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Clinical implications of human cytomegalovirus glycoprotein B among immunocompromised patients in South Korea

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Clinical implications of human cytomegalovirus glycoprotein B among immunocompromised patients in South Korea

Directed by Professor June Myung Kim

The Doctoral Dissertation
submitted to the Department of Medicine of
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the
degree of Doctor of Philosophy

Sun Bean Kim

June 2018

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<ABSTRACT>

Clinical implications of human cytomegalovirus glycoprotein B among immunocompromised patients in South Korea

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(Directed by Professor June Myung Kim)

Background:

Human cytomegalovirus glycoprotein B (gB) is highly immunogenic and essential for both *in vivo* and *in vitro* viral entry to the host. It is well known that HCMV plays an important role in viral-host interaction. gB genotypes vary based on geographical distributions of global HCMV strains. However, few studies have analyzed gB genotypes and their association with clinical outcome in patients with various underlying diseases. There are no data concerning gB genotypes and their clinical manifestations in South Korea. This study aimed to analyze the gB genotype distribution and its association with clinically significant factors.

Methods:

This study was performed on 138 HCMV strains obtained from blood samples of 138 patients presenting with various diseases under immunosuppression at a

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single tertiary center between January 2013 and March 2014. Polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing analysis were used to identify the gB genotypes. Patients' clinical data were obtained from electrical medical records.

Results:

The frequency of the various immunosuppressive diseases was as follows: 45.7% (63/138) were hematologic malignancies, 22.5% (31/138) were solid organ malignancies, 18.8% (26/138) were infectious diseases with severe sepsis, 10.1% (14/138) were HIV infection, and 3.6% (5/138) were autoimmune diseases. The distribution of HCMV genotypes was as follows: gB1, 98 of 138 (71%); gB2, 1 of 138 (0.72%); and gB3, 39 of 138 (28.3%). Results with respect to previous viremia, previous ganciclovir use, Eastern Cooperative Oncology Group (ECOG) performance scale, acute kidney injury (AKI), shock, and intensive care unit (ICU) stay were not different between gB1 and gB3 among hematologic and solid organ malignancies.

However, the duration from hematopoietic stem cell transplantation (HSCT) to HCMV viremia was significantly longer for patients with gB1 infection than for those with gB3 infection (219 (5–912) vs. 57 (20–2099), p=0.04). Regarding AKI, shock, and ICU stay, gB3 was significantly more common than gB1 among infectious diseases with severe sepsis (gB1 vs. gB3, 61.1% (11/18) vs. 75% (6/8), p=0.02; gB1 vs. gB3, 55.6% (10/18) vs. 75% (6/8), p=0.02; and gB1 vs. gB3, 44.4% (8/18) vs. 50% (4/8), p=0.03). No statistically significant difference was found between the occurrence of HCMV disease and genotypes among patients with various diseases under immune suppression (p=0.47). No statistically significant difference was noted between genotypes and all causes of in-hospital mortality (p=0.37). However, all-cause in-hospital mortality was significantly different between gB1 and gB3 among infectious diseases with severe sepsis (gB1 vs. gB3, 12 (66.7%) vs. 6 (75%), p=0.04).

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Conclusion:

We found that the most common genotypes were gB1 (71%, 98/138) and gB3 (28.3%, 39/138) in South Korea, and the duration from HSCT to HCMV viremia was significantly longer for gB1 than for gB3. Regarding AKI, shock, and ICU stay, gB3 was significantly more common than gB1 among infectious diseases with severe sepsis. Based on these results, further studies on viral toxicity assays should be considered for infectious diseases.

Key words: Human cytomegalovirus; Glycoprotein B; Genotypes; Severity; Association factor

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Clinical implications of human cytomegalovirus glycoprotein B among immunocompromised patients in South Korea

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I. INTRODUCTION

Herpesviruses are double-stranded DNA, enveloped viruses that cause lifelong latent infections. These viruses are divided into 3 subfamilies, alpha-, beta-, and gammaherpesviruses.[1] Human cytomegalovirus (HCMV), a betaherpesvirus, was identified in 1956 for the first time. The name is derived from the fact that it causes enlargement of the infected cell (cytomegaly) and induces characteristic inclusion bodies.[2] The HCMV genome consists of double-stranded DNA of approximately 230,000 bp in length. The mature viral particles have a diameter of 150–200 nm. Like all herpes viruses, HCMV is sensitive to low pH, lipid-dissolving agents, and heat. HCMV has a half-life of approximately 60 min at 37^{o}C and is relatively unstable at -20^{o}C. It needs to be stored at a temperature of at least -70^{o}C in order to maintain its infectivity.[2] HCMV of human Herpes has the tropism in fibroblasts *in vitro*.[3] In the blood, HMCV is predominantly

