Molecular prediction of interaction between the novel mutated Wiskott-Aldrich syndrome (WAS) protein L101P with WAS-interacting protein by molecular modeling

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Directed by Professor Dong Soo Kim

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Moon Kyu Kim

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This certifies that the Doctoral Dissertation of Moon Kyu Kim is approved.

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주를 두려워하는 자를 위하여 쌓아 두신 은혜 곧 인생 앞에서 주께 피하는 자를 위하여 베푸신 은혜가 어찌 그리 큰지요 (시편 31:19)
How great is your goodness, which you have stored up for those who fear you, which you bestow in the sight of men on those who take refuge in you. (Psalm 31:19)

한 주제를 놓고 깊이 생각하면 할수록 내 자신이 얼마나 모르고 있는지 깨닫게 됩니다. 벼가 무르익을수록 고개가 숙여진다는 말의 의미를 새삼 느끼게 하는 기회가 되었습니다. 실로 오랜 기간 동안 씨름하며 애썼지만 그에 따르는 결과가 없었을 때 나의 힘과 생각들을 내려놓고 주님께 나아가 기다리는 시간을 가져야 했습니다. 연구를 마무리하면서 마치 긴 터널을 지나 밝은 빛을 만난 것 같습니다. 이런 노력들이 국내에 생소한 Wiskott-Aldrich syndrome을 앓고 있는 환우들과 가족, 그리고 같은 분야의 연구를 하는 의학도에게 작은 도움이 되길 바랍니다.

먼저 학위를 시작할 수 있도록 해주신 김 길영 교수님께 감사드리며 석사 논문부터 계속 지도해 주시면서 힘들 때마다 격려를 아끼지 않으셨던 김 동수 교수님께 감사를 드립니다. 논문 구석구석 꼼꼼하게 심사해주신 심사위원께 감사드리며 특히 처음부터 마무리까지 함께 수고를 아끼지 않으신 신 전수 교수님과 김 은숙님에게 감사를 전하고 싶습니다. Molecular modeling을 통해 이 연구에 날개를 달아주신 KIST의 윤 창노, 문 태성 교수님께 다시 감사드립니다.

저를 낳아 주시고 의학의 길로 들어설 수 있도록 사랑하며 후원해주신 부모님께 이 논문을 드리며, 힘들 때마다 위로하며 격려하고 기도했던 아내와 공로를 나누고 싶습니다. 저를 생각할 때마다 이 연구의 완성을 위해 함께 기도해주신 목사님들, 성도님들, 직장 동료 여러분, 그리고 예수원 공동체에게 감사를 전하며 부족한 저에게 항상 좋은 것으로 채워 주시는 하나님께 영광을 돌립니다.

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김 문규

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ABSTRACT

Molecular prediction of interaction between the novel mutated Wiskott-Aldrich syndrome (WAS) Protein L101P with WAS-interacting protein by molecular modeling

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Wiskott-Aldrich syndrome (WAS) is an X-linked disorder characterized by eczema, thrombocytopenia, and increased susceptibility of infections, and mutations of *WAS* gene are responsible for WAS and X-linked thrombocytopenia. We here report two novel mutations of WAS at T336C (patient 1), exon 3, and at 1326-1329, a G deletion (patient 3), exon 10, resulting in L101P missense mutation and frameshift mutation 444 stop, respectively. The affected patients with either mutation showed severe suppression of WASP levels, T cell proliferation, and T cell viability after stimulation, suggesting classical WAS. The generation numbers of T cell division were decreased to 2-3 times, compared to 6-7 times of those of the normal controls, in CFSE-labeled cell division study after polyclonal activation, suggesting defect of mitotic event of T cells.

To understand the pathogenesis of WAS, we analyzed the molecular

interaction of L101P WASP with WASP-interacting protein (WIP) using computer modeling because L101 have no evidence of a direct nuclear Overhauser effect (NOE) contact with the WIP in NMR spectroscopy. It is presumed that P101 WASP induced a conformational change in the Q99 residue of WASP and made the side chain of Q99 move away from the WIP peptide, resulting in disruption of the hydrogen bond between Q99 WASP and Y475 WIP. We were able to propose a possible model for the molecular pathogenesis of WAS by analyzing interactions of WASP and WIP using a molecular modeling study.

Key words : Wiskott-Aldrich syndrome (WAS), WAS protein (WASP), WASP-interacting protein (WIP), molecular modeling

The molecular prediction of interaction between the novel mutated Wiskott-Aldrich Syndrome Protein (WASP) L101P with WIP by molecular modeling

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I. INTRODUCTION

Wiskott-Aldrich syndrome (WAS, 301000) is a rare X-linked disorder characterized by eczema, thrombocytopenia, and immunodeficiency[1,2] which was first reported by Wiskott in 1937 followed by Aldrich who realized that it is inherited as X-linked recessive trait. WAS is now more characterized by its small platelets, increased risk of autoimmunity and malignancy. Classical WAS is often associated with the absence of WAS protein (WASP) caused by mutations in the *WAS* gene[3], which consists of 12 exons containing 1823 base pairs and is located on the X-chromosome at Xp11.22-p11.23[4]. But mutation in the same locus can cause a much milder disease called X-linked thrombocytopenia (XLT, 313900) whose platelets are also small and decreased in number, and either free of eczema or mild and transient eczema[5,6]. The life expectancy used to be less than 1 year in 1930's but now it is about 11 years or

even more[7]. The only way to cure this disease is stem cell transplantation which has a 2-year survival rate about 40 to 90% depending upon the donor characteristics[8].

