SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

Molecular pathology of Candidiasis and Epstein-Barr infection in primary immunodeficiency

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The Examination takes place at the Lecture Hall of Building "C", Department of Internal Medicine, Faculty of Medicine, University of Debrecen at 11 a.m. on 18 April 2017.

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The PhD Defense takes place at the Lecture Hall of Building "A", Department of Internal Medicine, Faculty of Medicine, University of Debrecen at 1 p.m. on 18 April 2017.

1 INTRODUCTION

The immune system provides the appropriate protection of our body against pathogens (bacteria, viruses, fungi, parasites). After infection by viruses or fungi, the pathogens bind to the pattern recognition receptors (PRRs), and then the cells of innate immune system are activated forming effector cells of immune system and these cells eliminate the viruses and fungi (Mogensen, 2009; Swain, 2012).

Inborn error in the differentiation of naive CD4 + cells to different effector T cells leads to impaired or lack of immunity against pathogens, viruses or fungi and greater susceptibility to different diseases. Inborn errors of IL-17-mediated immunity lead to development of candidiasis (Puel, 2010; 2011; 2012).

Cytotoxic CD8+ T cells are formed in the presence of cytokines (IL-2) and then migrate to the infected areas where eliminate the virus infected cells (Swain, 2012). EBV-specific natural killer cells (NK), CD4 + and CD8 + T cells inhibit the proliferation of EBV-infected lymphocytes. After EBV infection, the natural killer cells (NK) are not able to remove the uncontrollably dividing B lymphocyte cells in patients with X-linked lymphoproliferative disease. IgG isotype switching is impaired, therefore the humoral immune response against EBV is not developed and the cytotoxic CD8 + T cells may lose their function (Seemayer, 1995; Maródi and Notarangelo, 2007).

In my research work, I analysed the molecular genetic background and pathomechanisms of two primary immunodeficiency diseases belong to the T cell defects, in the broader sense; the chronic mucocutaneous candidiasis (CMC) due to the *STAT1* gain of function (GOF) and the X-linked lymphoproliferative syndrome (XLP).

The chronic mucocutaneous candidiasis is characterized by persistent and recurrent fungal infections of various *Candida* species of the skin, mucous membranes and nails, especially *Candida albicans* (Maródi, 1997; Puel 2012; Soltesz, 2013). The CMC is sometimes the only phenotype in patients with isolated chronic mucocutaneous candidiasis disease (CMCD), the *STAT1* GOF mutations belong to this group (Puel, 2012). The susceptibility to *Candida* infections may due to the reduced IL-17-mediated immunity (Maródi, 2012; Puel, 2012; Cypowyj, 2012). The STAT1 molecule plays an important role in cytokine signaling pathway affecting the IL-17 + T cell differentiation, the IFN-α/β, IFN-γ and IL-27 cytokines inhibit the development of IL-17 + T cells from naive T lymphocytes

through the STAT1-dependent signaling pathways. The IL-17 + T cells and the cytokines secreted by cells have an important role in immunity against the fungus (Liu 2011; Maródi, 2012; Maródi, 2013; Soltesz, 2013).

The X-linked lymphoproliferative syndrome is associated with fatal infectious mononucleosis (FIM) due to the EBV infection or secondary, haemophagocytic lymphohistiocytosis (HLH) is developed. Seventy-five percent of patients with XLP1 die before the age of 10 years; the chance of survival is 4%, so it is very important to recognize the disease in time (Seemayer, 1995). The mutations in SAP protein coding *SH2D1A* gene are responsible for the development of XLP1, even mutations localised in the promoter and intron of the gene may also cause the XLP disease (Recher, 2013). The cytokine secretion in CD4 + T cells is controlled by SAP and these are necessary for CD8 + T- and NK cells-mediated cytotoxic removal of EBV-infected cells (Latour, 2003).

2 AIMS OF STUDY

1. To analyse the molecular genetic features of patients with chronic mucocutaneous candidiasis and their relatives.

2. To verify the pathogenicity of novel *STAT1* sequence variants, and to analyse the consequences in protein function of novel *STAT1* mutations.

3. To determine the in vitro differentiation of CD4+IL-17+ and CD4+IL-22+ T cells.

4. To measure the anti-*Candida* cytokine responses of patients with chronic mucocutaneous candidiasis.

5. To study the molecular genetic features of patients with X-linked lymphoproliferative disease and their relatives.

6. To verify the pathogenicity of novel, intronic *SH2D1A* mutation.

3 MATERIALS AND METHODS

All the studies were approved by the Regional Ethics Committee of the Faculty of Medicine, University of Debrecen. Informed consent was obtained from the patients or their relatives. Molecular genetic and biology examinations were allowed by Eastern- and Central-European countries.

