and sporadic cases of HIES (Holland, 2007; Minegishi, 2007). Mutations were mainly localized in either the DNA-binding domain or the SH2 domain of STAT3.
Autoimmune polyendocrine syndrome type 1 also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a monogenic primary immunodeficiency disorder affecting endocrine organs and the skin (Leonard, 1946; Perheentupa, 2006; Husebye, 2009). APS1 is typically characterised by the association of hypoparathyroidism, primary adrenocortical insufficiency and chronic mucocutaneous candidiasis. The clinical phenotype, however, is highly variable, including the failure of pancreatic β-cells, gonads, and gastric parietal cells, which results from autoimmune reactions towards endocrine and non-endocrine organs. APS1 is caused by mutations in AIRE (autoimmune regulator gene) and usually manifests in childhood or teenage years (Mathis, 2007; Nagamine, 1997; Perheentupa, 2002). More than 60 different mutations have been described so far which are scattered throughout AIRE and four mutational hotspots have been defined in exons 2, 6, 8, and 10 (www.hgmd.cf.ac.uk; Peterson, 2005). Various autoantibodies that target tissue specific autoantigens typically develop and their presence correlate with disease manifestation in the organ where the autoantigen is expressed (Blizzard, 1963; Gylling, 2000; Söderbergh, 2004; Alimohammadi, 2008). Whether the autoantibodies have a direct pathogenic role in tissue destruction is not currently known. Recently it has been found that virtually all APS1 patients develop high-titer antibodies against type I interferons (IFN), especially IFN-ω and IFN-α2, and assay of such antibodies has been shown to be a highly reliable diagnostic marker (Meager, 2006; Meloni, 2008; Ofstedal, 2008).
OBJECTIVES

1. To study the genetic and demographic features of X-linked agammaglobulinemia in Belarus, Croatia, Hungary, Poland, Republic of Macedonia, Romania, Russia, Serbia, Slovenia, and Ukraine.

2. To collect clinical, immunological, and genetic data of 122 X-linked agammaglobulinemia patients from 109 families.

3. To perform clinical, immunological and genetic studies of 12 hyper-IgE syndrome patients from 4 Hungarian, 2 Russian, 2 Ukraine, and one Polish families with AD or sporadic HIES patients.

4. To analyze pathogenicity of the novel (H332Y) STAT3 mutation found in one Hungarian family.

5. To study clinical, immunological, and genetic features of autoimmune polyendocrine syndrome type 1 in Hungarian patients.

6. To determine the correlation of IFN-ω serum concentration with laboratory and clinical findings in patients with autoimmune polyendocrine syndrome type 1.
PATIENTS AND METHODS

Patients

We analyzed 122 XLA patients from 109 unrelated families from 10 Eastern and Central European (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 \textit{BTK} gene. Fifty-two of the 122 patients studied underwent genetic diagnosis at the Jeffrey Modell Diagnostic Laboratory at the University of Debrecen, 29 were analysed 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).

Twelve individuals from 4 Hungarian, 2 Russian, 2 Ukraine, and 1 Polish families with AD or sporadic HIES were studied. The diagnosis of HIES was made by analysis of clinical, immunological, and radiological findings, and segregation of the disease in the families.

We recruited patients with APS1 by contacting Endocrinology Departments and Divisions at Hungarian University Hospitals, Children’s Hospitals and County Hospitals running endocrinology clinics. As a result, we could include in this study 9 APS1 patients from 6 unrelated Hungarian families. The diagnosis of APS1 was made by analysis of clinical manifestations and laboratory findings, segregation of the disease in the family, and genomic DNA sequencing of \textit{AIRE}. The patients reported in this study had at least two of the three major clinical criteria of APS1 (hypothyroidism, adrenal failure, and mucocutaneous candidiasis) or only one disease manifestation together with diagnosed APS1 in their family.

A control group of healthy asymptomatic individuals, aged 20-50 years were also studied.

