THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

THE MOLECULAR PATHOLOGY OF THE WISKOTT-ALDRICH SYNDROME

by Vera Gulácsy, MD

Supervisor: Beáta Tóth Lajszné, MD, PhD

UNIVERSITY OF DEBRECEN
GYULA PETRÁNYI DOCTORAL SCHOOL OF CLINICAL IMMUNOLOGY AND ALLERGOLOGY
DEBRECEN, 2014
The molecular pathology of the Wiskott-Aldrich syndrome

By Vera Gulácsy, MD

Supervisor: Beáta Tóth Lajszné, MD, PhD

Gyula Petrányi Doctoral School of Clinical Immunology and Allergology, University of Debrecen

Head of the Examination Committee: Margit Zeher, MD, PhD, DSc
Members of the Examination Committee: Kálmán Nagy, MD, PhD
István Szegedi, MD, PhD

The Examination takes place at the Lecture Hall of Bldg. C, Department of Internal Medicine
Faculty of Medicine, University of Debrecen
12.00 p.m., September 2., 2014.

Head of the Defense Committee: Margit Zeher, MD, PhD, DSc
Reviewers: Árpád Lányi, PhD
Miklós Garami, MD, PhD

Members of the Defense Committee: Kálmán Nagy, MD, PhD
István Szegedi, MD, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen
14.00 p.m., September 2., 2014.
INTRODUCTION

The Wiskott–Aldrich syndrome (WAS) is a rare X-linked recessive primary immunodeficiency disorder characterized by microthrombocytopenia, recurrent infections due to combined immunodeficiency and eczema. The incidence is approximately one to ten cases per 1 million live male birth. The three main symptoms of WAS are bleeding – caused by decreased platelet count and abnormal function of platelets -, increased susceptibility to bacteria which lead to recurrent infections and eczema. Generally these symptoms manifest during the first year of age. Typical case with WAS is a male newborn who shows up with petechias on the skin, then bloody diarrhea and nose bleeding can be seen, later recurrent respiratory infections manifest and at last the eczema appears. The risk of autoimmun and malignant diseases in older ages is also increased.

The three milder variant forms of WAS are X-linked thrombocytopenia (XLT), intermittent thrombocytopenia and X-linked neutropenia (XLN). In XLT the only finding is the microthrombocytopenia, the risk of recurrent infections and eczema is low. In intermittent thrombocytopenia patients have normal platelet production at times with episodes of thrombocytopenia, but the decreased platelet volume and increased bleeding tendency are both missing. In XLN the patients suffer from severe recurrent bacterial infections due to neutropenia and monocytopenia.

The gene responsible for WAS was found in 1994 on the short arm of the X-chromosome. The gene consist of 12 exons and encodes a 502 amino acids long protein, named the Wiskott-Aldrich syndrome protein (WASP). WASP is expressed by hematopoietic cells and osteoclasts. WASP is involved in actin polymerization, signaling events and it is essential for proper immuncell functions. The only curative treatment in WAS is stem cell transplantation. For those patients who lack of compatible donor, gene therapy could be an option in the future.
OBJECTIVES

1. To make diagnosis of Hungarian patients with Wiskott-Aldrich syndrome using immunological, hematological and genetic diagnostic tools. To analyze the genotype/phenotype correlations in this study population.

2. To verify carrier status of female family members during family screening. To perform prenatal genetic tests in pregnant carrier women by chorionic villus sampling at the 10-12. weeks of their pregnancies.

3. To study the genetic and demographic features of patient with Wiskott-Aldrich syndrome from Eastern and Central European countries, Russia, Turkey and Azerbaijan.

4. To analyze large deletion mutations of the Wiskott-Aldrich syndrome gene on the basis of own results and the literature.

PATIENTS, RUTIN LABORATORY, IMMUNOLOGICAL AND HAEMATOLOGICAL METHODS

All investigations were carried out after informed consent had been obtained from the patients or their parents, and were approved by the institutional ethical committee. Genetic and clinical investigations were allowed by competent authorities of involved countries.