3.1 Patients

Patients with chronic mucocutaneous candidiasis

We studied the mutational analysis of *STAT1* gene in nine patients (P1-P9) with CMC from Czech Republic (3 patients), Hungary (2 patients), Russia Federation (2 patients) and Ukraine (2 patients).The chronic mucocutaneous candidiasis diagnosis was based on the clinical and immunological results.

Patients with X-linked lymphoproliferative disease

We analysed the *SH2D1A* gene in Hungarian siblings. The XLP diagnosis was based on the immunological and clinical results.

The healthy controls were symptomless persons.

3.2 Routine laboratory examinations

Taking blood samples were performed under aseptic conditions. Clinical chemistry and immunology parameters were examined from freshly taken blood samples by routine laboratory tests.

3.3 DNA isolation

From EDTA anti-coagulated peripheral blood samples, the genomic DNA was isolated by Gen ELUTE Blood Genomic DNA mini kit (Sigma-Aldrich Ltd., St. Louis, Missouri, USA) according to manufacturer's instructions.

3.4 Amplification polymerase chain reaction

Protein coding exons and exon-intron boundaries of *STAT1* and *SH2D1A* genes were amplified by polymerase chain reaction using specific intronic primers.

3.5 Sequencing PCR

During the sequencing PCR, one of the primer pair was added to the amplified double-stranded DNA to amplify single stranded DNA. Bidirectional sequencing was performed by using the Big Dye Terminator Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI 3130 capillary DNA sequencer (Applied Biosystems).

3.6 Analysis of sequencing data

Sequence variants were determined compare to reference sequence, GenBank accession no. Ensembl ENSG00000115415 of the *STAT1*and Ensembl ENSG00000183918 of *SH2D1A* to identify the position of mutations. Mutations are nominated according to Dunnen and Antonarakis, 2001. The first (c.1) position corresponds to the A of the ATG translation initiation codon. We compared the mutations with the known mutations in the Human Gene Mutation Database Professional (HGMD).

3.7 Preparation of cDNA and real-time PCR

Total RNA was isolated from peripheral blood leukocytes using Trizol reagent (Invitrogen Life Technology, Carlsbad, California, USA). Superscript III first-strand synthesis supermix (Invitrogen) and random hexamer primers (Applied Biosystems) were used for reverse transcription reaction (RT-PCR). Quantitative real-time PCR was performed with TaqMan assay (Hs00158978_m1; Applied Biosystems) in triplicates.

3.8 Isolation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation (Ficoll-Paque PLUS, GE Healthcare Bio-Science AB, Uppsala, Sweden) from the heparinised blood samples of patients and healthy controls.

3.9 Generation of Epstein-Barr-transformed lymphoblasts

PBMCs were resuspended in RPMI supplemented with FBS, and cyclosporin A (CyA). They were then infected with the supernatant of B95–8 cells infected with EBV and incubated them at 37°C under an atmosphere containing 5% CO_2 for 3 weeks.

3.10 Preparation of *Candida* suspension and stimulation of PBMCs by *Candida*

Candida albicans (ATCC 10231) was maintained on Sabouraud dextrose agar, then transferred into Dulbecco's modified Eagle's Medium (DMEM; Sigma-Aldrich) supplemented with penicillin and streptomycin. Heat-inactivated yeast was prepared and then the density of *Candida* was adjusted to 1×10^6/ml with a McFarland densitometer.

After washing in Krebs-Ringer phosphate buffer, the cells were resuspended in DMEM. PBMCs were incubated either alone or in the presence of heat-killed *C albicans* and the supernatants were collected.

3.11 Concentration of secreted cytokines was determined by enzyme-linked immunosorbent assays

The concentrations of IFN-γ, IL-1β, IL-6, IL-17A and IL-22 in the supernatants of *Candida*-exposed PBMCs were determined by sandwich enzyme-linked immunosorbent assays (ELISA; Quantikine, R&D Systems, Minnesota, USA).

3.12 Luciferase reporter assay

U3C cells (STAT1-deficient fibrosarcoma cell line) were transfected with reporter plasmids (Cignal GAS Reporter Assay kit, SA Biosciences) and plasmids carrying the wt, c.537C>A,c.821G>A or c.854A>G alleles of *STAT1* or a mock vector, in the presence of Lipofectamine LTX (Invitrogen). The transfected cells were then stimulated by IFN-γ and analysed by Dual-Glo Luciferase assay system (Promega, Madison, Wisconsin, USA). This experiment was performed in collaboration with Professor Jean-Laurent Casanova, MD, PhD.

3.13 Production of IP-10 by ELISA

The EBV-transformed cells were stimulated with IFN-γ then the release of IP-10 was determined by ELISA (Quantikine, R&D Systems).