WASP is a signal transduction protein in the cytoplasm that is expressed exclusively in hematopoietic lineage cells and plays a central role in promoting actin polymerization[9] and has a possible role in motility[10], phagocytosis[11], and activation of T cells[12] and B cells[13]. WASP encodes a 502 amino acid protein which is composed of WASP homology 1 (WH1) domain, basic region (BR), GTPase-binding domain (GBD), polyproline (PP) domain, verprolin-cofilin homology (V/C, or WH2) domain, and a carboxy-terminal acidic (A) domain[1,2]. The domain organization of WASP has identified structurally similar proteins such as N-WASP, SCAR/WAVE proteins, Bee1p, now considered as WASP family[14]. WASP can bind to a variety of proteins[1, 2]; WH1 domain at N-terminal region of WASP interacts with the WASP-interacting protein (WIP)[15,16], which is important for NF-AT/AP-1-mediated gene transcription with cooperating with Vav[17] and for actin-tail formation[16], BR and GBD interact with GTP-bound Cdc42 and phosphatidylinositol-4,5-biphosphate (PIP_2), PP domain with several signaling and adaptor molecules, such as Nck, Grb2, Fyn, CrkL, Bruton's tyrosine kinase (Btk), and proline-serine-threonine phosphatase-interacting protein (PSTPIP). And V/C and A domains of WASP recruit actin-related protein (Arp) 2/3 complex for actin polymerization[1,18].

WASP WH1 domain is a small structural module with approximately 100 residues which is about 22% of the amino acids of WASP but contains over

50% of all known mutations[19]. Until now, more than 150 mutations has been identified in 300 patients. Recently NMR spectroscopy and computer modeling studies of WASP WH1 and N-WASP EVH1, which is highly homologous WASP WH1 domain, have been able to predict the structure of WASP WH1 and its molecular interaction with WIP peptide[19,20]. And the pathogenesis of WAS could be partially understood by analyzing the missense mutation events of WASP WH1 through the structural basis of WASP WH1 to WIP.

In the present study, we report two novel mutations of WASP gene, a missense mutation of L101P WASP in exon 3 (family A) and a deletion frameshift in exon 10 (family B), both of which caused defects in WASP expression and CFSE-stained cell division. And we have focused on why L101P WASP induced WAS by analyzing the molecular interaction of P101 WASP with WIP peptide using computer remodeling, because L101 WASP was not the direct binding site to WIP in NMR spectroscopy[20].

II. MATERIALS AND METHODS

1. Patients

Family A: Patient 1 (the index case), a 29-yr-old man, had chronic idiopathic thrombocytopenia diagnosed at 7-years of age. He got the splenectomy for the treatment of idiopathic thrombocytopenic purpura (ITP) at the age of 19. He was admitted due to pneumonia, and bacterial meningitis. *Streptococcus pneumoniae* type 6A was identified in blood culture and *Acinetobacter baumanii* and *Pseudomonas* were identified in sputum and vancomycin-resistant enterococci (VRE) in blood. Platelet counts were $30x10^9$/L and MPV was 8.5 fL. Absolute lymphocyte count was markedly decreased to be $0.23x10^9$/L (normal range: 1.4-4.0 $x10^9$/L). Platelet aggregation tests showed a depressed response to ADP, collagen, epinephrine, and ristocetin. No secondary response wave was observed, revealing a pattern of storage pool deficiency. The immune cells were 41% of CD4+ T cells, 23.6% of CD8+ T cells, and 11.2% of B cells. Serum IgG, IgA, and IgM levels were normal. His clinical score[21] was 4 and he died of intracranial hemorrhage. His brother with thrombocytopenia died at early age due to suspicious infections. His nephew (patient 2), a 4-yr-old boy, was admitted due to recurrent intussusception. His brother also had decreased platelet count and died at 8 years of age. The platelet count was $30x10^9$/L and the clinical score was 3.

Family B: Patient 3 was an 18-month-old boy of nonconsanguineous parents. He had eczema, intermittent petechiae with bruisings with clinical

score of 3. The platelet count was $14.7x10^9$/L and MPV was 7.7 fL of low-normal limit. Serum IgG, IgA, and IgM levels were normal, but the IgE level was highly elevated to 2,310 IU/mL (normal range; < 17 IU/mL). He had congenital cytomegalovirus (CMV) infection confirmed by positive anti-CMV IgM (2.08) in serum and by CMV DNA PCR of urine samples, but no chorioretinitis and hearing loss were observed. The immune cells were 11.7% of CD4+ T cells, 28.5% of CD8+ T cells, 3.3% of NK cells, and 53.8% of B cells. HIV RNA was not detected by PCR in serum sample. His bone marrow finding was increased myeloid to erythroid ratio with fully matured myeloid cells, abundant number of megakaryocytes, and with marked lymphocytosis. At the age of 9 months, he showed worsening of eczema in addition to positive anti-CMV IgM which was consistent.