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associated with granulocytes and macrophages. Similar to other herpes viruses, the HCMV double-stranded DNA is incorporated into the host chromosome after primary infection usually in early life and retained in a sleepless latent status during the lifetime in mainly CD34+ hematopoietic stem cell progenitors or CD14+ monocytes.[3,4] The temporary or sustained HCMV genetic replication through various mechanisms including immune evasion and epigenetic dysregulation can generate complete virions and lead to active cytolytic inflammation. This reactivation process can result in harmful effects categorized as direct tissue damage causing end-organ diseases and indirect immunomodulation or immune exhaustion or immunosenescence.[3,4]

Once an individual has contracted HCMV, the infection spreads throughout the body, where it can infect a variety of cell types encompassing nearly every organ system. This broad cellular tropism does not lead to the production of extracellular viral particles in the circulatory system during an acute infection. The mechanisms by which HCMV-infected cells transmit the virus to uninfected tissues are not well understood. One study suggested that interactions between viral glycoproteins on the surface of infected cells and cellular receptors on adjacent cells mediate cell to cell transmission.[5] In support of this hypothesis, it has been shown that direct contact between the plasma membranes of HCMV-infected and uninfected cells is required for cell to cell spread of the infection *in vitro*.[6] During infection, HCMV glycoproteins are expressed and presented on the cell surface.[7,8] The HCMV genome encodes many glycoproteins. Seven of these, gB, gH, gL, gO, UL 128, UL 130, and UL 131, are critical for cell entry.[9] HCMV gB is a type 1 transmembrane protein encoded by the UL 55 gene, with sequence variations in the open reading frame that exists as a proteolytically processed dimer on the surface of infected cells and the viral envelope.

Chou and Dennison invented a method of HCMV genotyping based on the *UL55* gene nucleotide sequence that encodes a variable region encompassing the protease cleavage site. They found that there were HinfI and RsaI restriction sites

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between nucleotides 1344 and 1440. Amplification of this region, using polymerase chain reaction (PCR) followed by restriction analysis, confirmed the existence of four different gB genotypes.[10] Binding of gB to cellular integrins or platelet derived growth factor receptor (PDGFR) has been proposed to initiate cellular signaling cascades necessary for viral internalization.[11,12] gB elicits a strong immune response in humans and induces the production of neutralizing antibodies, though most anti-gB antibodies are non-neutralizing.[13,14] gB is the most highly conserved of the HCMV entry glycoproteins and may represent a better antigenic target for monoclonal immunoglobulin therapy.[1] Several laboratories have reported that separate monoclonal antibodies to gB inhibit infection at different steps during viral entry.[15-17]

HCMV is acquired without symptoms at an early age in most healthy individuals and is maintained as a latent infection with continuous protein synthesis and intermittent activation throughout an individual's lifespan via various mechanisms including immune evasion and suppression of genes encoding immediate early protein synthesis.[18-21] This phenomenon can cause a wide range of indirect effects, including chronic inflammation as well as immunosenescence or immune exhaustion, even in immunocompetent populations.[22] The lytic replication of HCMV results in life-threatening tissue-invasive diseases affecting several organs including the lungs, retina, and gastrointestinal tract, particularly in severely immunocompromised patients, including those who have undergone solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT), or those with acquired immunodeficiency syndrome (AIDS).[23] The cytotoxic replication of HCMV in immunocompetent and immunocompromised individuals may induce severe inflammatory diseases in the lungs, upper or lower gastrointestinal tract, liver, central nervous system, and retina.[3] In particular, HCMV pneumonia and retinitis could be life-threatening or result in serious sequelae such as blindness in AIDS patients.[24] The indirect effects of HCMV can cause serious problems of increased