Analyzed patients and carriers

We analyzed 177 persons (114 males and 63 females) with WAS from 100 unrelated families from 11 Eastern and Central European (ECE) countries, Russian, Turkey and Azerbaijan. WAS diagnosis in all these patients was based on typical clinical and immunological findings, including microthrombocytopenia, eczema and recurrent infections. WAS diagnosis was confirmed genetically by screening for mutations of the WAS gene. Thirty-five of the 114 patients and 38 of the 63 female carriers studied underwent genetic diagnosis at the Jeffrey Modell Diagnostic Laboratory at the University of Debrecen, the rest of the patients and carriers were analyzed in other foreign laboratories. Prenatal genetic test
was performed in 5 Hungarian and one Russian family. In these cases amniocentesis was made at the 12 weeks of pregnancy and DNA was isolated from chorionic villus samples.

DNA amplification and sequencing

DNA isolation

Genomic DNA was isolated from EDTA peripheral blood samples using Gen ELUTE Blood Genomic DNA mini kit (Sigma–Aldrich Ltd., Germany).

Amplification polymerase chain reaction

Each one of the 12 exons of the WAS gene was amplified by polymerase chain reaction (PCR) using specific intronic primers.

Agarose gel electrophoresis

The size of amplified DNA fragments were checked by agarose gel electrophoresis with EasyLadder I (100 bp-2000 bp) (Bioline, London, UK), the agarose gel used was 2%.

Sequencing PCR and direct gene sequencing

PCR product was purified then sequencing PCR was performed to amplify single stranded DNA. Direct sequencing was performed by using the Big Dye Terminator Sequencing Kit (Applied Biosystems, Foster City, CA) and an ABI 3130 capillary sequencer (Applied Biosystems). Sequence variations were described with respect to a reference sequence, NCBI NG_007877.1 for WAS, cDNA and nucleotides were counted from the first ATG translation initiation codon. Mutations of the WASP gene were identified with the Human Gene Mutation Database (HGMD) (www.hgmd.cf.ac.uk) supported by ESID.

Flow cytometry

Cells from the EDTA whole blood were used. Intracellular WASP was stained with anti-human-WASP rabbit monoclonal antibody (Abcam, Cambridge, MA) and isotype-matched control and labelled by Alexa Fluor 488 conjugated anti-rabbit-IgG (Cell Signaling, Eugene, OR, USA) as secondary antibody.
Western blot

Total protein isolated from PBMC from heparin whole blood were electrophoresed on 10% sodium-dodecilsulfate–polyacrylamide gel and electro blotted into a polyvinylidene fluoride (PVDF) membrane (Millipore Corp, Billerica, MA). Next, anti-human-WASP rabbit monoclonal antibody (Abcam, Cambridge, MA), were added and incubated then, membranes were incubated with Horseradish peroxidase (HRP)–labeled affinity-purified goat anti–rabbit IgG (Sigma-Aldrich) as secondary antibody. WASP and actin bands were visualized by incubation with SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Scientific, Rockford, IL).

T cell proliferation assays

T cell proliferation assays were performed as described previously with minor modifications. In brief, T cell stimulation was performed using anti-CD3-coated 96 well plates with or without additional human interleukin-2 (IL-2) at. Cells were stimulated for 48 h before pulsing with 1 mCi (0.037 MBq) of $^{3}H$-thymidine (Perkin Elmer, Jügesheim, Germany) for 24 h and subsequent analysis of thymidine incorporation. This analysis was performed in collaboration with Prof. Dr. Klein.

Podosome staining

CD14+ cells were labeled with anti-CD14 magnetic beads (Miltenyi Biotech, Bergisch Gladbach, Germany) and isolated using an AutoMACS device (Miltenyi Biotech). Podosome analysis was performed as described previously in collaboration with Prof. Dr. Klein.

Molecular analysis

CD3+ lymphocytes from patients were sorted into fractions of WASp-positive and WASp-negative cells. Genomic DNA was extracted from both fractions, followed by polymerase chain reaction (PCR) amplification of all 12 exons of the WAS gene including intron–exon boundaries. DNA sequences were analyzed using an ABI Prism 3130 DNA Sequencer and the DNA Sequencing Analysis software version 3.4 (Applied Biosystems, Foster City, CA) and Sequencer version 3.4.1 (Gene Codes Corporation, Ann Arbor, MI). The WASp-positive CD3+CD56+NK cell fraction and B cell population (sorted as CD3+CD56+CD19+CD20+cells) was amplified using the primer pair described above. The product was gel extracted and subcloned into PCR-Scriptcloning vector (Stratagene,
Amsterdam, the Netherlands). Sequencing of clones was performed using M13 universal primers. This analysis was performed in collaboration with Prof. Dr. Klein.