2. Mutation analysis at the *WAS* gene

Genomic DNA was extracted from peripheral blood and amplifications of 12 exons containing flanking splice-sites at the WASP locus were performed as described[22]. Briefly, 25 ng of genomic DNA was mixed with 1 μL of 10X buffer, 0.25 μL for dNTP (10mM), 0.5 μL of each primers in Table 1, 1.25 U of Taq (Taqgold®, Perkin Elmer Applied Biosystems, Norwalk, CT, USA), and H_20 to adjust to 10 μL for PCR(polymerase chain reaction). Thermocycling was performed using following conditions: 94 °C for 10 min, 35 cycles of 95 °C for 35 sec, annealing temperature for 1 min (Table 1), 72 °C for 1min, followed by final extension at 72 °C for 10 min. DNA sequencing was performed in both

directions using ABI Prism 310 sequencer (Applied Biosystem, Foster city, CA, USA).

Table 1. Primer sequences of DNA amplification and annealing temperature.

Name of primer	Sequence	Exon	Temp (^{o}C)[a]	base pair
WASP E1	5': GCCCCTGGAGGACTTGTT	1	60	470
	3': ATGGGCCCAAAGGAAGAG			
WASP E2	5': TCTACTTGCCTTCCCTCTGG	2	60	407
	3': CTTTGGTTCTGGGGCTCA			
WASP E3/4	5': TGAAAATCTCCAAACCAGACTATG	3, 4	60	502
	3': AGGACCATGAGAGGGGACTT			
WASP E5/6	5': CCCTCGTGCAGGAGAAGATA	5, 6	60	504
	3': ACCCATCCATCAGTCCACAC			
WASP E7[b]	5': CCACCCACTTCTCCATAGACC	7	63	496
	3': CTAGACCCAGCCCTGCAC			
WASP E8/9	5': ATTCTGGCCCCTCAGAGTC	8, 9	60	601
	3': GGTGCTCCCATAAGGACTGA			
WASP	5': GCTTCAGTCAGGAGTTGGTCAGTG	10-1	57	321
E10-1	3': CAGTGGTCCAGAACGTCCAGTAGC			
WASP	5': TCCAGCTACTGGACGTTCTG	10-2	63	287
E10-2	3': TCCTGACTTAGACGGGACAC			
WASP E11[b]	5': GGGATTCAGTGATAGGGTTGA	11	56.4	404
	3': CAAAAACGAGGCTGACACAA			
WASP E12	5': GGAAACTCTAACTTGCCCTCCT	12	60	402
	3': TCCTTGAGTGAAGAGAACTGAGAA			

a: annealing temperature, *b*: 5% of DMSO (dimethyl sulfoxide) was added

3. Analysis of WASP expression

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-hypaque gradients. The cells were lysed using 1% Nonidet P-40 buffer containing protease inhibitor cocktail (Sigma, Saint Louis, MI, USA), and the concentrations of cytoplasmic protein were measured by Bradford assay (Biorad, Hercules, CA, USA). After normalization of concentrations of cytoplasmic protein, the protein lysates were run on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. Western blot analysis was performed using monoclonal anti-WASP to amino acids 1-250 of WASP of human origin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and HRP-labeled rabbit anti-mouse Ig was used as a secondary antibody. The signal was revealed with enhanced chemiluminescence (Pierce, Rockford, IL, USA).

4. Proliferation assay

PBMCs in 10% FBS-RPMI1640 (Gibco BRL, Grand Island, NY, USA) containing 2 mM L-glutamine and 100 μg/mL of streptomycin and 100 U/mL of penicillin were plated at $1x10^5$ cells/well in 96-well U-bottom plates. Phytohemagglutinin (PHA) was added to the wells at the concentration of 1 or 10 μg/mL and incubated for 72 h. The cells were labeled with [^{3}H]-thymidine for 18 h. Thymidine incorporation was measured in triplicates by liquid scintillation counting and the mean values were used for the analysis.

5. Analysis of T cell division

PBMCs were labeled with 5 mM CFSE dye (Molecular probes Inc., Eugene, OR, USA) for 10 min at 37°C[23]. PBMCs were washed three times with 0.1% BSA-PBS and cultured with PHA at 1 μg/mL for 90 h. The cells were washed and were incubated with PE-conjugated anti-CD3 (BD Biosciences, San Jose, CA, USA) at 4°C for 30 min. The cells were incubated with propidium iodide to exclude dead cells and analyzed by flow cytometry (Beckman Coulter Company, Miami, FL, USA).