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mortality, graft dysfunction or failure, and acute/chronic rejection in severely immunosuppressive SOT and HSCT recipients.[24] In addition, chronic HCMV-specific-immune activation in the general population can become a predisposing factor for chronic inflammatory diseases including cardiovascular diseases (CVDs) and diabetes.[25] Although HCMV is reactivated from the latent state, expression into overt disease is only partially possible, and most cases are inhibited by immune responses and exhibit a latent asymptomatic infection.[4] Eventually, HCMV causes repeated inflammation throughout life and reactivation is a strong stimulant for HCMV-specific T cells. Chronic inflammation due to HCMV promotes atherosclerosis, autoimmune disorder, and inflammatory bowel disease.[24] While the traditional risk factors of CVD such as diabetes mellitus (DM), dyslipidemia, hypertension, and smoking are decreasing due to the high level of attention paid to health care control and exercise these days, CVDs such as ischemic heart diseases, acute myocardial infarction and stroke have showed increasing trends.[25,26] One of the main explanations supporting this phenomenon is HCMV infection and many studies have suggested that the risk of CVD is increased due to the underlying mechanism of atherosclerosis by chronic inflammation, endothelial cell injury, and changing the lipid profile or smooth muscle cell via HCMV infection. Other reports describe that HCMV increases the risk of CVD and mortality associated with immunosenescence.[25,26] Therefore, the surveillance of HCMV seropositivity indicating past asymptomatic HCMV infection may have the epidemiologic importance in an aging era and may be useful information in personalizing tailored anti-HCMV preventive strategies in SOT recipients.[24]

Several studies analyzing anti-HCMV-immunoglobulin G (IgG) tests have shown that HCMV seroprevalence rates varied from 20–100% according to region, race, socioeconomic status, sex, and age.[23,27] The serostatus of HCMV assessed using anti-HCMV IgG is an important pre-transplant risk parameter for post-transplant HCMV diseases.[28] The spectrum of diseases caused by HCMV

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varies. Despite not being as highly contagious as varicella zoster virus, HCMV is most commonly transmitted by intimate person-to-person contact or through fomites.[3] HCMV transmission is mostly dependent on host immunity and is never fully cleared by the immune system. HCMV infection in immunocompetent hosts is generally asymptomatic, although the virus persists lifelong in the host.[29] Recently, HCMV reactivation in critically ill non-immunocompromised patients, especially those receiving intensive care unit care, has received increased attention and a randomized control study was performed to evaluate the efficiency of HCMV prophylaxis for clinical outcome in this population.[30-32] However, HCMV infection in immunocompromised hosts induces severe illness, such as leukopenia, hepatitis, nephritis, pneumonia, esophagitis, enterocolitis, encephalopathy, or encephalitis, which lead to substantial morbidity and mortality.[33] HCMV is an important pathogen in transplant recipients and is capable of causing life-threatening conditions, such as tissue-invasive diseases and graft failure, which results in increased mortality.[33] Another critical issue for HCMV is intrauterine fetal or congenital infection through vertical transmission from the primary HCMV-infected mother as it can result in irreversible sequelae including neurological abnormalities such as microcephaly or hearing loss and premature birth or intrauterine growth retardation.[34] Congenital HCMV infection is the leading cause of non-genetic birth defects in the United States.[35,36] It is estimated that over 5,500 newborns suffer from sequelae of congenital HCMV infection each year, with clinical manifestations of microcephaly, sensorineural hearing and/or vision loss, mental retardation, and psychomotor impairment.[36] In Korea, 49 neonates were identified as having symptomatic congenital HCMV infection and the estimated incidence was 0.06% (49/81,229) between January 2001 and February 2015 in 7 university hospitals.[37] The need for a prophylactic vaccine against congenital HCMV infection and disease is increasing.[35] Similarly, restoration or reconstitution of host anti-HCMV immunity could provide long-term control of HCMV post-transplantation.[38,39] However, despite numerous