RESULTS

Hungarian patients with WAS

Our main purpose was to study Hungarian families with WAS. We have indentified totally 9 male patients with WAS and 18 carrier females from 9 unrelated families since 2004. Prenatal diagnosis was also performed in many cases.

WAS phenotype in Hungarian patients

Thrombocytopenia

Thrombocytopenia was the first presenting symptom of all patients. Its most common clinical manifestations were the bloody diarrhea and petechia. Thrombocytopenia was discovered soon after birth in every cases except two. The lowest platelet count was around 20 G/L (Normal range: 150-400 G/L) in all patients. The mean platelet volume was also decreased. Thrombocyte transfusion was administered to all patients. In one patient the thrombocytopenia was the only manifestation of WAS.

Infections

Infections were common in Hungarian patients, the most severe manifestations were pneumonia and Salmonella sepsis. Skin abscess after BCG vaccination was seen in two boys. The respiratory tract was mostly involved, pneumonia occurred in many patients. Fatal infection was the cause of death in 3 patients, two of them died due to severe post transplant infection.

Eczema

Eczema was seen in every patient except one. Usually the skin rash appeared during the first year of age and was therapy resistant.
Autoimmunity/malignancy

Autoimmune or malignant disorders affected only one patient. This patient showed signs of myelodysplasia in his bone marrow 7 years after gene therapy.

WAS score

Eight of nine Hungarian patients had classical form of WAS, one patient was scored as XLT.

Laboratory features

Laboratory findings were mostly not typical for WAS. No severe lymphopenia was seen in either patients. The serum immunoglobulin isotypes (IgG, IgA, IgM, IgE) were measured in the normal range. In some cases increased level of IgA and IgE, in three patients decreased level of IgM was detected. Neither of our patients needed regular IVIG substitution therapy.

Therapy

Seven from nine boys went through HLA-identical allogenic stem cell transplantation, five of them were cured, two boys died. One patient had gene therapy. One patient died without any curative treatment.

Family history

Family history was positive for WAS in only 2 cases.

Family screening and prenatal genetic testing in Hungarian families with WAS

Genetic testing of Hungarian families with WAS verified totally 15 carrier women. Prenatal genetic test was performed in three families where the result was carrier female foetus. In another family chorionic villus sampling verified wild type twins. In one family the mother went through twice prenatal genetic testing: first the male foetus carried the mutant allele, in the second pregnancy the male foetus proved to be wild type. One prenatal genetic test was performed in a Russian family, the result was a healthy, wild type boy.
Genetic testing of Hungarian patients

Molecular genetic analysis was performed in every Hungarian male patients with WAS. The first patient had two thymines deletion in exon 1, that resulted in Stop codon at amino acid position 36. Two females (mother, grandmother) proved to be carrier in his family. Prenatal genetic diagnosis was also made which verified carrier female foetus. The second patients nonsense mutation caused cytosine/thymine exchange in exon 1. His mother was wild type. The genetic analysis verified also a missense mutation in exon 2 with thymine/cytosine exchange in our third patient. The genetic diagnosis in the fourth case showed a known missense mutation in exon 2. We found many carrier females in his family, prenatal genetic diagnosis was performed which result was another carrier female foetus. Our next patient had hotspot mutation in exon 2 with Arg86His exchange. We performed prenatal genetic testing in his two twin sibling at the 12 week of pregnancy, both of them was wild types. In the fifth family the proband died in early childhood, genetic testing was made in his mother and during her second pregnancy, carrier status was found in both cases. Our next patient had a novel nonsense mutation in exon 4 of the WAS gene. Prenatal testing was performed twice, at first the result showed the mutation in male foetus, the pregnancy was terminated. At second time genetic analysis verified healthy male foetus. The eighth boy with was had a small deletion mutation in exon 10, the mother was carrier, his sister was wild type. The last patients genetic analysis resulted in a known splice site mutation in intron 9.

Next the results of genetic tests of large WAS cohort is summarized.

Type and location of WASP mutations

A total of 77 different WASP mutations were identified, 22 of which are first reported here in a large cohort of patients from ECE countries, Russia, Turkey and Azerbaijan. We detected 34 single basepair changes (20 missense and 14 nonsense mutations), 20 small, 2 large deletions és one complex (InDel) mutation, 12 splice site defects and 8 small insertions.

