THESIS FOR DEGREE OF DOCTOR OF PHILOSOPHY

Primary immunodeficiency disorders: From bedside to genomic sequencing

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1. INTRODUCTION

Primary immunodeficiencies (PIDs) are inherited diseases of the immune system, predisposing individuals to different sets of infections, allergy, autoimmunity, and cancer. Most of these hereditary diseases are associated with single gene defects. Over the past 15 years, the genetic basis of more than 110 PIDs has been identified. Diagnosis can now be made more accurately, more precise genetic counseling can be given, and in some instances gene therapy as a new treatment has been designed. In many of these disorders, we understand better the underlying mechanisms as the function of various gene products. There are at least four reasons why evaluation of PIDs should include genetic analysis:

1) Genetic studies can confirm a suspected diagnosis of immunodeficiency, and can help to pinpoint a specific genetic etiology even when other available data are unusual, incomplete, or inconclusive.
2) Accurate, early genetic diagnosis offers the best opportunity for reduced morbidity and survival.
3) Precise understanding of the genetic basis and pathogenesis of immunodeficiencies can be gleamed as more disease genes are identified.
4) Diagnosing a genetic disorder in an index patient, or proband, has profound implications for the patient's family members, both affected and unaffected, living and as yet unborn. Prenatal genetic diagnosis conveys great benefits for family planning.

Mutation analysis has become part of the evaluation in a large number of immunodeficiencies. Application of genomic sequencing helps to speed up the diagnostic process, thereby supporting early recognition of PIDs, before serious infections have compromised the child's general health, reducing the chances of survival.

2. AIMS

I started to work at the Department of Infectious and Pediatric Immunology in 2000 and my research interest focused on the immunological phenotype, the mutation analysis, and the functional activity of mutated proteins in patients with different PIDs. I focus my discussion on the increasingly important role of genomic sequencing in the prenatal and postnatal diagnosis, and follow-up of immunodeficient children in clinical practice. The aims of these studies were as follows:

1) To analyze disease-causing genes in families with X-linked lymphoproliferative disease (XLP), X-linked hyper-immunoglobulin M syndrome (X-HIGM), and Shwachman-Diamond syndrome (SDS).
2) To analyze products of mutated genes in XLP and SDS.
3) To present the importance of prenatal genetic diagnosis in the complex management of families with XLP and SDS.

3. PATIENTS AND METHODS

Patients and family members with XLP, X-HIGM, and SDS were studied. Informed consent was obtained from the parents or patients.

3.1 Genomic DNA isolation

Ethylenediaminetetraacetic acid (EDTA) blood from the patients and family members was collected and genomic DNA (gDNA) was isolated using QIAamp DNA Blood Mini kit (QIAGEN GmbH, Hilden, Germany). gDNA was also isolated from chorionic villus sample taken from the pregnant mothers by transabdominal approach.

3.2 Sequence analysis

For sequencing, exons 1-4 of the *SH2D1A*, exons 1-5 of the *CD40L,* exons 1-5 of the *SBDS* and the flanking intron regions were amplified by polimerase chain reaction (PCR). The following reaction mix was used for the amplification of each exon: 12.5 μL JumpStart REDTaq ReadyMix PCR Reaction Mix (SIGMA-ALDRICH GmbH, Budapest, Hungary), 6.5 μL destillated water, 0.5 μL 50 pmol/μL primer F, 0.5 μL 50 pmol/μL primer R, and 5 μL gDNA. Amplified segments were purified using a MICROCON YM-100 Centrifugal Filter Devices (Millipore Co., Bedford, MA USA) and the PCR products were sequenced with the BigDye Terminator Cycle sequencing kit (Applied Biosystems, Foster City, CA) and compared to the published sequence of *SH2D1A, CD40L* or *SDS*. Exons were sequenced using reactions mixtures as follows: 4 μL 5X Sequencing Buffer, 4 μL BigDye Terminator v3.1, 3 μL destillated water, 1 μL 3.3 pmol/μL primer, and 8 μL PCR product in a 20 μL total reaction volume. Sequencing reactions were purified using SigmaSpin Post-Reaction Clean-Up Columns (SIGMA-ALDRICH GmbH, Budapest, Hungary). Mutational analysis was performed by using 310 or 3130 ABI PRISM Genetic Analyzer (Applied Biosystems, Foster City, CA). The DNA mutation numbering was based on the complementary DNA (cDNA) sequence and the cDNA numbering system we have used follows the standard convention that + 1 is the A of the initiator ATG codon.