3.14 Differentiation ofIL-17+ T cells

Peripheral mononuclear cells from patients and healthy controls were resuspended in RPMI supplemented with 10% FBS and 1% Pen-Strep and then non-adherent blood cells were cultured in anti-CD3 antibody-coated plates (Miltenyi Biotec, Bergisch Gladbach, Germany, Eu) in the presence of different cytokines. After five days, the cells were stimulated

with phorbol 12-myristate 13-acetate (PMA; Sigma- Aldrich) and ionomycin (IMC; Sigma-Aldrich) in the presence of GolgiPlug (Sigma-Aldrich) for flow cytometry analysis.

3.15 Analysis of STAT1 protein by Western blot

EBV-B cells were stimulated with IFN-γ, IFN-α or IL-27 and the nuclear extraction was extracted by Subcellular Protein Fractionation Kit for Cultured Cells (Thermo Scientific) according to the manufacturer's instructions. The EBV-B cells were stimulated with IFN-γ, and then incubated with staurosporine (Sigma-Aldrich). The effect of the phosphatase inhibitor, pervanadate on IFN-γ-induced STAT1 phosphorylation was assessed by incubating the EBV-transformed B cells. The nuclear proteins were extracted and subjected to immunoblot analysis. STAT1 rabbit IgG antibody (Santa-Cruz), phosphorylated human STAT1 (pY701) mouse IgG_1 antibody (BD Biosciencies), Lamin B1 mouse IgG_1 antibody (Santa-Cruz) were used as first antibody, and then the horseradish peroxidise-labelled goat anti-rabbit IgG or horseradish peroxidise-labelled sheep anti-mouse IgG (Sigma-Aldrich) were added as secondary antibody.

3.16 Detection of SAP proteins by Western blot

Mononuclear cells were incubated in the presence or absence of phytohaemagglutinin (Life Technologies, Carlsbad, CA, USA) in RPMI. SAP-specific affinity purified rabbit serum was used as first antibody, and then horseradish peroxidise-conjugated donkey anti-rabbit Ig antibody (GE Healthcare, Little Chalfont, United Kingdom) was added to reaction. The bands were visualized by enhanced chemiluminescence. Actin was used as a loading control. This experiment was performed in collaboration with Árpád Lányi, PhD.

3.17 Determination of IL-17+ T cells by flow cytometry

For flow cytometry analysis, the cells were incubated for the surface labelling with allophycocianin (APC)-conjugated anti-hCD4 IgG_1 monoclonal antibody (mAb) (BD, San Jose, CA, USA) and fluorescein isothiocyanate (FITC)-conjugated anti-hCD3 IgG_1 monoclonal antibody (BD) And then the cells were stained with phycoerythrin (PE)-conjugated mouse anti-human IL-17A IgG_1 mAb (R&D Systems, Minneapolis, MN, USA) and fluorescein isothiocyanate (FITC)-conjugated anti-human IL-22 IgG_1 mAb (R&D Systems) antibodies. Cells were analysed with an Accuri C6 flow cytometer (BD).

3.18 Detection of SAP protein expression by flow cytometry

The cells were incubated for the surface staining with peridinin chlorophyll protein-conjugated mouse anti-CD3 (BD), phycoerythrin-conjugated mouse anti-CD8 (Sigma Aldrich) and allophycocyanin-conjugated mouse anti-CD56 (BD). After fixation and permeabilization steps, the cells were incubated with purified rat immunoglobulin G_1 (IgG_1) isotypic control (BD) or rat anti-SH2D1A unconjugated monoclonal antibody (clone: KST-3).The cells were analysed by FACS Calibur flow cytometer (BD Biosciences). This experiment was performed in collaboration with Regional Immunology Laboratory of 3rd Internal Medicine, University of Debrecen.

3.19 Analysis of phosphorylated-STAT1 by flow cytometry

EBV-transformed lymphocytes were incubated with IFN-γ and then staurosporine. Anti-pSTAT1 antibody (pY701, BD) was used as first antibody and for secondary antibody Alexa Fluor 488-conjugated anti-mouse IgG (Cell Signaling) was added. Cells were analysed with an Accuri C6 flow cytometer (BD).

4 RESULTS

4.1 IL-17-mediated immunity and chronic mucocutaneous candidiasis

4.1.1 Molecular genetic analysis of *STAT1* gene

We analysed the nucleotide sequence of *STAT1* gene in patients with suspicion of CMCD by Sanger sequencing. We identified heterozygous, disease-causing mutations in nine patients with CMCD affected the coiled-coil domain (CCD) and DNA-binding domain (DBD) of STAT1.