6. Molecular modeling

Primary sequences of human WASP and WIP were retrieved from Swiss-Prot[24]. In order to search the template for homology modeling, PSI-BLAST was carried out with WASP and WIP sequences against the Protein Data Bank (PDB, http://www.rcsb.org/pdb)[25]. Homology modeling was performed using the InsightII2000 Homology module (Accelrys Inc.). WASP L101P mutant was constructed by replacement of the corresponding residue. For the refinement of model structures, each structure was energy-minimized using the steepest descents for 500 steps and conjugate gradients for 5000 steps with a distance-dependent dielectric constant (4*r). All molecular mechanics calculations were carried out using the Discover2.98 program (Accelrys Inc.) with AMBER forcefield using nonbonded cutoff of 12 Å running on a Silicon Graphics O2 workstation.

III. RESULTS

1. Mutation analysis

Patients 1 and 2 showed T336C nucleotide substitution in exon 3 of the *WAS* gene, resulting in L101P missense mutations (Fig. 1 and 2). Heterozygosity for this mutation was detected in three sisters, the mother, and one niece of patient 1. Patient 3 showed a G deletion in 1326-1329 exon 10, resulting in a frameshift mutation to eventually stop at amino acid 444 (Fig. 1 and 2). Both mutations are novel findings and three patients in this study had classical WAS phenotypes.

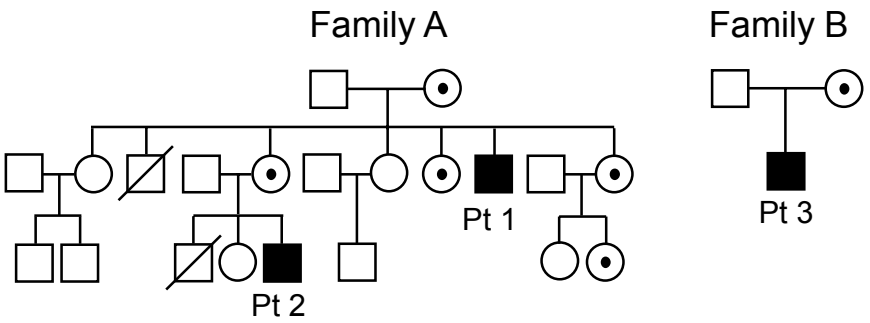

Fig. 1. Pedigree of family A and B. One brother of patient 1 and one brother of patient 2 died at early ages because of infection.

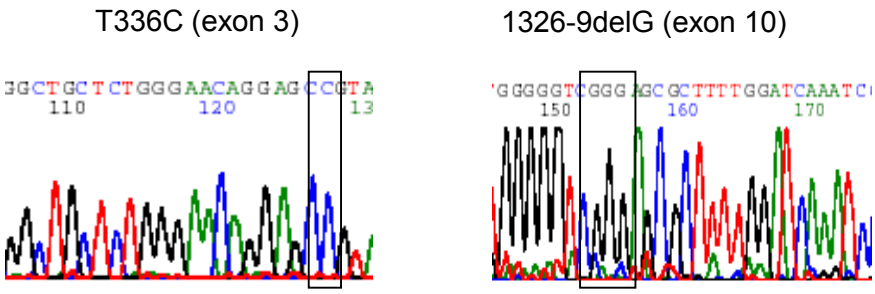

Fig. 2. Mutation analysis of genomic DNA for *WAS* gene of patient 1 and 2 (left) and of patient 3 (right). Patient 1 and 2 showed missense mutation of T336C at exon 3 and patient 3 showed frame shift mutation at 1326-9delG at exon 10.

2. Analysis of WASP expression

The expression of WASP in unstimulated PBMCs was severely suppressed in patient 1 which was decreased 58% compared to normal control and undetected in patient 3 (Fig. 3).

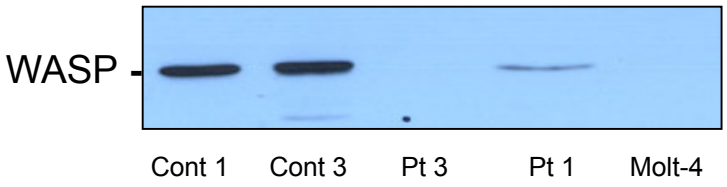

Fig. 3. The Western blot analysis of WASP. PBMCs were derived from patient(Pt) 1, 3 and age-matched normal control (Cont) 1 and 3, and Molt-4 .

3. Proliferation assay

In vitro lymphocyte proliferation to 1 μg/mL PHA was markedly decreased to 5-15% (patient 1) and 2-10% (patient 3) of those of age-matched normal controls and low proliferative response even at high concentration of 10 μg/mL PHA (Fig. 4), confirming previous result[26]. Contrast to the patients, mother of the patient 1 showed normal result (data not shown).