All investigations were carried out after informed consent had been obtained from the patients or their parents, and were approved by the institutional ethnical commited.
**Immunochemistry**

The serum total IgG, IgA, IgM concentrations were measured according to the manufacture’s instructions by nephelometry.

**Flow cytometry**

Peripheral blood mononuclear cells were isolated from heparinized blood by Ficoll Hypaque centrifugation. Fc receptors were blocked using rabbit IgG, and cells were stained with phycoerythrin labeled anti-CD19 (BD Pharmingen) or an isotype control. The percentage of cells positive in the isotype control (usually 0.01% or less) was subtracted from the percentage of positive cells in the CD19 assay.

**Mutational analysis**

Genomic DNA was extracted from blood leukocytes according to standard protocols. For sequencing, exons 1-19 of the \textit{BTK}, exons 1-23 of the \textit{STAT3}, exons 1-14 exons of the \textit{AIRE} and the flanking intronic regions were amplified by polymerase chain reaction (PCR).

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 \textit{BTK} cDNA, GenBank accession no. ENSG00000168610 for \textit{STAT3} cDNA; GenBank accession no. ENST00000291582 for \textit{AIRE} 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).

**Restriction fragment length polymorphism (RFLP)**

The presence of the c.994C>T mutations of \textit{STAT3} in family members and in healthy individuals were analyzed by using the Mph11031 restriction enzyme (Fermentas, Rockford, IL). Exon 9 of the \textit{STAT3} gene was amplified in 230 bp products. In homozygous wild type samples, digestion with Mph11031 yielded 130 bp and 100 bp restriction fragments. However, due to the c.994C>T heterozygous mutation, genomic DNA was not digested by Mph1103.
Antibody assays

Antibodies against IFN-ω was assayed by radio-immunoprecipitation, based on the antigens synthesised by in vitro transcription and translation as previously described by Oftedal et al.

In vitro cytokine assays

Peripheral blood mononuclear cells (PBMC) were isolated from K-EDTA-anticoagulated blood by using Ficoll-Paque PLUS (GE HealthCare, Uppsala, Sweden) and density gradient centrifugation. Cells were cultured at 5x10^6/ml density in AIMV medium in 96-well round-bottom plates and treated with 200 ng/ml lipopolysaccharide (LPS) from *Escherichia coli* (Sigma-Aldrich, St. Louis, MO), 2.5 µg/ml of the TLR7/8 agonist CL075 (Invivogen, San Diego, CA), or 40 ng/ml interleukin (IL)-6 (PeproTech, Rocky Hill, NJ). After 48 hours of incubation at 37 °C in humidified atmosphere containing 5% CO₂, supernatants were harvested and cytokine levels were determined with a sandwich enzyme-linked immunosorbent assay (ELISA).
RESULTS

Types and location of BTK mutations

In a cohort of 122 XLA patients from 15 referral centers, we identified 98 different BTK mutations, 46 of which were previously unknown. Diverse mutations, including missense or nonsense base substitutions, splicing mutations, large and small deletions and insertions were detected. 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%). We also identified four large deletions affecting at least two domains of BTK and one large deletion affecting exon 1. No missense mutation was detected in the SH3 domain.

Novel mutations in BTK

We defined 46 new mutations, by comparison with the online BTK database www.bioinf.uta../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%). The new mutations identified included missense (17), splicing (11), frameshift (9) and nonsense (7) mutations, and two large deletions.

Recurrent mutations in BTK

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

Demographics of patients with XLA and BTK mutations in ECE countries

Based on the data for nine countries (excluding Russia), we estimate that XLA may affect at least 1 in 1,399,000 individuals, with significant differences in prevalence between countries. These data are likely 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.
Mutations in STAT3

We have sequenced the entire coding region of STAT3 of 12 HIES patients and found five heterozygous mutations, four in the DNA binding domain (H332Y, R382W, K370fs, and H410Y), and one in the SH2 domain (V637M).