We analysed seven previously known and two novel mutations (P1, P7). The results in case of P4, P5 (Tóth, 2012) and P9 patients (Liu, 2011) were earlier submitted. The identified heterozygous cytosine-adenine mutation in P1 led to asparagine-lysine amino acid change at position 179 within coiled-coil domain of STAT1. We determined a novel mutation in P7 patient, the adenine-guanine sequence variant led to glutamine-arginine change at position 285 affected the coiled-coil domain of STAT1.

4.1.2 Verification of pathogenicity of novel*STAT1* sequence variants

We verified the pathogenicity of the identified two novel mutations (N179K, Q285R). These mutations were not found in mutational databases: NCBI SNP (https://www.ncbi.nlm.nih.gov/snp) and ExAc (Exome Aggregation Consortium; http://exac.broadinstitute.org/gene/ENSG00000115415). The N179K and Q285R amino acid changes were predicted as “benign” by PolyPhen-2 software (Adzhubei, 2010; http://genetics.bwh.harvard.edu/pph2/). The N179 and Q285 positions were highly conserved.

4.1.3 The effect of N179K and Q285R mutations for STAT1 protein function (Luciferase assay)

We analysed the γ-activated factor (GAF)-dependent cellular responses to IFN-γ of N179K and Q285R mutant STAT1 protein by luciferase assay. We studied the cellular responses by measuring the luciferase activity of the reporter gene under the control of the γ-activated sequence (GAS) promoter. The responses were two to three times stronger in cells transfected with the two new mutant alleles or with the CMCD-causing R274Q allele (positive control) than in those transfected with WT or MSMD-causing Y701C alleles (Ives, 2013; Hirata, 2013). These data suggested the mutant STAT1 alleles were gain-of-function

mutations. This experiment was performed in collaboration with Professor Jean-Laurent Casanova, MD, PhD.

4.1.4 Production of IP-10 in CMCD patients

The production of IFN-γ-induced IP-10 was higher from EBV-transformed B lymphocytes of P4 (R274W), P5 (R274W) and P8 (T385M) patients than in healthy control's cells. The data suggested the mutations were gain-of-function because the IP-10 is the downstream target of STAT1. Higher IP-10 secretion was confirmed in cells of CMCD patients with *STAT1* GOF mutations (Takezaki, 2012).

4.1.5 Dephosphorylation and phosphorylation of STAT1 in CMCD patients by Western blot

The EBV-transformed lymphocytes (P4 and P8) were activated by different cytokines (IFN-γ, IFN-α and IL-27, these activate the STAT1 protein; Liu, 2011) and the amount of expressed STAT1 and phosphorylated STAT1 (pSTAT1) proteins were analysed. Stronger STAT1 phosphorylation in the nuclear extracts of cells carrying R274W and T385M alleles was determined, suggesting that the dominant phenotype of gain of STAT1 phosphorylation.

The treatment with staurosporine, as tyrosine-kinase inhibitor showed that the dephosphorylation of IFN-γ-treated EBV-B cells heterozygous for the STAT1 T385M or R274W alleles was impaired.

After phosphatase inhibitor, pervanadate treatment, the phosphorylation of T385M STAT1 and R274W STAT1 was similar to that of WT STAT1.

These data suggested that the T385M and R274W amino acid substitutions in STAT1 result in a gain of STAT1 phosphorylation due to a loss of dephosphorylation.

4.1.6 Detection of phosphorylated-STAT1 by flow cytometry

Higher IFN-γ- induced pSTAT1 intensity was detected in cells from CMCD patients (R274W and T385M), than in healthy controls by Accuri C6 flow cytometer. Consistent with the Western blot results, the treatment with staurosporine decreased the pSTAT1 expression in healthy controls, but the intensity of pSTAT1 did not change after staurosporine stimulation in CMCD patients. We suggested the phosphorylation was prolonged in the patients, so the dephosphorylation might be impaired.

4.1.7 Cytokine secretion by *Candida*-stimulated mononuclear cells

The release of various inflammatory cytokines was measured by freshly isolated PBMCs after stimulation with heat-killed *Candida*. Similar secretion of IFN-γ, IL-1β and IL-6 was analysed by the mononuclear cells carrying the T385M (P8) allele and control cells, whereas negligible amounts of IL-17A and IL-22 were detected by *Candida*-exposed cells from CMCD patient (P8) in contrast to healthy controls. Smaller release of IL-17A and IL-22 cytokines was measured by cells from P4 and P5 carrying the R274W allele, by contrast, the IFN-γ secretion was similar to the healthy controls. Our results suggested a normal 'Th1-type' response, however, an impaired 'Th-17-type' reaction to *Candida* in patients (P4, P5 and P8) due to the small secretion of IL-17A and IL-22 cytokines.