3.3 Cell lines and antibodies

COS-7 cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies, Grand Island, NY) supplemented with 10% fetal calf serum (FCS) as described. The Jurkat T cell line was obtained from American Type Culture Collection. Anti-human SLAM monoclonal antibody, rabbit anti-SHP-2 polyclonal antibody, horseradish peroxidase-conjugated goat anti-mouse antibody, and anti-rabbit IgG polyclonal antibody were

purchased from Santa Cruz Biotechnology. The anti-mouse 2B4 monoclonal antibody was from Pharmingen, and the anti-FLAG monoclonal antibody M5 was from Eastman Kodak Corporation, anti-myc antibody recognizing the EQKLISEEDL peptide was from Invitrogen Corporation (Carlsbad, CA).

3.4 Plasmid construction and transfection

cDNAs coding for *SH2D1A* (p.G16D) and *SBDS* (p.N121T and p.R175W) mutants were generated by site-directed mutagenesis in PCR using oligonucleotide primers incorporating the point mutation. Wild type (wt) SAP or SBDS, and p.G16D or p.N121T and p.R175W were cloned in vector pCMV-FLAG (Eastern Kodak Co.) to generate fluorescence labeled antigen (FLAG)-SAP or (FLAG)-SBDS constructs. The human SLAM cDNA in vector pJFE14-SR was a gift from DNAX Research Institute. Human 2B4 cDNA was expressed by a pcDNA3.1 vector, human FynT was expressed as myc-FynT fusion protein. COS-7 cells (1×10^6) were transfected with different expression vectors containing the appropriate cDNA insert by the FuGene 6 as recommended (Stratagene). Cells were harvested 72 hours after transfection.

3.5 Immunoprecipitation and Western blotting

After lysis of the cells with 0.5% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), immunoprecipitations were performed by using different antibodies and 30 µl of protein A-agarose beads (Invitrogen) for 2 h at 4 °C. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVD) membrane (Immobilon, Millipore Co.). Filters were blocked for 1 h with 5% skim milk and then probed with the indicated

antibodies. Bound antibody was revealed using horseradish peroxidase-conjugated secondary antibodies by enhanced chemiluminescence (Supersignal, Pierce).

3.6 Pulse-chase assay

Transfected COS-7 cells were starved for methionine and cysteine for 1 h and then pulse-labeled for 3 h with [^{35}S]methionine and [^{35}S]cysteine using Tran^{35}S-label (ICN Radiochemicals, Cleveland, OH). Newly synthesized proteins were chased for various times in complete medium containing cycloheximide (final concentration, 25 µg/ml). Cell aliquots (5 x 10^6 cells) were lysed, and the radiolabeled wt and mutant SAP and SBDS protein was analyzed by immunoprecipitation with the M5 antibody to FLAG. SDS-PAGE was performed using 15% density (BioRad, Hercules, CA). Gels were transferred to Immobilon membrane or blotted to PVD nylon membrane before autoradiography.

3.7 Densitometry and statistical analysis

Densitometry analysis of the Western blot bands was performed by using the TotalLab Analysis Software program. After autoradiography, films were scanned on a FOTO/Analyst® Investigator Workstation (Fotodyne, Hartland, WI). Each experiment was repeated three times, and statistical analysis was done by applying the Student's paired *t* test.

3.8 PCR-restriction fragment length polymorphism (PCR-RFLP)

The presence of the c.362A>C mutation in the exon 3 of *SBDS* was analyzed by MaeIII enzyme digestion of PCR-amplified products in the family members and in 50 healthy individuals. Exon 3 of the *SBDS* gene was amplified in an 899 bp (base pare) PCR product.