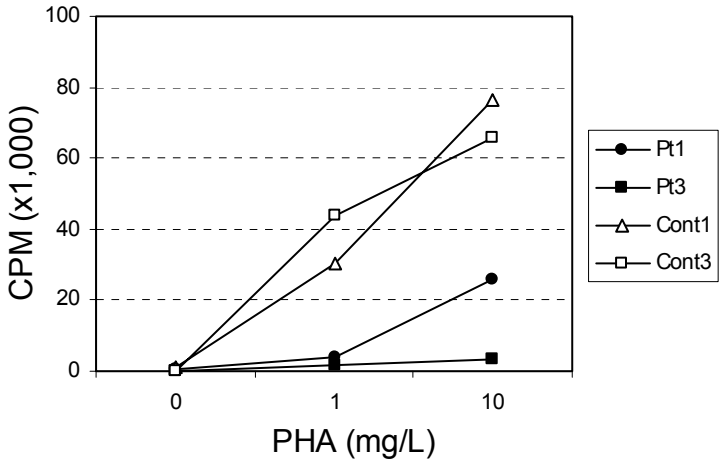

Fig. 4. T cell proliferation assay to 1 and 10 μg/mL PHA. Patient (Pt) 1 and 3 showed markedly decreased proliferation compared to normal age-matched controls (Cont).

4. Analysis of T cell division

To investigate the cell division processing of T cells, PBMCs were cultured with 1 μg/mL of PHA for 90 h and the cells were stained with anti-CD3 Ab and propidium iodide. The numbers of live CD3+ T cell division

generation were severely decreased to 2-3 times in patient 1 and 3, compared to 6-7 times of controls (Fig. 5). The live CD3+ T cells after polyclonal activation were severely decreased to 11.6% (Pt 1) and 8.1% (Pt 3), compared to 44.4% (Cont 1) and 52.5% (Cont 2) of total CD3+ T cells (Fig. 5). The numbers of mitotic events of live T cell were 944 (patient 1) and 627 (patient 3) among a total of 20,000 CD3+ T cells gated, compared to age-matched normal controls of 6,061 (control 1) and 12,437 (control 3), and they were 15.6% and 5.0% of those of normal controls, respectively.

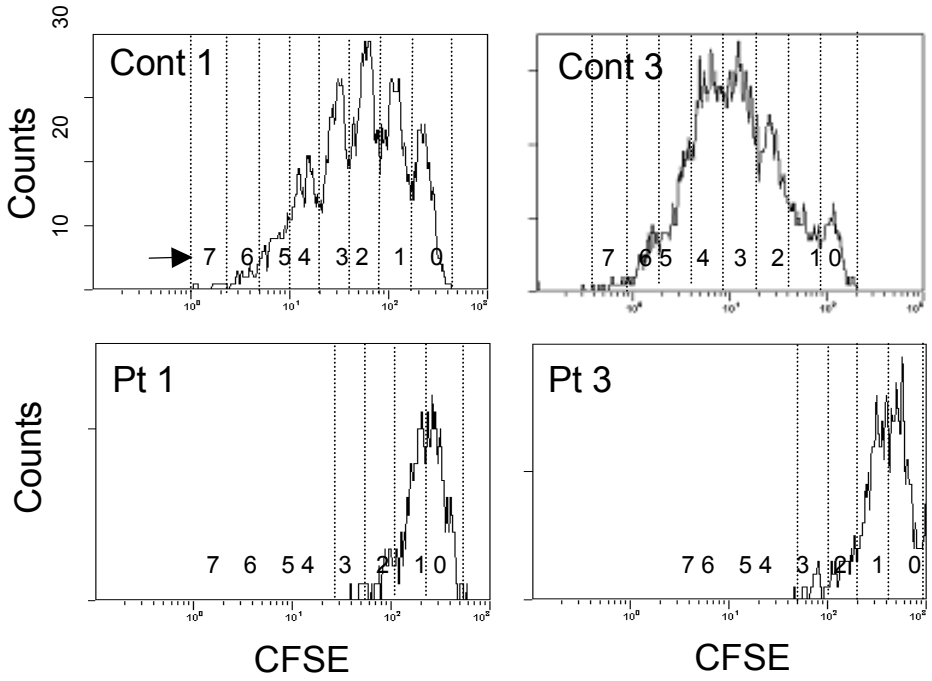

Fig. 5. CFSE-labelled T cell division analysis**.** CFSE-labeled PBMCs (Pt1, Pt3, Cont1, and Cont3) were cultured with 1 μg/mL PHA for 90 h, and the cells were harvested and stained with PE-conjugated anti-CD3. The cells were

stained with propidium iodide and live CD3+ T cells were analyzed by flow cytometry. The arrow indicates the number of cell divisions.

5. Molecular modeling

L101 of WASP is identified as a highly conserved hydrophobic amino acid in multiple sequence alignment of WH1 domains of WASPs from several different species[19]. However, L101 did not show direct NOE contact with the WIP peptide, although the neighboring amino acids of Q99, E100, Y102, and S103 showed interactions with WIP peptide in NMR spectroscopy[20]. We analyzed how the novel L101P missense mutation influences the interaction with the WIP peptide using molecular modeling, based on the NMR structure of the N-WASP EVH1-WIP complex[20] (pdb code of 1mke). The sequence alignment of human WASP with 1mke (sequence identity of 47.5%) was obtained from a PSI-BLAST search (Fig. 6). This alignment suggested that 1mke could be a good template for homology modeling[27,28].