4.1.8 Ex vivo differentiation of CD4+IL-17+ and CD4+IL-22+ T cells

Impaired development of „IL-17-producing" and „IL-22-producing" T cells were obtained for the cells with heterozygous R274W and T385M mutant alleles in contrast to the healthy control, these data was consistent with the negligible IL-17A and IL-22 cytokine secretion.

4.2 X-linked lymphoproliferative disease

4.2.1 Molecular genetic analysis of*SH2D1A* gene

A novel mutation in intron 1 (c.137+5G>A) on the *SH2D1A* gene from genomic DNA of P10 patient were determined in hemizygous form. Sequencing of the *SH2D1A* gene revealed heterozygosity for this mutation in the mother and maternal grandmother. The father of P10 was wild-type for this sequence variant. We detected the same intronic mutation from prenatal sample of P11 in hemizygous form. The father of P11 was not available for genetic testing. Complete *SH2D1A* cDNA amplification from leukocytes of P11 patient showed a smaller band (352 bp) compared with that of the mother and healthy control (374 bp). Sequencing of the cDNA confirmed a 22 bp deletion (116_137del22bp) in exon 1 of P11 and this mutation was not detected in cDNA isolated from a healthy control and from the mother.

The c.137+5G>A nucleotide change probability may result in altered donor splice site, change of cDNA sequence and leading to a truncated SAP protein.

Real-time-PCR analysis revealed a decreased expression of *SH2D1A* relative expression in the mother and the complete lack of expression in P11.

4.2.2 Analysis of SAP expression by Western blot and flow cytometry

SAP protein could not be detected with Western blot in the P11's cells in contrast to that seen in the healthy control. SAP protein expression in the carrier mother's blast cells was decreased as indicated by the thickness of the bands. Actin was used as housekeeping protein. This experiment was performed in collaboration with Árpád Lányi, PhD.

Cytotoxic $CD8^+$ T cells (CD3+CD8+CD56-cells); natural killer (NK) cells (CD3-CD56+ cells); and CD56 T cells (CD3+CD56+ cells) cells from P11 contained negligible amounts of SAP expression, much smaller than those in the corresponding populations from a healthy control. This experiment was performed in collaboration with Regional Immunology Laboratory of 3rd Internal Medicine, University of Debrecen.

5 CONCLUSIONS

1. We identified disease-causing mutations in the Signal transducer and activator of transcription (*STAT1*) gene in nine patients with chronic mucocutaneous candidiasis.

2. We have detected two novel mutations which were previously unknown and not described in mutational databases. We proved the gain-of-function in the case of two novel mutations.

3. The identified mutations in the *STAT1* gene in two patients led to gain-of-phosphorylation and impairment in dephosphorylation.

4. The development of IL-17+ T and IL-22+ T helper cells was impaired in CMCD patients.

5. We presented the secretion of IL-17A and IL-22 was decreased in CMCD patients suggesting the responsible for the greater susceptibility to mucosal fungus infections.

6. We identified a novel intronic mutation in the *SH2D1A* gene in a Hungarian sibling and we confirmed the pathogenicity of this variant by functional tests.

7. The c.137+5G>A mutation is predicted to result in splicing defect leading to reduction of SAP protein expression. The lack of SAP is responsible for the development of X-linked lymphoproliferative disease.

6 SUMMARY

The frequent and recurrent intracellular pathogens (e.g. *Candida albicans*) and viral (e.g. Epstein-Barr virus) infections may cause the lack or impaired function of cellular immune responses (T cell deficiency) (Cooper, 2003). The IL-17+ T cell immunity is impaired due to the gain-of-function STAT1 genetic defects, leading to the reduction of host defense against fungal infections and the development of chronic mucocutaneous candidiasis disease (CMCD) (Puel, 2012). In X-linked lymphoproliferative disease (XLP) after Epstein-Barr virus infection, the natural killer cells are not able to remove uncontrollably multiplied B cells, and CD8 + T and NK cells – mediated cytotoxicity may not be developed. Both CMCD and the XLP can be classified as primary immunodeficiency diseases (Maródi and Notarangelo, 2007).

In my research work, the molecular pathological mechanisms of CMCD and XLP were examined. In both cases, the genetic alterations responsible for the development of diseases were confirmed by bi-directional DNA sequencing and the pathogenicity of mutations was proven by cell assays and various molecular biology methods.