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active vaccine studies in the past 40 years, no approved vaccines are available.[40,41] Therefore, these detrimental effects of HCMV infection have prompted the development of well-tailored post-transplantation preventive strategies in SOT and HSCT recipients as well as routine national maternal screening programs.[42-44] As mentioned above, host immunity to HCMV infection is associated with numerous viral proteins and glycoproteins.[45] HCMV gB is a multifunctional envelope glycoprotein and is used for genotyping.[46] gB is highly immunogenic and is essential for both *in vivo* and *in vitro* viral entry and replication, and plays a role in viral–host interaction.[47] Based on the sequence variations, HCMV is classified into 4 major gB genotypes (from gB1 to gB4) and additional non-prototypic genotypes.[48] Nucleotide polymorphisms of the HCMV gB gene can affect the cell tropism of the virus and host immune response and are believed to have important roles in the pathogenesis of HCMV.[49] Recently, it has been proposed that HCMV disease and pathogenesis may be related to the genetic diversity of the virus.[50]

A previous study has suggested that different viral strains are related to various abilities to replicate in endothelial and smooth muscle cells that have different abilities to cause immunosuppression.[51] Nonetheless, the exact mechanisms through which these events occur remain poorly understood.[51] In addition, many studies have reported the association between different gB genotypes and their virulence, cell tropism, viral pathogenesis, and clinical manifestations.[52-58] Relying on the functions of gB, any alteration in the gB gene might predispose individuals to HCMV disease, either by promoting viral replication or provoking a immunopathological response, which has been linked with adverse outcomes[52,59] It remains unclear whether particular gB genotypes are related to a greater risk of developing HCMV disease. gB genotypes varies based on geographical distributions of global HCMV strains. In western countries, gB2 and gB3 are predominant,[54,55,58] whereas in eastern, gB1 is a predominant strain.[53,56,57] Considering regional differences in the frequency of gB genotypes,

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understanding the role of gB genotypes in virulence can be useful.[60,61] However, various studies have shown that the local incidence of individual genotypes may differ,[62,60,63] and most genotypes identified to date are probably distributed worldwide.[64-67] Data concerning gB genotypes and their clinical manifestations are not yet available in South Korea. Therefore, this study aimed to analyze the gB genotype distribution and its association with clinically significant factors, such as survival rate and occurrence of tissue-invasive diseases.

II. MATERIALS AND METHODS

1.Patients

A prospective cohort study was conducted at Severance Hospital, a 2000-bed, tertiary-care, university-affiliated referral center located in Seoul, Republic of Korea. A total of 138 blood samples from 138 patients who visited a single tertiary-care center for various diseases under immunosuppression, including hematologic malignancies with or without hematopoietic stem cell transplantation, solid organ malignancies, autoimmune disease, infectious diseases with severe sepsis, and human immunodeficiency virus (HIV), were examined from December 2012 to March 2014. Written informed consents were obtained from all patients before study enrollment. This study was approved by our local Ethics Committee and Institutional Review Board.

2. Definitions

A. Definition of immunocompromised patients

Patients whose immune systems are deficient because of congenital or acquired immunologic disorders (e.g., HIV infection, congenital immune deficiency syndromes), chronic diseases such as diabetes mellitus, cancer, emphysema, or cardiac failure, ICU care, malnutrition, and immunosuppressive therapy for another disease process [e.g., radiation, cytotoxic chemotherapy, anti-graft

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rejection medication, corticosteroids, monoclonal antibodies directed against a specific component of the immune system]).[68]

B. Definition of the high-risk group among patients with hematologic malignancies

The high-risk group was defined as comprising of patients with leukemia and lymphoma with the following features: (1) acute leukemia (AL) with the t(9;22), Flt3-ITD mutation or complex cytogenetics regardless of the disease stage; acute myeloid leukemia (AML) during complete remission (CR)1 after 3 or more cycles of induction, acute lymphoblastic leukemia (ALL) in CR1 beyond 4 weeks of induction, or AL in CR1 with positive minimal residual disease (MRD) after 2 cycles of consolidation; (3) AL beyond CR2 or in non-remission (NR) or chronic myeloid leukemia (CML) beyond the 1st chronic phase; (4) T-cell lymphoblastic lymphoma resistant to chemotherapy or autologous transplantation.[69] The high-risk group for multiple myeloma was characterized by the presence of at least 1 of the following chromosomal abnormalities; any of deletion(17p), translocation t(4;14)(p16;q32), or t(14;16)(q32;q23), [70] and the revised international staging system (R-ISS) over stage III.[71] The high-risk group for myelodysplastic syndromes (MDS) was defined as revised international prognostic scoring system (IPSS-R) over 4.5.[72] In terms of hematopoietic stem cell transplantation (HSCT), the high-risk group was defined as not in 1st CR among AL, not in CR among lymphoma or multiple myeloma, and advanced stage MDS (IPSS-R > 4.5), as described above.[73]

C. Definition of HCMV infection

HCMV infection was defined as virus isolation or detection of viral proteins (antigens) or nucleic acid in any body fluid or tissue specimen.[33]

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D. Definition of HCMV detection in blood

Several specific definitions for HCMV detection in the blood were recommended.[33]

Viremia. "Viremia" was defined as the isolation of HCMV using culture methods that involve the use of either standard or shell vial techniques.