The most common sequence variants were small deletions (25.9%) and missense mutations (25.9%), followed by nonsense (18.2%), splice site (15.6%) mutations, insertions (10.4%), large deletions (2.6%) and one complex mutation (1.3%). We detected mutations in all of the 12 exons except exon 9. In addition, 12 splicing mutations were located at six intronic parts of the WASP gene.

Exonic mutations were the most common types (83%). Thirty-eight exonic mutations (59.4%) were localized in the first four exons, less mutations (12.5%) involved the middle part of the
gene (5-8 exons), the last 3 exons carried more gene defects (26.5%) and in one case the whole WAS gene was deleted (1.5%). The intronic part of the gene was less affected, only 15.6% of mutations localized there, mostly in intron 6.

Mutations in all of the six previously described hotspots were found in our cohort (T45M (2), R86H (5), R86C (2), R211X (4), IVS6+5g>a (4), IVS8+1g>n (1), IVS8 + 1-6 del gtga (3). These mutations affected 21 patients from 17 families, totally 18.4% of all patients had hotspot mutations. The most common involved triplet was in exon 2 that encodes Arg86. Although occurring at the same rate as small deletions, missense mutations were detected in the largest group of patients (42), particularly due to a presence of the same mutation in several unrelated families. The most commonly affected amino acids were Arg86 and Asp224 (in five and 7 males). Most of the missense mutations (16) were found in the first four exons of the WASP gene and altogether only four missense mutations were detected in exons 7, 11 and 12. Small deletions were also common in the WAS gene. These deletions caused the lack of 1–22 basepairs, except for two patients who had a large deletion of 322 nucleotide and the deletion of the whole gene, respectively. Most of the deletions occurred in the first six exons of the WAS gene, five deletions were found in exon 10 and one in each of exons 11 and 12. In one case the large InDel mutation involved both of exons 10 and 11. We found nonsense mutations in 21 patients localized in 9 exons of the WAS gene. Insertions and splicing defects affected the middle regions of the WAS gene. In most cases one cytosine or thymine was inserted. Splicing defects occurred mostly in introns 6 and 8.

The 22 novel mutations were deletions (8), insertions (6), nonsense (4) and missense (2) mutations, splice site defects (1) and complex (InDel) mutations (1). Most of the new mutations were found in exon 10 (5), 3 novel mutations were located in each of exons 1, 2, two novel gene defect occurred in both of exons 4 and 11. One novel mutation was identified in each of exons 3, 5, 7, 8, intron 7 and at the intron 3-exon 4 boundary.

Small deletions of WAS gene were found to be common in our cohort. Known large deletion mutations caused severe phenotype occurred in only 2 cases. We described a novel complex mutation in a Romanian boy which consisted of a large deletion and an adenine insertion.

Large deletion mutation of WAS gene

PCR was performed for the 12 exons of WASP from the patient by using genomic DNA. According to agarose gel electrophoresis on exon 1-9 and 12 we detected appropriate length bands and normal order of nucleotides was analyzed by sequencing. In case of exon 10
and 11 the amplicons were undetectable; this data suggests a large deletion in samples of patient. In order to determine the boundaries of the deletion gDNA between exons 9 and 12 was amplified. In case of normal sequence the PCR product of exon 9-12 is 2816 bp length, in sample of patient a smaller, about 1000 bp length amplicon was analyzed. Bidirectional sequencing was used to analyze the order of nucleotides. On the chromatogram a 1595 bp deletion and an adenine insertion were determined. We performed the experiments in sample of mother, after PCR checking by agarose gel electrophoresis two bands were identified due to the normal and mutant alleles. By sequencing we found a heterozygous g.5247-6841del1595insA mutation. The InDel mutation located in the exon 10-11 and the intron 10-11, the mutation caused a frame shift, the synthesis of protein was damaged (R439fs)

WASP expression in blood cells of the patient, his mother and healthy controls was examined by flow cytometry after intracellular staining. We determined the WASP expression compared to isotype control. Cells isolated from the mother and healthy controls expressed WASP, whereas negligible amount of WASP was evaluated in PBMC of the patient. Western blot analysis showed that PBMC derived from the mother contained full length WASP (66 kDa) similarly to that of travel and healthy control cells. In contrast, WASP was not detectable in PBMC of the patient.

Somatic reversion is rare, but known event in WAS. Here we describe the cases of somatic reversion in Ukrainian siblings from our cohort.