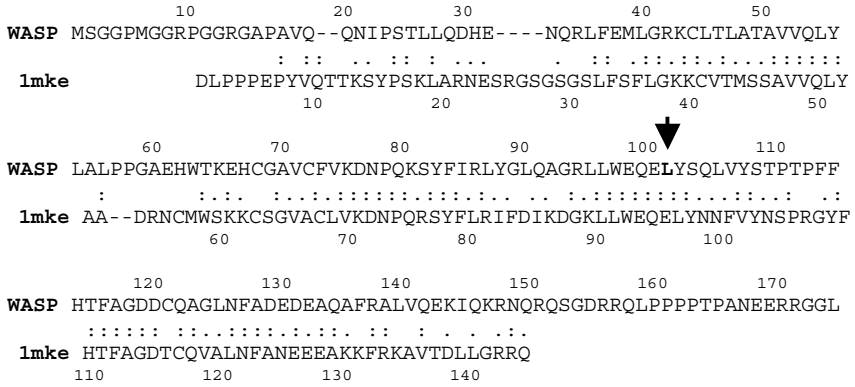

Fig. 6. Sequence alignment of human WASP with pdb(protein data bank) code of 1mke. Identities are shown with the (**:**) symbol, and similarities (or conservative replacements) with the (**.**) symbol. The arrow is the mutation site of WASP L101.

The human WASP-WIP peptide complex structure was built by homology modeling using 1mke as a template (Fig. 6), ranging from R34 to R148 of WASP WH1 and from WIP peptide from D461 to P476. It seems that L101P does not have an effect on the WASP-WIP interactions since L101 does not contact with WIP. The model structure of human WASP-WIP complex showed that the amino acid residues around L101 (Q99, E100, Y102, and S103) have interactions with WIP but not L101 (Fig. 7).

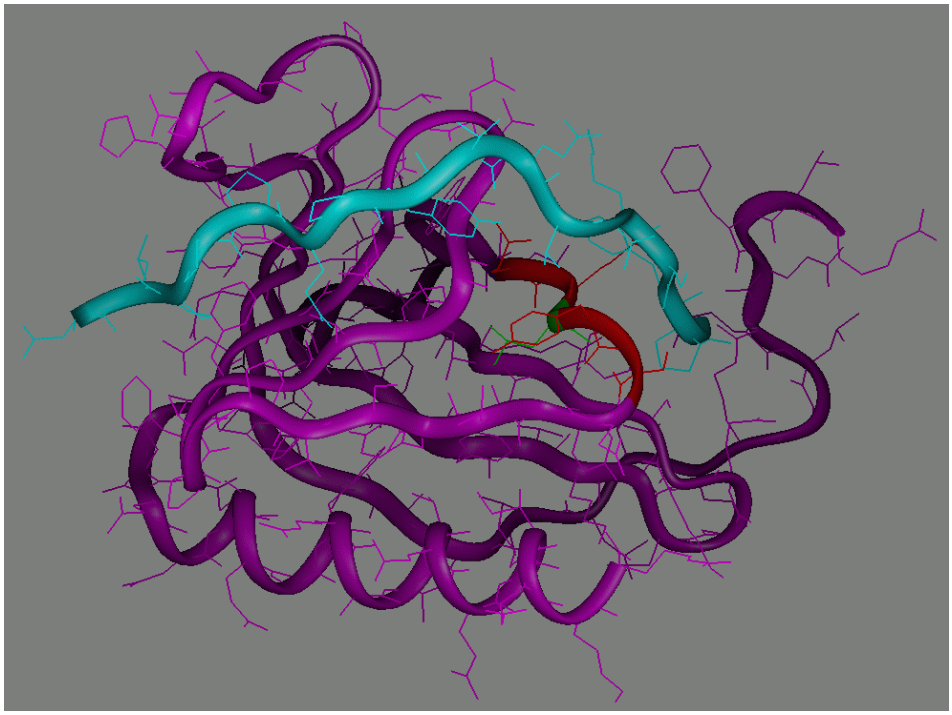

Fig. 7. Molecular modeling of human WASP-WIP peptide complex. Purple; human WASP (residues R34-R148), cyan; WIP peptide (residues D461-P476), green; residue L101, red; residues Q99, E100, Y102, and S103.

In order to investigate the effect of L101P mutation, the molecular modeling studies were carried out on wild and L101P mutant WASP. The models achieved by molecular modeling suggest that L101P mutation makes the side chain of Q99 move away from the WIP peptide and disrupts hydrogen bond between Q99 of WASP and Y475 of WIP (Fig. 8B), although the wild WASP maintain the hydrogen bond (Fig. 8A). The distances between side chain nitrogen of Q99 and the side chain oxygen of Y475 are 3.01 Å and 5.39 Å in wild WASP and L101P mutant, respectively (Fig. 8A and 8B).