Antigenemia. "Antigenemia" was defined as the detection of HCMV pp65 in leukocytes.[74]

DNAemia. "DNAemia" was defined as the detection of DNA in samples of plasma, whole blood, and isolated peripheral blood leukocytes or in buffy-coat specimens. There are several techniques available for the detection of DNAemia, including PCR-based techniques, hybrid capture, and branched-chain DNA analysis.[75]

E. Definitions of HCMV disease

The definitive diagnosis of tissue-invasive disease depends on the detection of HCMV in the tissue specimen, with the exception of central nervous system disease and retinitis. Identification of inclusion bodies or viral antigens in biopsy material using immunohistochemistry[76] is the preferred method for the diagnosis of tissue-invasive disease. Cultures (either more rapid shell vial or routine viral culture) should routinely be performed on gastrointestinal biopsies given the diagnostic challenges resulting from potentially negative blood testing. Culture or quantitative nucleic acid amplification testing (QNAT) results of a tissue specimen may be difficult to interpret, particularly in the setting of active viremia, as they could reflect shedding as well as active disease; however, if the tissue immunohistochemistry and blood DNAemia are negative, a positive tissue culture or QNAT can support the diagnosis of tissue-invasive disease.[77] The diagnosis of tissue-invasive HCMV disease, such as hepatitis and gastrointestinal infection, should be confirmed using immunohistochemistry or in situ DNA hybridization.[33] HCMV pneumonia was defined by the presence of signs and/or

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symptoms of pulmonary disease combined with the detection of HCMV in bronchoalveolar lavage fluid or lung tissue samples.[33] HCMV can also cause disease in other organs, and the definitions of these additional disease categories include the presence of compatible symptoms and signs and documentation of HCMV using biopsy (detection of HCMV using PCR alone is insufficient), with other relevant causes excluded.[33]

3. Study protocol

A. HCMV DNA extraction

HCMV DNA was extracted from plasma using the Qiamp Blood Kit (Qiagen, Chatsworth, CA) according to manufacture recommendations. DNA samples were re-suspended in 200 μL of diethylpyrocarbonate-treated water.

B. gB genotyping

For HCMV genotyping, PCR-based restriction fragment length polymorphism (PCR-RFLP) analysis was performed. The gB region was amplified using nested PCR with external primers (sense: 5'-GGC ATC AAG CAA AAA TCT-3', anti-sense: 5'-CAG TTG ACC GTA CTG CAC-3' with production of 482–488 bp) and internal primers (sense: 5'-TGG AAC TGG AAC GTT TGG C-3', antisense: 5' -GAA ACG CGCGGC AAT CGG-3' with production of 299–305 bp). DNA amplification was carried out starting with 50 μl PCR mixture containing 5 μl 10× PCR buffer [100 mM Tris-HCl, 20 mM $MgSO_4$, 100 mM KCl, 80 mM $(NH_4)SO_4$, 0.5% NP-40, pH 9.0], 200 uM dNTP, 2.5 U DNA polymerase, 25 pmol primers, and 5 μl extracted DNA. The thermocycling conditions of the first round consisted of 30 cycles of denaturation at 94℃ for 50 s, annealing at 60℃ for 50 s, and extension at 72℃ for 1 min; this was preceded by an initial denaturation step at 94℃ for 5 min and followed by a terminal extension at 72℃ for 5 min. The second round differed from the first in that 35 cycles were

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performed. The first round of amplification was conducted with primers gB 1043 and gB 1724 and the second round of PCR was performed with internal primers gB 1319 and gB 1604. The amplified gB products were digested with restriction enzyme *Hin*fI and *Rsa*I (Invitrogen, USA) at 37℃ for 3 h and separated using electrophoresis at 80 V in 7% polyacrylamide gel. Digested DNA was analyzed on 4% agarose gel and visualized with ethidium bromide. From the results of this analysis, 4 gB genotypes could be distinguished by their different patterns of fragment lengths, as described in a previous study,[10] and mixed gB genotypes were identified. If the results showed different patterns from these 4 genotypes, it was classified as "untype" (Figure 1).