Second site mutations and somatic reversion

Patient with classic WAS were proved to carry novel 58C>T nonsense mutation in exon 1, that resulted in Stop codon instead of glutamine at amino acid position 20 of WASP. Further investigations started in collaboration in Germany.

Patients’ routine laboratory results verified thrombocytopenia, decreased B cell count, inverted CD4/CD8 ratio. T cell proliferation assays in both patients showed decreased proliferative responses upon CD3 stimulation, which could be partially rescued by additional stimulation with human IL-2. Podosome analysis in peripheral blood CD14+ monocytes showed complete absence of podosomes in both patients.

Flow cytometric analysis of WASP expression in PBMCs revealed the presence of fractions of WASP-positive and WASP-negative cells among CD3+ lymphocytes and NK cells. We performed flow cytometric sorting of WASP-positive and WASP-negative CD3+ lymphocytes from both patients, extracted DNA and sequenced all 12 exons of the WAS
gene. The WASP-negative cells harbored the nonsense mutation (58C>T) verified earlier, WASP-positive cells showed different second-site mutations within the same triplet.

At the clinical follow up of the two patients 18 months later, both patients had decreased bleeding and eczema, their thrombocyte counts had risen to normal values. The younger boy results showed improvement in CD8+ lymphocyte- and NK cell count, but both patients had persistent B cell lymphopenia. The CD4/CD8 ratio remained inverted.

**DISCUSSION**

In this study we analyzed and presented genetic features of patients with WAS. Both molecular genetic tests and examination of WAS protein expression was made. Nine Hungarian male patients from 9 unrelated families with WAS were described.

Hungarian patients and carriers with WAS

One case of the 9 Hungarian patients referred to XLT, where the only clinical manifestation was thrombocytopenia. The other eight patients suffered from classical WAS. The most severe phenotype complicated with autoimmunity. Thrombocytopenia was the most common symptom in every patient, which usually was treated with transfusions. The mean platelet volume was decreased in most of our patients. Recurrent infections were also common in all patients except two boys. Eczema was a general symptom in eight of our patients. Autoimmunity or malignancy was seen only in one case. This phenomena could be due to the young age of our patients and the success of curative treatment that decreases the risk of autoimmune and malignant complications of WAS. The average WAS score was between 3 and 4. The immunological laboratory findings (lymphocyte count, immunoglobulin levels) were not typical in either of our cases. So these parameters could be less important than the clinical symptoms and the result of genetic analysis. Stem cell transplantation was performed in seven cases in the Hungarian group of patients, two boys died due to severe infections in the post transplantation period. One patient went through gene therapy procedure.

Molecular genetic analysis was performed in all of the Hungarian patients and gene defects of the WAS gene were revealed in all of the 9 cases. Known mutations of the WAS were found in 8 patients. Hotspot mutation was identified in single case. The type of the known mutations were deletions in 3 cases, missense mutations also in 3 cases and nonsense
mutation is one case. Novel mutation was found in one boy, the gene defect resulted in guanine to adenine exchange at 397 nucleotide position in exon 4. Triplet encoding glutamate at amino acid position 133 was changed to Stop codon, which led to early termination of protein synthesis. The gene defect resulted in classic WAS phenotype. Analysis of WAS protein expression was not routinely performed in the Hungarian group.

Carrier females were also tested in the Hungarian families and 18 carrier females were identified. Prenatal genetic testing was performed in 5 Hungarian and 1 Russian family and totally eight foetuses were analyzed.

Mutations of the WAS gene in large cohort of patients with WAS

We collected patients with WAS from 11 ECE countries, Russia, Turkey and Azerbaijan. In those foreign countries where the molecular genetic testing was not available, their patients were analyzed in Debrecen. Totally 46 persons, 26 males and 20 females were analyzed from Ukraine (8-8), Russia (8-5), Turkey (3-2), Romania (3-2), Azerbaijan (2), Poland (1-2) and Latvia (1-1). Genetic data of patients from other seven countries were also summarized.

Most of the mutations were found in the first four exons of the WAS gene, according to literature. Hotspot mutations account for nearly 20% of all patients included in this study, our data was similar to the result of earlier study. The most common hotspot mutation, (Arg86His) resulted in severe classic WAS phenotype in a Hungarian boy in contrast to the literature, where this mutation mostly appeared in milder XLT phenotype.