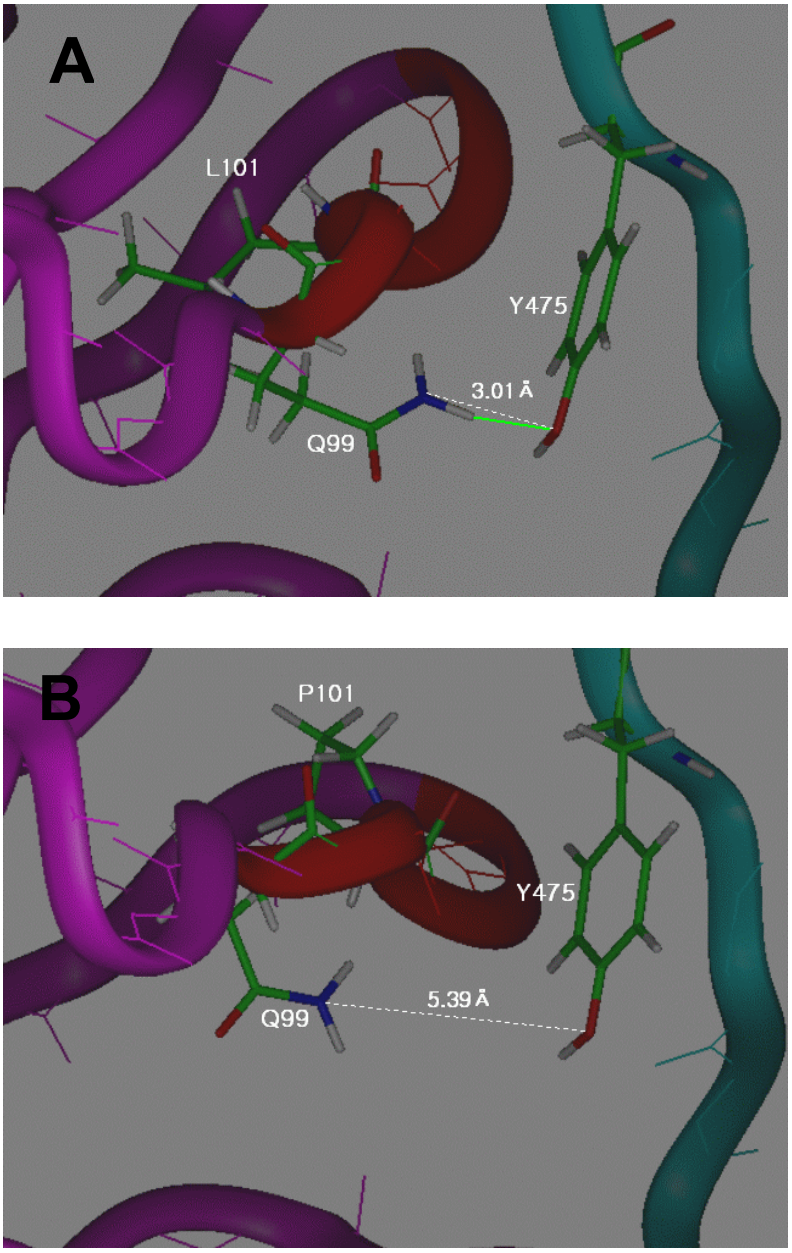

Fig. 8. Molecular modeling of L101 wild (A) and P101 mutant (B) WASP with WIP peptide. The hydrogen bond between Q99 in wild WASP and Y475 in WIP is shown in green line (B) and distances (white dotted line) between

side chain nitrogen of Q99 and the side chain oxygen of Y475 were 3.01 Å and 5.39 Å in wild WASP and L101P mutant respectively. Blue; nitrogen, red; oxygen, and green; carbon.

IV. DISCUSSION

Classically WAS was defined as a symptom triad of immunodeficiency, thrombocytopenia, and eczema, not all patient express these 3 symptoms. There are some patients who have a partial or variant phenotypic expression. Before the era of genetic studies for WAS, most of the clinical researchs were focused on the immunologic manifestations[7]. But the abnormalities of immune response were inconsistent and varied even within a single patient with time[29,30]. As the WASP was identified in the context of a search for the gene defect responsible for WAS, researchers are trying to find correlation between phenotype and genotype by mutational analysis of WASP gene. Recently, mutation in the GTP-binding domain (GBD) of WASP resulting in failure of autoinhibition was identified to cause X-linked neutropenia[31]. In this case, microthrombocytopenia was not present.

In Korea, only few cases of WAS are reported and none of them was a novel mutation[32-34]. We here report two novel mutations of L101P WASP missense and frameshift mutation of WASP 444 stop, respectively. WASP 444 stop by a G deletion in 1326-1329, which deleted partial region of V/C and whole A domain of WASP, is predicted to interfere the Arp2/3-mediated actin polymerization[1,18]. This effect is similar to that seen with 1030-1035 del G, 1109-1113 del C, or 1301-1305 del G, each of which has a stop codon at the 444th residue[35].