C. DNA Sequencing

If RFLP failed to identify the genotype, the PCR products were subjected to sequencing analysis (O isolates). Products were sequenced using the Big Dye Terminator Kit (Applied Biosystems) in a 5% acrylamide gel automatic sequencer (ABI model 373, Applied Biosystems). The nucleotide sequences were edited using the BIOEDIT program (www.mbio.ncsu.edu/BioEdit/bioedit. html) and aligned using the NCBI (National Center of Biotechnology Information) sequence database.

D. Statistical analysis

Statistical analyses to correlate HCMV gB genotypes and patients' demographic data were performed using SPSS software, version 20. The Mann-Whitney *U* test, Fisher's exact test, and χ^2 test were applied, and a p-value < 0.05 was regarded as statistically significant.

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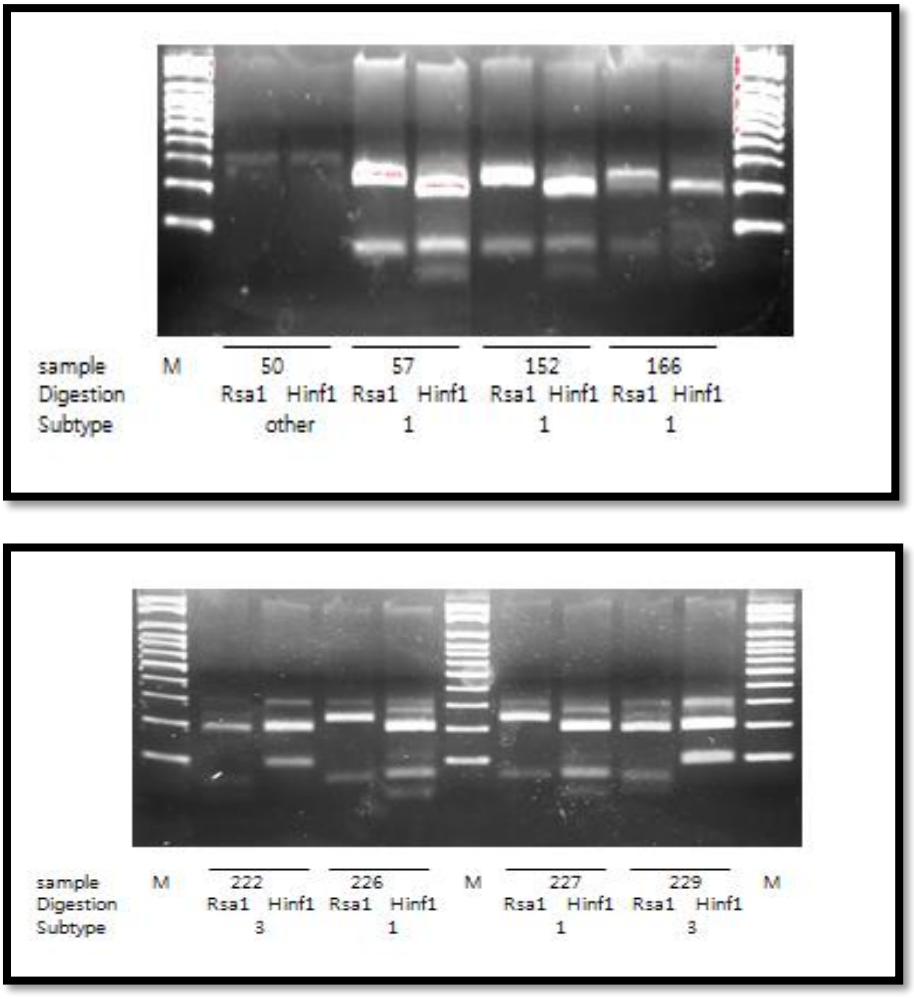

Figure 1. RFLP analysis of the glycoprotein B gene sequences.