This most common type of mutations was the missense form, which affected the first 4 exons of the WAS gene. Large group of patients suffered from deletions mutations as well, these mutations were mostly small deletions. Large deletions were rare, as reported previously, 2.6% of patient had this type of gene defect. Among these large deletions, one novel complex mutation was described in detail. Few insertions were found, but most of them (75%) proved to be novel mutations. We detected mutations in all of the 12 exons except exon 9. Exon nine seems to be not the mostly affected part of the WAS gene according to literature. Nearly half of the splice site defects were hotspot mutations.

A total of 77 different WAS gene mutations were identified, 22 of which were novel. The novel mutations were deletions (36.3%), insertions (27.2%), nonsense (18.1), missense (9%), splice site defects and complex mutations (4.5-4.5%), respectively. Eighteen out of 22 novel mutations were frame shift deletions or frame shift insertions or nonsense mutations resulting in a premature termination of protein synthesis, and thus can be considered as
pathogenic ones. The huge WAS database which contains more than 300 different mutations of the WAS gene was enriched with our novel 22 mutations described here.

Approximately 80% of our WAS cases based on known mutations of the WAS gene. The most severe phenotypes were caused by nonsense mutations or deletions. Large deletions of the WAS gene are rare, here we identified a novel InDel mutation in a Romanian boy and were summarized the large deletions of WAS gene described earlier.

Large deletions of the WAS gene

Deletions in WAS were in most cases identified as short (1-12 bp) whereas the occurrence of large deletions have been rare. These genetic aberrations can span from a single exon to larger regions involving two or more exons with the encompassed introns or, in certain cases, the entire gene can be deleted. Here we reviewed the literature describing large deletions together with their genomic and clinical features and extend the list with a newly identified deletion mutation.

We report here the identification of a mutation affecting WAS in a Romanian patient based on laboratory and clinical findings. His mutation proved to be a novel InDel localized to the 3’ end of the WAS gene. Genetic analysis revealed that a breakpoint occurred in exon 10 at nucleotide position 5246 of WAS and resulted in a 1595 bp deletion with a single adenine insertion at the site of the deletion. The aberration causes the loss of part of exon 10, lack of the entire intron 10 and exon 11 and the partial loss of intron 11.

Among all the mutations that have been identified in WAS, large deletions constitute an extremely small fraction. Based on this limited number of known cases no preferentially affected gene regions can be identified. All of the large deletions led to either the total loss of WASP or to the expression of a nonfunctional truncated variant. These were manifested without exception in severe classical WAS phenotype. Therefore, early detection of large deletion mutation is crucial in order to initiate therapies including stem cell transplantation or gene therapy.

Rare case of second-site mutations and spontaneous clinical reversion was seen in two brothers and described.
Second-site mutations and somatic reversion

Ukrainian siblings with classical WAS phenotype were proved to carry novel nonsense mutation in exon 1 of the WAS gene. After genetic diagnosis allogenic stem cell transplantation was planned but no donor was found in either cases. So patients travelled to Germany where they were admitted to gene therapy program and further examinations were made in collaboration with Prof. Dr. Cristoph Klein. WAS protein expression was found in both patients in contrast to their nonsense mutation, that result led to the theory of somatic reversion which is a known but rare phenomena in WAS.

While the majority of cases of somatic mosaicism in WAS is caused by true back reversions, more rare scenarios of second-site mutations leading to altered but functional gene products have also been observed. The study of somatic mosaicism due to secondary somatic mutations is of considerable interest for the prospect of gene therapy, as it supports the concept of a potential proliferative advantage of gene-corrected cells.

The cases presented here are remarkable in several aspects. Our analyses show that both affected siblings have somatic mosaicism for WAS with the large majority of CD8+ lymphocytes and NK cells expressing WAS protein. Increasing expansion of WASp-positive lymphocytes over time in a WAS patient with revertant mosaicism, is due to proliferative advantage. The precise mechanism underlying these findings is unclear at present. An alternative hypothesis could be environmental influences that the patients may have experienced.

SUMMARY

The diagnosis of WAS should be considered in every case of male patients with thrombocytopenia and early death of male infants in the family. The typical clinical triad of WAS consist of microthrombocytopenia, recurrent infections and eczema. Molecular genetic analysis is essential in WAS beside the clinical and laboratory findings. Genetic analysis helps to find out the background of milder forms (XLT, iXLT) as well. As in every X-linked genetic disorder, analysis of potential carrier females is also important in WAS. WAS is a life threatening disease and at present the only curative treatment is stem cell transplantation. Due to the limitations (donor problem, severe immunosuppression) of this procedure, new curative treatments, as gene therapy are urgently needed in WAS.