The novel heterozygous mutation (c.994C>T) at amino acid position 332 was found in a Gypsy family from Hungary. The mutation was located in exon 9 and within the DNA binding domain of STAT3. In the family the affected female and both of her sons carried a nucleotide change from CAT to TAT that caused amino acid change from histidine (H) to tyrosine (Y).

To exclude the possibility of polymorphism, and to reveal the pathological relevance of the c.994C>T mutation, RFLP analysis of the STAT3 gene was performed. This mutation was not present in 150 controls (25 Hungarian, 25 Gypsy, 50 Lebanese and 50 Swedish). These data together with the absence of mutation in the parents without disease manifestations suggested that these genetic changes were disease-associated.

Concentrations of TNF-α were 2-3 fold higher in the supernatant of LPS stimulated PBMCs from the two children with the novel H332Y mutation, than in supernatants of control cells from a 5-year-old healthy boy. These findings were in concert with previous data showing that leukocytes of patients from HIES produce a higher amount of TNF-α than those from healthy individuals when stimulated with the TLR4 receptor agonist, LPS. In addition, we found that TNF-α were also elevated in supernatants of HIES children’s cells when stimulated with the TLR-7/8 receptor agonist CLO75 compared to control cells. In contrast to results with TLR4 and TLR7/8 stimulation, IL-6 induced a negligible secretion of MCP-1 protein by PBMCs from HIES patients than by cells from controls suggesting an impaired IL-6-mediated signaling in HIES. These data further indicated that the H332Y mutation was disease causing in affected members of the Gypsy family showing characteristics of multisystemic HIES phenotype.

Mutations in AIRE

All APS1 patients carried one or two alleles with the major Finnish mutation c.769C>T (p.R257X). In the heterozygotes, the second mutation was either c.44_66dup23bp (p.R15fsX19), c.965_977del13bp (p.L323fsX373), or c.1344delC (p.C449fsX479). The latter
has not been reported previously and was present in two patients. The single nucleotide C deletion was localized to exon 11 and resulted in frameshift in the *AIRE* mRNA.

*Antibodies to IFN-ω*

Titers of antibodies to IFN-ω were 15-20 folds higher in the serum of genetically defined APS1 patients than in serum of healthy carriers or of those with multi-organ autoimmune diseases. Repetitive measurement of autoantibodies in Patient B1 unveiled a marked elevation of antibodies to type I IFN-ω between ages of 6 weeks and 7 months. This patient has remained clinically healthy at 2 years of age.
DISCUSSION

PIDS have recently been recognized as a global public health problem affecting at least 1 in every 250 individuals (Casanova, 2007). However, patients with these conditions remain largely neglected, particularly in countries with poor socioeconomic conditions in this research. We have focused in particular on XLA, the prototype immunodeficiency disorder, because this condition is reasonably easy to diagnose on clinical and immunological bases and to treat with regular immunoglobulin infusions. We provide here 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 slow number of circulating CD19+ B cells and low or undetectable concentrations of serum immunoglobulin isotypes. In addition, all but the p.F10Y and p.A367E missense mutations described here affected conserved amino acids (Lindvall, 2005). The cosegregation of missense mutations in 7 of the 17 families provided further confirmation that these missense mutations were involved in disease. In this study, we aimed to estimate the prevalence of XLA in ECE countries (excluding Russia) 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. 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 also indicate that awareness of XLA is greater in these countries than in Eastern Europe and the USA. 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.