The PCR products were digested by Hinf I and Rsa I, respectively. M = 20-bp DNA ladder marker (20 bp, 40 bp, 60 bp, 80 bp, 100 bp, 120 bp, 140 bp, 160 bp, 180 bp, 200 bp, 300 bp, 400 bp, and 500 bp).

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III. RESULTS

Characteristics of study participants

A total of 138 blood samples and clinical data were evaluated during the study period. The mean age (±SD) was 58.7 (±16) years, ranging from 21 to 95 years. Among the 138 patients, 84 (60.9%) were men. The most common comorbidities were hypertension (49.3%, 68/138), followed by diabetes (26.8%, 37/138), cardiovascular disease (18.8%, 26/138), and chronic renal disease (14.5%, 20/138). The frequency of the various immunosuppressive diseases was as follows: 45.7% (63/138) were hematologic malignancies, 22.5% (31/138) were solid organ malignancies, 18.8% (26/138) were infectious diseases with severe sepsis, 10.1% (14/138) were HIV infection, and 3.6% (5/138) were autoimmune diseases. The most common hematologic malignancies were malignant lymphoma (22.5%, 31/138), followed by AML (8.7%, 12/138), ALL (7.25%, 10/138), and multiple myeloma (5.1%, 7/138). The most common solid organ malignancies were lung cancer (8%, 11/138), followed by liver (1.4%, 2/138), breast (1.4%, 2/138), head and neck (1.4%, 2/138), and colon cancers (2.2%, 3/138). The most common infectious diseases with severe sepsis were pneumonia (14.5%, 20/138), followed by urinary tract (4.3%, 6/138), bone and soft tissue (2.2%, 3/138), and biliary tract (1.5%, 2/138) infections. HCMV diseases comprised 42% (58/138) of all cases. All-cause in-hospital mortality was 28.9% (40/138) (Table 1).

Distribution of HCMV genotypes

The distribution of HCMV genotypes was as follows: gB1, 98 of 138 (71%); gB2, 1 of 138 (0.72%); and gB3, 39 of 138 (28.3%). A total of 28 samples (9 with gB1, 19 with gB3) were sequenced. The distribution of gB1 and gB3 was not statistically different between age, sex, and underlying comorbidities.

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Table 1. Baseline characteristics of 137 patients included in this study according to glycoprotein B genotypes.

	gB1 (n=98)	gB3(n=39)	p- value
Age, median	59 (21-95)	57 (25-79)	0.56[a]
Sex, male	59 (60.2%)	24 (61.5%)	0.71[b]
Co-morbidities			
Diabetes mellitus	29 (29.6%)	7 (17.9%)	0.09[c]
Hypertension	51 (52%)	16 (41%)	0.3[b]
Chronic renal disease	12 (12.24%)	7 (18%)	0.77[c]
Chronic liver disease	6 (6.1%)	2 (5.1%)	0.94[c]
Chronic lung disease	9 (9.2%)	5 (12.8%)	0.9[b]
Cardiovascular disease	18 (18.4%)	8 (20.5%)	0.9[c]
Underlying disease related to immunocompromised conditions			
Hematologic malignancy	44 (44.9%)	19 (48.7%)	0.58[b]
Solid organ malignancy	20 (20.4%)	10 (25.6%)	0.14[b]
Auto-immune disease	4 (4.08%)	1 (0%)	0.89[b]
Infectious disease on severe sepsis	18 (18.4%)	8 (20.5%)	0.45
Human immunodeficiency virus	12 (12.2%)	2 (5.1%)	0.16[b]
Laboratory data			
Leukocyte count (uL)	6430 (30-46780)	5810 (190-22940)	0.52[a]
Hemoglobin (mg/dL)	9.1 (6.5-15.6)	9.4 (6.6-14.5)	0.64[a]
Serum CRP (mg/L)	41.5 (0.42-352.2)	32.7 (1-333.4)	0.001[a]
Serum ESR (mm/h)	42 (2-120)	48.5 (2-122)	0.04[a]
Serum Albumin (mg/dL)	2.8 (1.3-5.1)	2.9 (1.9-4.2)	0.25[a]
Viral loads (log copies/mL)	3.6 (2.4-6.2)	3.3 (2.7-5)	0.49[a]
Quantitative HCMV IgG (AU/mL)	46 (11-112)	42 (35-81)	0.5[a]
Acute kidney injury	28 (28.6%)	12 (30.8%)	0.03[c]
Shock	24 (24.5%)	14 (35.9%)	0.82[c]
ICU stay	20 (20.4%)	10 (52.6%)	0.34[c]
Evidence of previous viremia	22 (50%)	10 (52.6%)	0.85[b]
Previous ganciclovir use	9 (9.2%)	6 (15.4%)	0.48[b]