In this review we presented the genetic diagnosis of 9 Hungarian families with WAS. Family screening revealed 18 carrier females and prenatal diagnosis was made in 5 cases in
the Hungarian group and in 1 case of a Russian woman. Results of genetic analysis of patients and carriers from 11 ECE countries, Russia, Turkey and Azerbaijan were also summarized. Disease causing mutations of WAS gene were analyzed with bidirectional DNA sequencing in patients and their family members. Totally 114 male patients and 63 carrier females were involved, 77 different types of WAS gene mutations were found with 22 novel gene defects. The features of mutations fitted to literature. Large deletion mutations of WAS gene were also reviewed in conjunction with the analysis of novel complex InDel mutation. Somatic reversion due to independent, second-site mutations were find in Ukrainian brothers and presented. This study has covered the largest group of patients with WAS from ECE countries according to our knowledge.
Candidate: Vera Gulácsy
Neptun ID: NKAWMO
Doctoral School: Doctoral School of Clinical Immunology and Allergology

List of publications related to the dissertation

   DOI: http://dx.doi.org/10.1016/j.molimm.2010.11.013
   IF: 2.897

   DOI: http://dx.doi.org/10.1111/j.1399-0004.2008.01019.x
   IF: 3.206
List of other publications

    DOI: http://dx.doi.org/10.1556/OH.2011.28940

4. Liu, L., Okada, S., Kong, X., Kreins, A.Y., Cypowyj, S., Abhyankar, A., Toubiana, J., Itan, Y., Audry,  
    M., Nitschke, P., Masson, C., Tóth, B., Flatot, J., Migaud, M., Chrabieh, M., Kochetkov, T.,  
    Bolze, A., Borghesi, A., Toulon, A., Hiller, J., Eyrierch, S., Eyrierch, K., Gulácsy, V., Chernyshova,  
    L., Chernyshov, V., Bondarenko, A., Cortés Grimaldo, R.M., Blancas-Galicia, L., Madrigal Beas,  
    I.M., Roesler, J., Magdorff, K., Engelhard, D., Thumerelle, C., Burgel, P., Hoernes, M., Drexel, B.,  
    Seger, R., Kusuma, T., Jansson, A.F., Sawalje-Belohradsky, J., Belohradsky, B., Jouanguy, E.,  
    Bustamante, J., Bué, M., Karin, N., Wildbaum, G., Bodemer, C., Lorholary, O., Fischer, A.,  
    Blanche, S., Al-Muhsen, S., Reichenbach, J., Kobayashi, M., Espinosa Rosales, F., Lozano,  
    C.T., Sebnem Kilic, S., Matias, O., Etzioni, A., Traudl-Hoffmann, C., Renner, E.D., Abel, L.,  
    STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis.  
    DOI: http://dx.doi.org/10.1084/jem.20110958  
    IF:13.853

    Bokréta G., Müller V., Katona M., Medvecz M., Fiedler O., Széchey R., Varga E., Rudas G.,  
    Kertész A., Molnár S., Kárpáti S., Nagy V., Magyar P., Mohamed M., Rákóczi É., Németh K.,  
    DOI: http://dx.doi.org/10.1556/OH.2010.28796


    DOI: http://dx.doi.org/10.1556/OH.2008.28377

H-4032 Debrecen, Egyetem tér 1.  
e-mail: publikaciok@lib.unideb.hu
Total IF of journals (all publications): 19.956
Total IF of journals (publications related to the dissertation): 6.103

The Candidate's publication data submitted to the Publication Database of the University of Debrecen have been validated by Kenezy Life Sciences Library on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

25 November, 2013
Other publication related to the dissertation:

Article in press.
ACKNOWLEDGEMENTS

I am grateful to my supervisor, Beáta Tóth, PhD and the leader of the Department of Infectious and Pediatric Immunology, Prof. Dr. László Maródi for their professional advice, scientific guidance and support.

I gratefully acknowledge Prof. Dr. Christoph Klein and his colleagues for their research collaboration.

I would like to express my thanks to Gabriella Csorba, Beáta Soltész for their help during my research.

I would like to offer my special thanks to co-authors for their collaboration and helpful discussion.

I wish to acknowledge the help provided by all of my colleagues at the Department of Infectious and Pediatric Immunology.

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

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