We described here 4 recurrent and one novel mutation in the \( STAT3 \) gene causing AD and sporadic cases of HIES in eight families from different ethnic groups. The disease-causing role of the novel H332Y mutation was supported by the lack of mutated alleles in
healthy controls and family members without HIES phenotype, and the impaired IL-6-induced release of MCP-1 in affected individuals. The H332Y mutation is predicted to damage DNA binding according to bioinformatics analysis with sift and polyphen (Ramensky, 2002). The essential importance of His 332 (as well as Arg 382) in the DNA binding of STAT3 (and STAT1), where both residues are in close contact with the phosphates of DNA is clearly demonstrated in previous studies on the three-dimensional structure of STAT3 and STAT1 bound to DNA (Becker, 1998; Chen, 1998). The three other (R382W, K370fs, and H410Y) mutations have the same deleterious effects on the DNA binding of STAT3 and, as a result, on the disease development. It seems that all STAT3 mutation carriers showed HIES clinical features often including skeletal/dental abnormalities, which is consistent with the heterozygous mode inheritance. HIES is a complex multisystem disorder and other genetic or nongenetic factors are likely involved in the disease development. Furthermore, identified mutations have a potential role in a panel development for clinical use, aiding diagnosis (for example prenatal diagnosis and genetic counseling) and ultimately even for individualizing therapy based on genetic variation profiles.

APS1 is characterized by three major disease manifestations -hypoparathyroidism, adrenal failure, and mucocutaneous candidiasis- and patients usually present with at least 2 or 3 of these clinical diseases before the age of 20 (Leonard, 1946; Perheentupa, 2006; Huseby, 2009; Mathis, 2007). However, patients may present with an incomplete clinical phenotype even beyond the first decade of life as found in our cohort. In such cases identification of a family member with typical syndrome and family screening for AIRE mutation allows the extension of the diagnosis to affected patients with incomplete or atypical presentation. For example hypoparathyroidism occurring in the younger patient was considered to be the presenting manifestation of APS1 as full blown disease was observed in his brother and genetic screening in the family revealed disease-causing AIRE mutations. In addition to family screening and sequencing AIRE, analysis of serum antibodies to IFN-ω is a valuable diagnostic tool to identify patients with APS1 as it is present in 100% of patients as found in this research and elsewhere. A number of patients also exhibit other autoimmune manifestations including hepatitis, thyroiditis, alopecia, celiac disease, and short stature. These secondary features differ widely from patient to patient even within one family despite carrying the same genetic lesion which suggests a role of environmental factors and stochastic events, or other disease modifying conditions as seen in our cohort.

APS1 has a high prevalence in certain genetically isolated populations like Iranian Jews (1:9,000), Finns (1:25,000), and Sardinians (1:14,400) (Zlotogora, 1992; The Finnish-
German APECED Consortium, 1997, Husebye 2009). In other populations such as in Norway and Ireland, prevalences around 1:100,000 has been reported (Dominguez, 2006; Wolff, 2007). In Hungary the prevalence of APS1 is unknown and only sporadic cases have been reported before (Cihakova, 2001). We present seven patients with clinical phenotypes and genotypes of APS1 representing a frequency of 1:1,148,714, indicating that more patients have yet to be diagnosed. It is unclear whether patients with APS1 remain under-diagnosed or under-reported and our data suggest that suspicion should be high and all suspected cases must be screened by determination of IFN-ω antibodies and sequencing AIRE. APS1 is a difficult diagnosis to make only on the basis of clinical criteria. Overlapping features between APS1 and other multi-organ autoimmune diseases like those described in this thesis make clinical diagnosis especially difficult underpinning the importance of serologic markers and genetic analysis in the diagnosis of this disease.

In our cohort we detected three recurrent and one novel mutations of AIRE. Intriguingly, the frequency of the p.R257X mutation was 75% which is close to the frequency of this sequence variant in the Finnish patient population (82%) and much higher than that found in non-Finnish patients in Great Britain, North America, and Northern Italy (Björses, 2000, Pearce, 1998; Wang, 1998; Heino, 1999). In contrast, p.L323fsX373 the most prevalent mutation found among British, Irish, North American, and Norwegian patients with APS1 was detected only in one patient of our cohort (Scott, 1998).