In homozygous wild type samples digestion with MaeIII yielded a 63 bp and an 836 bp restriction fragments.

4. RESULTS

4.1 Characterization of a new disease-causing mutation in a family with X-linked lymphoproliferative disease

A novel missense mutation in the *SH2D1A*, which caused a single p.G16D amino acid replacement of the protein SAP is presented here. To reveal the pathological relevance of this mutation the half-life and binding characteristics of the mutated SAP to SLAM and 2B4, two members of the SLAM family of immune receptors was analyzed. It was found that the p.G16D protein did not manifest a reduced half-life, but was defective in binding to its physiologic receptors compared to that of wt SAP.

4.1.1 Mutation in the *SH2D1A*

FIM was diagnosed in two related male patients of a Hungarian family. Given the severe clinical and histological phenotypes, mutation in the *SH2D1A* was thought. gDNA from the relatives of the two patients was extracted from blood cells and the *SH2D1A* exons and flanking intron regions were amplified and sequenced. First, I sequenced the *SH2D1A* in the mother and grandmother of P2, and found them heterozygous for a c.47G>A transition in exon 1. The same mutation was then detected in the gDNA isolated from stored lymph node specimen of P2. Mutation analysis in P1 was not applicable. However, based on the clinical phenotype, the X-linked inheritance, and the histological findings it is proposed that he had the same hemizygous mutation as his nephew did. During this study, the aunt of P2 became pregnant and sex determination disclosed a male fetus. Sequencing the

SH2D1A in this woman showed no mutation, and analysis of DNA isolated from amnionic fluid cells indicated wild type sequences in the fetus. The c.47G>A missense mutation resulting in p.G16D replacement in the amino acid sequence of the SAP protein has not been reported before in patients with XLP. Therefore, we analyzed various characteristics of the mutant protein to find out whether this mutation could lead to the clinical phenotype observed in the two patients.

4.1.2 Half-life of the mutant p.G16D protein

In a previous study, it has been proposed that two distinct mechanisms may underlie the pathogenesis of XLP. Limited amount of intracellular SH2D1A protein attributable to severely decreased half-life may be one of these mechanisms, the second being the disruption of binding of SH2D1A to the SLAM family of immunoreceptors. We hypothesized that the p.G16D mutation might affect the stability of SAP and as a result, the decreased SAP level in T lymphocytes and NK cells leads to disease phenotype. To probe this hypothesis, the half-life of the mutant SAP expressed in COS-7 cells by using pulse-chase assay was studied. The autoradiographs of [^{35}S]methionine- and [^{35}S]cysteine-labeled SAP at different times during a period of 24 hours showed that half-life of p.G16D protein was comparable to that of wt SAP. Half-life of the wt SAP in Jurkat cells and COS-7 cells labeled under the same condition was comparable. These data suggested that a decreased half-life of the p.G16D SH2D1A could not be responsible for the severe clinical phenotype in this family.

4.1.3 Abnormality of p.G16D protein binding to physiologic receptors

We hypothesized that the replacement of glycine by aspartic acid might alter the binding capacity of SAP to physiologic receptors. Therefore, we assessed in vitro binding of wt

SAP and p.G16D to SLAM and 2B4. All COS-7 cells cotransfected with plasmids coding for SLAM or 2B4 expressed these receptors 72 hours after transfection. However, binding to physiologic receptors could only be detected with wt SAP, whereas the mutant p.G16D protein lost its ability to bind to both SLAM and 2B4.

4.2 Invasive *Cryptococcus laurentii* disease in a nine-year-old boy with X-linked hyper-immunoglobulin M syndrome

We described here a patient with invasive *C. laurentii* infection and the X-linked form of HIGM. Flow cytometry showed that T cells activated with PMA plus ionomycin failed to express functional CD40L suggesting X-HIGM.