Heterozygous missense mutations were identified on *STAT1* gene in nine patients with CMCD. Two mutations to DNA binding domain (DBD) and eight mutations were affected to the coil-coiled domain (CCD) of STAT1. Two genetic alterations were not described earlier in mutation databases, so novel sequence variants were determined. In two patients, decreased level of IL-17+ T cells after treatment of PMA (phorbol 12-myristate 13-acetate) and IMC (ionomycin) was measured by flow cytometry and negligible secretion of IL-17A and IL-22 cytokines were analysed by ELISA after stimulation of heat-killed Candida compared to healthy controls. The differentiated IL-17+ T cells treated with PMA/IMC secreted low levels of IL-17A and IL-22 cytokines in the patient carrying the novel allele compared to healthy control. Epstein-Barr virus (EBV) - transformed cell lines from CMCD patients and healthy controls were stimulated with IFN-γ and treated with tyrosine kinase inhibitor, the nuclear extract extracted from cells higher phosphorylated STAT1 protein was deteced due to the loss of dephosphorylation. Higher luciferase activity was detected after IFN-γ stimulus from U3C cell line transfected with plasmid carrying novel mutation by Luciferase reporter assay compared to wild-type plasmid which is confirmed the fact of gain-of-function.

In the present work, we identified a novel and previously not described intronic mutation in siblings with siblings; the mother and maternal grandmother were heterozygous

for this mutation. The *SH2D1A*relative expression was completely missing in the patient, and was decreased in the mother compared to healthy control. Expression of the SAP protein was not detected by Western blot or flow cytometry from the sample of patient, decreased protein expression was detected from the sample of the mother, indicating she is a carrier.

These results show the mutation occurred on *STAT1* gene has enhances the function of STAT1, leading to the inhibition of IL-17 + T cell differentiation. The low percentage of IL-17+ T cells increases the patient's susceptibility to Candida infections.

The consequence of *SH2D1A* gene mutation showed no SAP protein expression was found thereby the cytotoxicity of EBV-infected B cells by NK and CD8 + T cells was diminished contributing to the severe phenotype XLP.

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Registry number: DEENK/298/2016.PL
Subject: PhD Publikációs Lista

Candidate: Beáta Soltész
Neptun ID: KP9JNT
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List of publications related to the dissertation

1. Lajszné Tóth, B., **Soltész, B.**, Gyimesi, E., Csorba, G. É., Veres, Á., Lányi, Á., Kovács, G., Maródi, L., Erdős, M.: Severe XLP Phenotype Caused by a Novel Intronic Mutation in the SH2D1A Gene.
J. Clin. Immunol. 35 (1), 26-31, 2015.
DOI: http://dx.doi.org/10.1007/s10875-014-0117-1
IF: 3.094

2. **Soltész, B.**, Tóth, B., Sarkadi, A. K., Erdős, M., Maródi, L.: The Evolving View of IL-17-Mediated Immunity in Defense Against Mucocutaneous Candidiasis in Humans.
Int. Rev. Immunol. 34 (4), 348-363, 2015.
DOI: http://dx.doi.org/10.3109/08830185.2015.1049345
IF: 4.438

3. **Soltész, B.**, Lajszné Tóth, B., Shabashova, N., Bondarenko, A., Okada, S., Cypowyj, S., Abhyankar, A., Csorba, G. É., Taskó, S., Sarkadi, A. K., Méhes, L., Rozsíval, P., Neumann, D., Chernyshova, L., Tulassay, Z., Puel, A., Casanova, J. L., Sediva, A., Litzman, J., Maródi, L.: New and recurrent gain-of-function STAT1 mutations in patients with chronic mucocutaneous candidiasis from Eastern and Central Europe.
J. Med. Genet. 50 (9), 567-578, 2013.
DOI: http://dx.doi.org/10.1136/jmedgenet-2013-101570
IF: 5.636

List of other publications

4. Gulácsy, V., **Soltész, B.**, Petrescu, C., Bataneant, M., Gyimesi, E., Serban, M., Maródi, L., Lajszné Tóth, B.: A novel large deletion and single nucleotide insertion in the Wiskott-Aldrich syndrome protein gene.
Eur. J. Haematol. 95 (1), 93-98, 2015.
DOI: http://dx.doi.org/10.1111/ejh.12424
IF: 2.544

Total IF of journals (all publications): 15,712
Total IF of journals (publications related to the dissertation): 13,168

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

10 November, 2016

Presentations in English

1. 79th J Project Meeting
   A novel large deletion and single nucleotide insertion in the Wiskott-ALdrich syndrome protein gene
   Beáta Soltész, Alexandra Bársony, Vera Gulácsy, Carmen Petrescu, Mihaela Bataneant, Margit Serban, László Maródi
   Timisoara, Romania, October 25 – 26, 2012

2. 84th J Project Meeting
   STAT1 mutation in chronic mucocutan candidiasis
   Beáta Soltész, Beáta Tóth, László Maródi
   Munkachevo, Ukraine, April 18, 2013