The novel p.C449fsX479 mutation in association with the p.R257X sequence variant was found in two brothers and IFN-ω was high in sera of affected individuals. This deletion mutation of a single C nucleotide causes frameshift in the AIRE mRNA probably leading to the synthesis of inactive protein because of the likely loss of function of the PHD domain responsible for the transcription activation (Aasland, 1995). A similar but not identical mutation at position c.1344 of the AIRE cDNA was published before (Sato, 2004). In this latter report a single C deletion occurred together with insertion of TT which resulted in frameshift at codon 449 and premature truncation at codon 502. The insertion-deletion mutation destroyed the second PHD-type zinc finger domain and eliminated the carboxyl terminus including the third LXXLL motif.

To our knowledge patient B1 is the youngest patient ever verified with APS1. To make the diagnosis we applied early genetic analysis and measurement of anti-IFN-ω antibodies. We propose that anti-IFN-ω antibodies may be elevated in early infancy and childhood as a heralding marker of APS1 before the occurrence of clinical manifestations.
allowing early diagnosis and disease monitoring. The high frequency of Finnish mutation in the Hungarian patient population with APS1 may indicate population genetic relationship.
CONCLUSIONS

1. We identified disease-causing mutations in 143 patients with primary immunodeficiency.

2. The BTK gene mutations may be present in the entire sequence in XLA patients from 10 Eastern and Central European countries.

3. We expanded the existing mutation database with 46 novel BTK mutations.

4. The prevalence of XLA in Eastern and Central European countries (total population 145,530,870) was found to be 1 per 1,399,000 individuals.

5. Dominant negative mutations of the DNA-binding and SH2 domains of STAT3 cause AD and sporadic cases of HIES in different ethnic groups.

6. Functional and genetic data support that the novel H332Y mutation may result in the loss of function of STAT3 and may lead to the hyper-IgE syndrome phenotype.

7. We identified homozygous and heterozygous AIRE mutations in 9 APS1 patients. We have detected a previously unknown sequence variant of AIRE in two patients.

8. Anti-IFN-ω antibodies may be elevated in early infancy as heralding markers of APS1, before the occurrence of clinical manifestations, allowing early diagnosis and disease monitoring.

9. The high frequency of the Finnish mutation in a Hungarian patient population with APS1 may indicate population genetic relationship.
Clinical manifestations of primer immunodeficiencies usually occur in infancy and early childhood. In many cases confirmation of the diagnosis is now possible by molecular genetic testing. The first clinically identified immunodeficiency is XLA, which is caused by mutation of the Bruton tyrosine kinase (BTK). For the development of HIES and APS1 the STAT3 and AIRE gene defects, respectively, are responsible.

In my research the molecular genetic background of XLA, HIES and APS1 were studied. The nucleotide sequence of BTK, STAT3, and AIRE responsible for the diseases were identified by bidirectional DNA sequencing. The inheritance of the disease was examined by screening of family members; the pathogenic role of the new, previously unknown mutations was proved by restriction fragment analysis and functional tests.

Samples were collected from XLA patients from 10 countries of Eastern and Central Europe. 98 different BTK mutations including 46 earlier unknown, novel sequence variant were identified by genomic sequencing. The results showed that BTK mutations in Eastern and Central Europe were similar to those found in other countries and continents.

Three known and 1 new mutations were identified on the STAT3 gene in patients from Hungary and Eastern and Central Europe. The pathogenic role of the new STAT3 mutation (H332Y) was confirmed by the lack of this genetic variation in the control population; cells from patients with this mutation showed decreased IL-6 responsiveness and increased TNF-α production to TLR7/8 stimulation.