4.2.1 Mutation in the *CD40L*

Sequence analysis of the *CD40L* gene in the patient revealed a hemizygous C to A transversion at nucleotide position 216 in exon 2 (c.216C>A), and heterozygosity for this mutation in the gDNA from his mother. This mutation resulted in stop codon at amino acid 72 (p.C72X). Mutation analysis in the brother of the patient was not applicable. However, based on the clinical phenotype and the X-linked inheritance it is proposed that he had the same hemizygous mutation as the patient.

4.2.2 Identification of *Cryptococcus laurentii*

Fungi cultured on Sabouraud's agar and on yeast extract containing agar at 26 ^{0}C. Fungal growth could not be achieved by cultivation at 36 ^{0}C. Colonies were slimy and cream-colored and the size of the ellipsoidal- or spherical-shaped yeast cells were 2.5-7.0 x 2.0-5.5 μm. The subculture on bird seed agar was black. Cryptococcus antibody titer was 1/1000 and 1/400 in acute and reconvalescent serum, respectively. Cryptococcus Latex

Agglutination Test (Ramco) was positive and specific for cryptococci. Identification with Auxacolor (BioRad) resulted in the code of *C. laurentii.* Identification was repeated by standard methods. The assimilation of lactose and melibiose as well as low thermo-tolerance clearly differentiated this species from *C. neoformans*.

4.3 New disease-causing missense mutations in Shwachman-Diamond syndrome

I presented here a Hungarian patient with severe SDS phenotype and two previously unknown *SBDS* mutations occurring in exons 3 and 4, respectively. These missense mutations resulted in p.N121T and p.R175W amino acid replacements in the SBDS structure. Restriction-fragment analysis and gDNA sequencing revealed that the patient inherited these mutations from his parents, and that the c.362A>C and c.523C>T mutations did not occur in 50 healthy individuals. I propose that SDS may result from compound heterozygosity for missense mutations without evidence of gene conversion between *SBDS* and its pseudogene.

4.3.1 Mutation in the *SBDS*

Given the severe SDS phenotype in the proband, mutation in the *SBDS* gene was thought. gDNA from blood samples of the patient and his relatives was extracted and the *SBDS* exons and flanking intron regions were amplified and sequenced. Two novel missense mutations of the *SBDS* gene were identified in the patient; he proved to be compound heterozygous for the c.362A>C and the c.523C>T mutations in exons 3 and 4, respectively. These mutations are predicted to result in p.N121T and p.R175W replacements in the amino acid sequence of the SBDS protein, respectively. These mutations were previously not identified among SDS patients. Analysis of *SBDS* genomic sequences in the parents disclosed heterozygosity for both the mother (c.523C>T) and the

father (c.362A>C). The brother of the patient was also heterozygous for the c.362A>C mutation. During this study, the mother became pregnant and she requested, after genetic counseling, prenatal diagnosis. Chorionic villus sampling was performed at 11 weeks of gestation and gDNA was isolated. Sex determination disclosed a female fetus. Sequencing data showed that the fetus was carrier for the c.523C>T mutation but did not carry the c.362A>C mutation. As a result of sequence analysis and genetic counseling, the mother could decide to carry on with her pregnancy and she gave birth recently of a healthy girl.

4.3.2 Restriction-fragment digests of PCR-amplified *SBDS* exon 3

The missense mutations reported here have not been detected before in patients with SDS. To exclude the possibility of polymorphism, and to reveal the pathological relevance of the c.362A>C mutation RFLP analysis of the *SBDS* gene was performed. The results showed that this mutation was not present in 50 healthy individuals representing 100 alleles, suggested that the c.362A>C mutation was not present in the normal population.

Due to the lack of appropriate restriction enzyme for the mutation site in *SBDS* exon 4, PCR-RFLP was not applicable to study the c.523C>T in family members and controls. By genomic sequencing, the c.523C>T mutation could not be detected in 50 healthy control individuals. These data together with the presence of both mutations in the patient and the lack of disease manifestations in carriers suggested that these genetic changes were disease-associated.