In case of L101P missense mutation of WASP, how the P101 WASP influences the interaction was predicted with WIP at the molecular level, although the L101 WASP is found not to be the site of the nuclear Overhauser effect (NOE) with the WIP peptide in the NMR spectroscopy study[20]. Considering that human WIP residues 461-485 wraps around the N-WASP EVH1 domain amino acids 26-147, a highly homologous protein to the human WASP WH1 domain[14], the L101P missense mutation induces WAS by weakening the interactions between WASP and WIP, even if residue L101 (or P101) has no direct interaction with WIP. Therefore, it is suggested that the hydrogen bond between Q99 WASP and Y475 WIP is one of important interactions, and its breakage by the mutation at Q99, or the neighboring amino acid which influences its side chain, can disrupt the WASP-WIP interaction. And we observed in our modeling study that the Q99R WASP mutation[36] weakened the WASP-WIP interactions (data not shown). In the study of the WASP-WIP interaction using the yeast two-hybrid system and in vitro WASP-WIP binding assay[37], WASP missense mutants such as R86H, Y107C, and A134T impaired the interaction with WIP in quantitative level, suggesting that the configuration is necessary for the interaction. So for the future work, the functional studies such as co-transfection or co-immunoprecipitation in cells will be helpful to show the consequences of L101P mutation with WIP.

Almost all missense mutations are located in exon 1 to 4 and most of the nonsense, frameshift, and splice site mutations are found in exons 6 to 11.

The genotype-phenotype correlation in WAS/XLT was reported independently by several investigators, and is still controversial. Greer et al.[36] and Schindelhauer et al.[22] suggested no strict correlations. In contrast, Zhu et al.[35] showed that missense mutations in exons 1-3 caused mild disease permitting WASP expression. Recently, Imai et al.[38] analyzed the clinical phenotype and course of 50 WAS/WLT patients longitudinally. They demonstrated that WASP-positive WAS patients had better hematological and immunological findings, such as bleeding tendency, episodes of infection and severity of eczema, and also had a longer survival time than WASP-negative patients. In L101P missense mutation, detectable WASP expression was shown but the patient showed a clinically WAS phenotype. It is presumed that the low level of WASP expression and the weakening of WASP-WIP interaction by the disruption of hydrogen bond are involved in the disease progression.

T cells of the affected patients with either mutation had dysfunctions of the continuous cell divisions, and T cell viabilities were severely decreased after mitogenic stimulation. The several reasons for defective T cell division and T cell viability were probably due to the decreased IL-2 production[39,40] or the increased apoptosis by Fas upregulation[41]. In the regulation of T cell activation, NF-AT/AP-1 gene transcription is important in mediating normal signals from the T cell receptor and Vav is a potent regulator of the IL-2 promoter by regulating the activity of the NF-AT/AP-1 transcriptional factor complex[42]. Savoy et al.[17] demonstrated that a functional WASP-WIP complex is

required to enhance Vav-mediated activation of NF-AT/AP-1 gene transcription. So it could be helpful to observe the NF-AT/AP-1 activity that is triggered upon WIP interaction with WH1 domain of WASP.

V. CONCLUSION

We found two novel mutations and we were able to propose a possible model for the molecular pathogenesis of WAS by analyzing interactions of WASP and WIP using a molecular modeling study. Even WASP L101 residue is not directly involved the interaction between WASP and WIP, L101P WASP cause disruption of hydrogen bond between Q99 of WASP and Y475 of WIP which resulted in a typical disease spectrum of WAS.

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ABSTRACT(IN KOREAN)

Wiskott-Aldrich syndrome 환자의 L101P WASP 돌연변이에 의한 WASP-WIP의 분자구조학적 변화

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김 문 규

Wiskott-Aldrich 증후군 (Wiskott-Aldrich syndrome, WAS)은 성염색체 열성으로 유전되는 드문 질환으로 습진, 혈소판감소증, 면역저하를 동반한다. 그 원인은 WAS단백 (WASP) 유전자의 돌연변이에 기인하며 성염색체 유전성 혈소판감소증 (X-linked thrombocytopenia)도 동일한 유전자의 이상에 의해 발생한다. 저자는 증례를 통해 새로이 발견된 돌연변이를 보고하는 바이다. 증례 1은 exon 3에 T336C로 변환되어 missense 돌연변이로 인해 101번 염기서열이 leucine에서 proline으로 치환되었다 (L101P). 증례 3은 exon 10에 1326-1329delG로 삭제되어 frameshift 돌연변이로 인해 444번 염기서열에서 멈추게 되었다. 증례 모두 WASP이 심하게 감소 내지

발견되지 않았고 T세포가 PHA 자극에 대해 반응하지 저하되어 전형적인 WAS를 나타내었다. CFSE-labelled T cell division assay를 통해 확인한 T세포의 분열은 정상의 6-7회에 비해 2-3회로 증례에서 현저하게 감소되어 있었다.

NMR spectroscopy 상에서 L101P WASP는 WASP-interacting protein (WIP)과 직접 수소 결합하여 nuclear Overhauser effect (NOE)가 나타나는 위치가 아니기 때문에 질환의 발병 기전을 밝히기 위해 컴퓨터를 이용한 단백질의 3차구조를 분석하였다. 그 결과 P101이 WASP의 Q99를 WIP의 Y475와 수소 결합하지 못하게 만들 수 있는 것을 확인하였다. 저자는 이 연구를 통해 WAS의 발병 기전에 있어 WASP-WIP의 결합을 3차구조의 분석으로 가능한 것을 보았다.

핵심되는 말 : Wiskott-Aldrich syndrome (WAS), WAS protein (WASP), WASP-interacting protein (WIP), molecular modeling