In 9 Hungarian patients with APS1, AIRE mutations (3 known and 1 new) were responsible for the disease. The titer of anti-IFNo, the earliest detectable and specific finding in this disease, was increased patients. Genetic examination of family members and occurrence of APS1 in the family, the disease was diagnosed well before the appearance of clinical symptoms.
LIST OF PUBLICATIONS

Candidate: Beáta Tóth
Neptun ID: CUS experts
Doctoral School: Doctoral School of Clinical Immunology and Allergology

List of publications related to the dissertation

DOI: http://dx.doi.org/10.1111/j.1365-2265.2009.03740.x
IF: 3.201 (2009)

DOI: http://dx.doi.org/10.1016/j.molimm.2009.03.012
IF: 3.202

DOI: http://dx.doi.org/10.1016/j.molimm.2008.07.007
IF: 3.555

Impact factor: 9.958
DOI: http://dx.doi.org/10.1016/j.molimm.2010.11.013
IF: 3.202 (2009)

DOI: http://dx.doi.org/10.1556/Oh.2010.28795

DOI: http://dx.doi.org/10.1556/Oh.2010.28796

DOI: http://dx.doi.org/10.1556/Oh.2010.28851

8. Erdős, M., Lajszné Tóth, B., Veres, I., Kiss, M., Remenyik, E., Maródi, L.: Nijmegen breakage syndrome complicated with primary cutaneous tuberculosis
DOI: http://dx.doi.org/10.1097/INF.0b013e3181faa941
IF: 2.854 (2009)

   DOI: http://dx.doi.org/10.1016/j.juro.2008.11.091 
   IF: 4.016

11. Erdős M., Lajszné Tóth B., Almássy Z., Tímár L., Maródi L.: Porc-haj hypoplasia. 
   DOI: http://dx.doi.org/10.1556/OH.2008.28256

   Focus Med. 10 (4), 14-16, 2008.

   DOI: http://dx.doi.org/10.1016/j.clim.2008.08.005 
   IF: 3.606

   DOI: http://dx.doi.org/10.1016/j.ymgme.2008.09.002 
   IF: 2.629

   DOI: http://dx.doi.org/10.1111/j.1600-0609.2009.01388.x 
   IF: 2.237


Impact factor: 22.706
Total impact factor: 32.664
Presentations in English

1. The 49th J-Project Meeting Future Strategies in the Management of the Primary Immundeficiencies
   Gene expression investigations for the identification of the ethiopathogenetical role of missense mutations.
   Oradea, Romania, 2010.

2. The International Immunocompromised Host Society
   Dectin-1-mediated immunity is redundant for host defense against mucocutaneous candidiasis

Presentations in Hungarian

1. Magyar Immunológiai Társaság XXXIX. Vándorgyűlése
   AIRE gén szekvencia variánsok autoimmun poliglanduláris szindrómában.
   Szeged, 2010.

2. Magyar Gyermekgyógyászati Társaság és a Magyar Gyermekgyógyász Társaság
   Gyermekimmunológiai Munkacsoport 2010. évi ülése
   AIRE gén szekvencia variánsok autoimmun poliglanduláris szindrómában.
   Tolna, 2010.

3. Magyar Gyermekorvosok Társaság 2010.évi Nagygyűlése
   AIRE gén szekvencia variánsok autoimmun poliglanduláris szindrómában.
   Esztergom, 2010.

4. Magyar Humángenetikai Társaság VIII. Kongresszusa
   AIRE gén szekvencia variánsok autoimmun poliglanduláris szindrómában.

5. Magyar Humángenetikai Társaság VIII. Kongresszusa
   Mutáció analízis és génexpressziós vizsgálatok Niemann-Pick „A” szindrómában.

6. Magyar Dermatológiai Társaság Továbbképző Napok
   Primer immundefektusok bőrgyógyászati manifesztáció
   Budapest, 2010.

7. Magyar Gyermekgyógyászati Társaság és a Magyar Gyermekgyógyász Társaság
   Gyermekimmunológiai Munkacsoport 2009. évi ülése
   Congenitalis neutropenia molekuláris patológiája.
   Budapest, 2009.

8. Magyar Gyermekgyógyászati Társaság és a Magyar Gyermekgyógyász Társaság
   Gyermekimmunológiai Munkacsoport 2009. évi ülése
   STAT3 szekvencia variánsok hyper-IgE szindrómás betegekben.
   Budapest, 2008.
10. Erdélyi Múzeum-Egyesület Orvos- és Gyógyszerésztudományi Szakosztály XVIII. tudományos Ülésszaka
Az X-kromoszómához kötött agammaglobulinemia (XLA) molekuláris patológiája.
Nagyvárad, 2008.