4.3.3 Stability of mutant SBDS proteins

To examine whether the p.N121T and p.R175W amino acid replacements might affect the stability of the mutant protein, wild type and mutant SBDS cDNAs were expressed in COS-7 cells. Specifically, COS-7 cells were transiently transfected with SBDS cDNAs (wt or mutant) in a FLAG-tagged vector and metabolically labeled with [^{35}S]methionine and

[^{35}S]cysteine for 3 h. The stability of the mutant SBDS proteins were decreased compared to that of wt SBDS. These data suggest that a decreased half-life of the mutant SBDS proteins could be responsible for the severe clinical phenotype in our patient.

4.3.4 Alignment of cDNA sequences for genes orthologous for *SBDS*

Alignment analysis of SBDS proteins were carried out using ClustalW of MacVector 7.2.2 program. The p.N121T and p.R175W amino acid changes in the SDBS protein occurred at highly conserved residues, so they were likely to produce protein changes causing the disease.

5. DISCUSSION

5.1 X-linked lymphoproliferative disorder

Males with an expressed mutation in the *SH2D1A* gene that encodes an SH2 domain protein named SH2D1A or SAP have an X-linked syndrome characterized by an increased vulnerability to infection with EBV. We described here two male patients with severe clinical phenotype and histopathological characteristics of XLP. Sequence analysis of family members revealed c.47G>A missense mutation in exon 1 of the gene coding for SAP. This mutation resulted in a p.G16D single amino acid replacement in the protein. Missense *SH2D1A* mutations affecting an amino acid residue may occur throughout the gene. Such mutations may either lead to protein instability manifested by decreased half-life, or may result in impaired binding to SLAM or SLAM-related receptors while maintaining protein stability. To analyze the effect of this missense mutation on protein function cDNA was generated by site-directed mutagenesis and expressed in COS cells. The mutant (p.G16D) SH2D1A protein was relatively stable. We found that half-life of the

p.G16D protein was comparable to that of wt SAP. However, the mutant protein was defective in binding to its physiological receptors SLAM and 2B4. These results suggest that a defect in receptor binding contributes to the loss of function of the SAP protein in patients carrying p.G16D mutation. The defect in SAP/SLAM binding may be responsible for the deficiency in the regulation of interferon-γ production, and the impairment of the SAP/2B4 interaction may affect the function of cytotoxic T cells and NK cells. These combined defects could be responsible for the severe clinical phenotype observed in these patients. Nevertheless, further research is needed to define the precise correlation between missense *SH2D1A* mutations and clinical phenotypes of XLP. Remarkably, phenotype-genotype correlation of mutations of the *SH2D1A* and clinical outcome of EBV infections has not been clearly defined. It was suggested that missense mutations may lead to mild disease phenotype, in contrast to mutations that remove or truncate the SAP protein. Our data does not support this suggestion.

Analysis of the mutant p.G16D protein indicated that a conformational change, introduced by the amino acid transition, could affect the tyrosine-binding pocket of SAP and interferes with binding to SLAM and 2B4. Classical SH2 domains bind phosphopeptides in a “two-pronged” fashion. The phosphotyrosine binds in the pocket central and 3-5 residues C-terminal to it. SAP has another binding site for the tyrosine-motif, N-terminal to the Tyr residue. The N-terminal “prong” fits into a well-defined binding site formed by Arg13, Glu17, Ile51, and Thr53. Although Gly16 is not directly involved in the peptide binding its vicinity to Glu17 may explain the defective SLAM and 2B4 binding. Alternatively, the glycine in position 16 may assure protein structure necessary for SAP to bind to its receptors. Whatever the mechanisms, our findings are consistent with an important role of Gly16 in the downstream effect of the SAP-SAP receptor interactions during activation of T cells evoked by EBV-infected B lymphocytes.

In conclusion, a novel missense mutation in the *SH2D1A*, in a family with FIM affecting two males is described here. The p.G16D mutation in SAP did not result in significant half-life reduction, but it abolished completely binding ability to at least two of the SLAM family of immune receptors. Taken together, these results suggest that p.G16D is a disease-causing mutation, leading to XLP phenotype.