3. 85th J Project Meeting
   STAT1 mutations in chronic mucocutan candidiasis
   Beáta Soltész, Beáta Tóth, László Maródi
   Bratislava, Slovakia, April 24, 2013

4. IMPULSE EFIS-EJI symposium
   Impaired development of IL-17-mediated immunity in patients with *STAT1* gain-of-function mutation
   Beáta Soltész, Beáta Tóth, László Maródi
   Mátraháza, Hungary, August 31 - September 4, 2013

5. "Celebration of Hungarian Science", Hungarian Academy of Sciences
   Impaired development of IL-17-mediated immunity in patients with *STAT1* gain-of-function mutations
   Beáta Soltész, Beáta Tóth, László Maródi
   Budapest, Hungary, November 22, 2013

6. 1st J Project Congress
   Novel *STAT1* GOF mutations in ECE patients with CMCD
   Beáta Soltész, Beáta Tóth, László Maródi
   Antalya, Turkey, March 11 – 15, 2014

7. 5th Symposium on Primary Immunodeficiencies
   Impaired development of IL-17-mediated immunity in a patient with a novel *STAT1* mutation
   Beáta Soltész, Beáta Tóth, Gabriella Csorba, Szilvia Taskó, Adrien Katalin Sarkadi, László Maródi
   Bratislava, Slovakia, April 22, 2015

8. ESID Spring School
   A novel *STAT1* mutation and impaired development of Th17 immunity in a Hungarian patient
   Beáta Soltész, Adrien Katalin Sarkadi, Szilvia Taskó, Gabriella Csorba, Melinda Erdős, Beáta Tóth, László Maródi
   Slavonice, Czech Republic, May 13 – 17, 2015

9. ESID Summer School
   Impaired development of IL-17+ T cells in a Hungarian patient with a novel *STAT1* mutation
   Beáta Soltész, Adrien Katalin Sarkadi, Szilvia Taskó, Gabriella Csorba, Melinda Erdős, Beáta Tóth, László Maródi
   Pisa, Italy, September 15 – 20, 2015

10. 44th Congress of Hungarian Society for Immunology
    Impaired development of IL-17-mediated immunity in a patient with a novel *STAT1* mutation
    Beáta Soltész, Beáta Tóth, Gabriella Csorba, Szilvia Taskó, Adrien Katalin Sarkadi, Eszter Jakobicz, Zsuzsanna Bata-Csörgő, Lajos Kemény, László Maródi
    Velence, Hungary, October 14-16, 2015

11. 44th Congress of Hungarian Society for Immunology
    Molecular characterization of a novel intronic mutation in the *SH2D1A* gene
    Beáta Soltész, Beáta Tóth, Edit Gyimesi, Gabriella Csorba, Ágota Veres, Árpád Lányi, Gábor Kovács, Melinda Erdős, László Maródi
    Velence, Hungary, October 14–16, 2015

12. 2nd J Project Congress
    Splicing mutations causing XLP
    Beáta Soltész, Beáta Tóth, Edit Gyimesi, Gabriella Csorba, Ágota Veres, Árpád Lányi, Gábor Kovács, Melinda Erdős, László Maródi
    Antalya, Turkey, March 2 – 5, 2016

13. TÁMOP DEFENSE-NET Scientific Meeting
    STAT1 mutations in chronic mucocutan candidiasis
    Beáta Soltész, Adrien Katalin Sarkadi, Szilvia Taskó, Vera Gulácsy, Melinda Erdős, Beáta Tóth, László Maródi (group leader: László Maródi)
    Debrecen, Hungary, May 24, 2013

14. TÁMOP DEFENSE-NET Scientific Meeting
    Novel sequence variants in patients with primary immunodeficiencies
    Beáta Soltész, Adrien Katalin Sarkadi, Szilvia Taskó, Vera Gulácsy, Melinda Erdős, Beáta Tóth, László Maródi (group leader: László Maródi)
    Debrecen, Hungary, June 13, 2014

15. TÁMOP DEFENSE-NET Scientific Meeting
    STAT1 gain of function mutations in patients with chronic mucocutaneous candidiasis
    Beáta Soltész, Adrien Katalin Sarkadi, Szilvia Taskó, Vera Gulácsy, Melinda Erdős, Beáta Tóth, László Maródi (group leader: László Maródi)
    Debrecen, Hungary, January 29, 2015

Presentations in Hungarian

16. Magyar Infektológiai és Klinikai Mikrobiológiai Társaság 41. kongresszusa
    STAT1 mutáció krónikus mukokután candidiasis betegségben
    Soltész Beáta, Tóth Beáta, Sarkadi Adrien Katalin, Taskó Szilvia, Csorba Gabriella, Maródi László
    Szolnok, Hungary, October 05, 2013