11. Magyar Gyermekorvosok Társaság 2008. évi Nagygyűlése
Az X-kromoszómához kötött agammaglobulinemia (XLA) molekuláris patológiája.
Szeged, 2008.

12. The J-project Meeting in Debrecen
Genetic analysis of BTK in countries patients with X-linked agamaglobulinemia

Poster presentation in English

1. XIV. Meeting of the European Societies for Immunodeficiencies
   Beáta Tóth, Péter Gogolák, Szilvia Taskó, Alexandra Bársny, László Maródi
   Dectin-1-mediated immunity is redundant for host defense against mucocutaneous candidiasis.
   Istanbul, Turkey, 2010.

2. The International Immunocompromised Host Society
   Beáta Tóth, Péter Gogolák, Szilvia Taskó, Alexandra Bársny, László Maródi
   Dectin-1-mediated immunity is redundant for host defense against mucocutaneous candidiasis.

3. The International Immunocompromised Host Society
   Melinda Erdős, Beáta Tóth, Imre Veres, Mária Kiss, Éva Remenyik, László Maródi
   Nijmegen breakage syndrome complicated with primary cutaneous tuberculosis.

4. XIV. Meeting of the European Societies for Immunodeficiencies
   Melinda Erdős, Beáta Tóth, Imre Veres, Mária Kiss, Éva Remenyik, László Maródi
   Nijmegen breakage syndrome complicated with primary cutaneous tuberculosis.
   Istanbul, Turkey, 2010.

5. XIII. Meeting of the European Societies for Immunodeficiencies
   Melinda Erdős, Miklós Garami, Éva Rákóczi, Attila Zalatnai, Daniel Steinbach, Ulrich Baumann, Gabrielle Kropshofer, Beáta Tóth, László Maródi
   Neuroendocrine carcinoma associated with X-linked hyper-immunglobulin M syndrome.
   Hertogenbosch, Netherlands, 2008

6. XIII. Meeting of the European Societies for Immunodeficiencies
   Hong Jiao, Beáta Tóth, Melinda Erdős, Ingegerd Fransson, Éva Rákóczi, István Balogh, Zoltán Magyaries, Beáta Déřfalvi, Gabriella Csorba, Anna Szaflikaska, Andre
Megarbane, Carlo Akatcherian, Ghassan Dbaibo, Éva Rajnavölgyi, Lennart Hammarström, Juha Kere, Gérard Lefranc, László Maródi
Novel and recurrent \textit{STAT3} mutations in hyper-IgE syndrome patients from different ethnic groups.
Hertogenbosch, Netherlands, 2008

\textbf{Poster presentation in Hungarian}

1. Magyar Gyermekorvosok Társaság 2010.évi Nagygyűlése
Reiger Zsolt, Varga Gergely, Tóth Beáta, Maródi László, Erdős Melinda
Felnőtt korban diagnosztizált dysceratosis congenita
Esztergom, 2010.
ACKNOWLEDGEMENTS

I am grateful to my supervisor, Prof. Dr. László Maródi for his professional advice, scientific guidance, and support.

I would like to express my thanks to Prof. Dr. János Sümegi, Prof. Anette Wolff, Prof. Anne Puell, Prof. Éva Rajnavölgyi, Dr. István Balogh, Dr. Melinda Erdős, Dr. Zoltán Magyarics, Szilvia Taskó for their help during my research.

I am grateful to co-authors for their research collaboration and helpful discussion.

I thank my colleagues at the Department of Infectious and Pediatric Immunology for the relaxed and stimulating environment they provided to my research.

I thank the patients and their families for understanding, and helpful collaboration.

I am most grateful to my family for their help and support.
REFERENCES