5.2 X-linked hyper-immunoglobulin M syndrome

We reported here a 9-year-old boy with X-HIGM syndrome and invasive *C. laurentii* infection identified on the basis of morphologic and biochemical characteristics and serologic tests. Fluconazole treatment resulted in complete recovery. Genomic sequencing of the *CD40L* gene revealed a nonsense mutation in exon 2 (c.216C>A) in the patient. The *CD40L* gene has been mapped to Xq26 and consists of five exons. A variety of mutations (missense, nonsense, deletions, insertions, splice-site mutations) are scattered throughout the gene, although they are more common in the TNF-homology domain. The human CD40L is a 261-amino acid type II. transmembrane molecule with a short intracellular tail, a transmembrane region, and an extracellular domain, that shares homology with TNF. Up to date nonsense mutations affecting 12 distinct codons have been identified in 18 families. In some cases the premature termination of the protein synthesis is compatible with expression of the mutated molecule at the surface. The p.C72X mutation in exon 2 leading to a premature termination affects the extracellular domain of the CD40L protein with the inability to express functional CD40L molecules on the activated T cells.

C. laurentii has recently been recognized as an opportunistic fungal pathogen in immunocompromised patients. This fungus was previously considered saprophytic and non-pathogenic to humans, but it has been isolated as the etiologic agent of skin infections, keratitis, endophthalmitis, lung abscess, peritonitis, meningitis, and fungemia. Individuals

receiving chemotherapy for leukemia or cancer, or immunosuppressive treatment of autoimmune diseases, and those who require intravenous catheters, parenteral nutrition, and broad spectrum antibiotics appear to be at increased risk to develop invasive *C. laurentii* infection. The unique case reported here demonstrates that patients with hereditary immunodeficiency disorders may also be susceptible to invasive *C. laurentii* infection. It is proposed that *C. laurentii* infection should be included in the differential diagnosis of opportunistic fungal infections in patients with primary T cell immunodeficiencies, like the CD40L deficiency.

There is no validated standard treatment for *C. laurentii* infection. Most isolates were found to be susceptible to amphotericin B, and minimal inhibitory concentrations for fluconazole range from 4 to 64 μg/ml. On the basis of susceptibility data and the lower toxicity of fluconazole than amphotericin B, we used fluconazole in this patient. Our experience and that of others suggest that fluconazole may be a first-line drug in immunodeficient patients with fluconazole-susceptible *C. laurentii* infection.

5.3 Shwachman-Diamond syndrome

SDS was diagnosed in a 5-month-old male infant who presented with an early onset of severe hematological manifestations and pancreatic insufficiency. Mutational analysis of the *SBDS* gene revealed missense mutations in exon 3 (c.362A>C) and in exon 4 (c.523C>T). Most of the previously identified *SBDS* mutations were conversion mutations to *SBDSP*. Here we first describe that compound heterozygous missense mutations in the *SBDS* gene may also result in SDS phenotype. That the mutations we found were disease-causing was proved by cosegregation with the disease in the family, genetic screening of the *SBDS* gene from healthy individuals, and by RFLP analysis. This study suggests that severe SDS may result from compound heterozygosity for missense mutations without

evidence of gene conversion events between *SBDS* and its pseudogene, *SBDSP*.

Alignment of cDNA sequences for genes orthologous for *SBDS* showed that both mutations occurred at highly conserved residues. Analysis of the entire human genome revealed that chromosome 7 contained the largest amount of intra-chromosomal duplications [14]. Segmental duplications (duplicons) on chromosome 7 can be targets for gene conversion events [60]. Gene conversion is a recognized mechanism of mutation in a number of human genetic diseases including SDS [11, 12, 56]. In the original report on the disease-causing gene, SDS was proposed to result from gene conversion due to recombination between *SBDS* and its pseudogene located 5.8 Mb distally in a paralog duplicon [8]. Sequence analysis of exon 2 indicated that most conversion events including the dinucleotide mutation 183-184TA→TC, as well as the 201A→G and the 258+2T→C mutations were confined to this region. Similar data were reported in smaller series of patients of Japanese and Italian ancestries [35, 45, 47]. In a few patients with no mutation in the *SBDS* gene expression of SBDS was normal suggesting that SDS is a genetically heterogeneous disease [71]. Frameshift or missense mutations are rare, and no clinical disease associated with missense mutations on both alleles has been reported before.