17. Magyar Gyermekimmunológiai Konferencia
    STAT1 funkciónyerő mutáció patológiai szerep krónikus mukokután candidiasisban
    Soltész Beáta, Tóth Beáta, Sarkadi Adrien Katalin, Taskó Szilvia, Csorba Gabriella, Maródi László
    Bükfürdő, Hungary, December 06 – 07, 2013

18. Magyar Immundeficiencia Társaság tudományos ülése
    Luciferáz assay alkalmazása funkciónyerő mutációkban
    Soltész Beáta, Tóth Beáta, Maródi László
    Debrecen, Hungary, April 29, 2016

Posters in English

1. IMPULSE EFIS-EJI symposium
   Impaired development of IL-17-mediated immunity in patients with *STAT1* gain-of-function mutation
   Beáta Soltész, Beáta Tóth, Nadejda Shabashova, Anastasia Bondarenko, Satoshi Okada, Sophie Cypowyj, Avinash Abhyankar, Gabriella Csorba, Szilvia Taskó, Adrien Katalin Sarkadi, Leonóra Méhes, Pavel Rozsíval, David Neumann, Liudmyla Chernyshova, Zsolt Tulassay, Anne Puel, Jean-Laurent Casanova, Anna Sediva, Jiri Litzman, László Maródi
   Mátraháza, Hungary, August 31– September 04, 2013

2. 1st J Project Congress

a. Novel *STAT1* GOF mutations in ECE patients with CMCD
   Beáta Soltész, Beáta Tóth, Nadejda Shabashova, Anastasia Bondarenko, Satoshi Okada, Sophie Cypowyj, Avinash Abhyankar, Gabriella Csorba, Szilvia Taskó, Adrien Katalin Sarkadi, Leonóra Méhes, Pavel Rozsíval, David Neumann, Liudmyla Chernyshova, Zsolt Tulassay, Anne Puel, Jean-Laurent Casanova, Anna Sediva, Jiri Litzman, László Maródi
b. Autosomal dominant *STAT3* mutation in a Hungarian HIES patient
   Ildikó Csürke, Anett Kassay, Beáta Soltész, Zsuzsanna Pistár, Beáta Tóth, Ferenc Dicső, László Maródi
   Antalya, Turkey, March 11 – 15, 2014

3. EFIS-EJI Tatra Immunology Conference
   A novel *STAT1* mutation and impaired development of IL-17-mediated immunity in a Hungarian patient with CMCD
   Beáta Soltész, Beáta Tóth, Gabriella Csorba, Szilvia Taskó, Adrien Katalin Sarkadi, László Maródi
   Strbske Pleso, Slovakia, September 06 – 10, 2014

4. 16th Biennial Meeting of the European Society for Immunodeficiencies (ESID 2014)

a. Impaired development of IL-17-mediated immunity in a patient with *STAT1* gain-of-function mutation
   Beáta Soltész, Beáta Tóth, Gabriella Csorba, Szilvia Taskó, Adrien Katalin Sarkadi, László Maródi

b. *STAT3* mutational spectrum of newly diagnosed patients with HIES from Eastern and Central European Countries
   Zsuzsanna Pistár, Irina Kondratenko, Zoltán Maszárovics, Lyudmila Chernyshova, Anastasiia Bondarenko, Mariana Guseva, Larissa Kostyuchenko, Ygor Romanyshyn, Beáta Soltész, Adrien Katalin Sarkadi, László Maródi, Beáta Tóth

c. Molecular characterization of a novel intronic mutationin the *SH2D1A* gene
   Beáta Tóth, Beáta Soltész, Edit Gyimesi, Gabriella Csorba, Ágota Veres, Árpád Lányi, Gábor Kovács, Melinda Erdős, László Maródi

d. A novel insertion mutation in the *IL2RG* gene
   Zsuzsanna Pistár, Beáta Soltész, László Maródi, Beáta Tóth
   Praha, Czech Republic, October 28 – November 01, 2014

5. 2nd J Project Congress

a. Novel *BTK* mutations in patients with XLA from Eastern and Central Europe
   Beáta Soltész, Beáta Tóth, Melinda Erdős, Larysa Kostyuchenko, Alla Volokha, Anastasiia Bondarenko, Irina Tuzankina, Nasyrina Tatiana, Jadranka Kelecic, Marina Guseva, Liudmyla Chernishova, Igor Savchak, Zoltán Ellenes-Jakabffy, Capilna Brindusa, Svetlana Cemirtan, the J Project Study Group, László Maródi

b. A novel insertion mutation in the *IL2RG* gene
   Zsuzsanna Pistár, Beáta Soltész, László Maródi, Beáta Tóth
   Antalya, Turkey, March 2 – 5, 2016