Genotype-phenotype correlation in SDS has not been clearly defined [35, 42]. Recent reports suggested that hematologic findings are variable even among patients with identical genotypes [35]. A lack of concordance in hematologic findings may occur even among affected siblings carrying the same mutation. Missense mutations may result in synthesis of proteins which are misfolded and targeted for degradation via the lysosomal proteolytic system or the cytoplasmic degradative system. Therefore, limited amount of intracellular SBDS attributable to decreased protein half-life could be responsible for the disease phenotype in this patient. Accordingly, we investigated whether p.N121T and p.R175W missense mutations could affect the stability of the SBDS protein. Data

presented here suggest that protein instability of SBDS mutants could be responsible for the disease in this patient. In addition, impaired binding of these proteins to their as yet undefined physiologic ligand(s) could also contribute to the disease phenotype described here.

To interpret the molecular consequences of the two missense mutations reported here remains challenging. Missense mutations may modify surface epitops and may change the electrostatic surface potential. The crystal structure of SBDS homologues revealed three-domain architecture with an N-terminal and a central domain which harbors the majority of disease-linked mutations, and a C-terminal domain, which shares structural homology with known RNA-binding domains [59]. Mutation in the C-terminal domain is extremely rare. The p.N121T mutation resides in the central domain which corresponds to residues 98-169 in human SBDS [59]. The R175W mutation resulting in replacement of a hydrophilic, basic residue by a hydrophobic, non-polar amino acid is located in the C-terminal domain (residues 170-250) of the SBDS protein. The two mutations we found may change the basic electrostatic charge of the central and C-terminal domains [62]. Such a change may have implication, as nucleic-acid binding proteins often use basic residues to contact the sugar-phosphate backbone.

One of the major outcomes of the present study is the identification of two novel missense mutations of the *SBDS* gene in a patient with SDS. The second major finding is that the pN121T and R175W mutant proteins that were selectively expressed in this patient were less stable compared to that of wt *SBDS*. These results are consistent with the severe clinical phenotype in the patient. In conclusion, we report here that the instability of proteins synthesized in patients with the c362A>C and c.523C>T missense mutations in the *SBDS* gene were associated with severe SDS phenotype.

6. NEW FINDINGS

- A new disease-causing missense mutation (c.47G>A) in *SH2D1A* resulting in p.G16D replacement in the amino acid sequence of the SAP protein, was discovered.
- The p.G16D protein is not able to bind to physiological ligands like SLAM and 2B4. It is proposed that he defect in ligand binding may contribute to the loss of function of the SAP protein in patients carrying p.G16D mutation.
- In a patient with severe CD40L deficiency a c.216C>A sequence variant was found that resulted in stop codon of CD40L synthesis in amino acid position 72 (p.C72X).
- Association of X-HIGM syndrome with invasive *C. laurentii* infection is first reported here.
- The first patient with severe SDS phenotype and two previously unknown *SBDS* mutations occurring in exon 3 (c.362A>C, p.N121T) and in exon 4 (c.523C>T, p.R175W) is presented.
- Severe SDS phenotype caused by compound heterozygous missense mutations without evidence of gene conversion events is first reported here.

LIST OF PUBLICATIONS

Publications related to the thesis

1. **Erdős M**., Maródi L.: Hyper-IgM szindróma.
   Gyermekgyógyászat 2003; 54:117-25.
2. Rákóczi É., **Erdős M**., Bartyik K., Kemény É., Nemes Z., Maródi L. X-kromoszómához kötött lymphoproliferatív betegség.
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Total IF: 17.29

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