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ECOG performance status			0.43[b]
<2	30 (30.6%)	12 (30.8%)	
>=2	68 (69.4%)	27 (69.2%)	
Evidence of HCMV disease	40 (40.8%)	14 (35.9%)	0.47[b]
All-causes of in-hospital mortality	28 (28.6%)	12 (30.8%)	0.37[d]

Abbreviations: CRP=C-reactive protein; ESR= erythrocyte sedimentation rate; HCMV= human cytomegalovirus; ICU= intensive care; ECOG= Eastern Cooperative Oncology Group. Data are expressed as the median (interquartile range) and number (percent). [a]Mann-Whitney *U* test, [b]chi-square test, [c]Fisher's exact test, [d]Kaplan-Meier estimator

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They were not statistically different between gB1 and gB3 among hematologic malignancies, solid organ malignancies, and infectious diseases with severe sepsis ($p > 0.05$) (Table 1). Results with respect to previous viremia, previous ganciclovir use, Eastern Cooperative Oncology Group (ECOG) performance scale, acute kidney injury (AKI), shock, and intensive care unit (ICU) stay were not different among hematologic and solid organ malignancies (Tables 2 and 3). However, the duration from HSCT to HCMV viremia was significantly longer for gB1 than for gB3 (gB1 vs. gB3, 219 (5–912) vs. 57 (20–2099), $p = 0.04$) (Table 2). Regarding AKI, shock, and ICU stay, gB3 was significantly more common than gB1 among infectious diseases with severe sepsis (gB1 vs. gB3, 61.1% (11/18) vs. 75% (6/8), $p = 0.02$; gB1 vs. gB3, 55.6% (10/18) vs. 75% (6/8), $p = 0.02$; and gB1 vs. gB3, 44.4% (8/18) vs. 50% (4/8), $p = 0.03$) (Table 4). For gB2, the patient was an 80-year-old man with hypertension, diabetes, chronic renal disease, and lung cancer. His ECOG performance scale was over 2. The HCMV viral load was 2.65 log copies/mL.

HCMV disease, Graft-versus-host-disease, and gB genotype

The frequency of HCMV disease was 39.1% (54/138), the frequency of the gB1 genotype was 40.8% (40/98), and that of gB3 was 35.9% (14/39) (Table 1). There was no statistical difference between gB1 and gB3 among the hematologic malignancies ($p = 0.53$) (Table 2). However, among the solid organ malignancies, gB1 was much more common than gB3 (gB1 vs. gB3, 33.3% (6/20) vs. 25% (2/10), $p = 0.04$) (Table 3). There was no statistical difference between gB1 and gB3 for HCMV pneumonia, colitis, and retinitis ($p > 0.05$). Among the 19 patients undergoing allogeneic HSCT, graft-versus-host-disease (GVHD) occurred in 9 of 12 (75%) patients with gB1 and in 6 of 7 (85.7%) patients with gB3 (Table 2). In the univariate analysis, differences among patients with gB genotypes with respect to the GVHD were statistically insignificant ($p > 0.05$).

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Table 2. Baseline characteristics of patients with hematologic malignancies according to glycoprotein B genotypes.

	gB1 (n=44)	gB3(n=19)	p- value
Age, median	56.5 (21-93)	48 (25-77)	0.48 [a]
Sex, male	28 (63.6%)	10 (52.6%)	0.4 [b]
Co-morbidities			
Diabetes mellitus	10 (22.7%)	3 (15.8%)	0.74 [c]
Hypertension	22 (50%)	6 (31.6%)	0.18 [b]
Chronic renal disease	0 (0%)	1 (5.3%)	0.30 [c]
Chronic liver disease	3 (6.8%)	0 (0%)	0.55 [c]
Chronic lung disease	2 (4.5%)	0 (0%)	>.99 [b]
Cardiovascular disease	5 (11.4%)	3 (15.8%)	0.69 [c]
Underlying hematologic malignancies			
AML	7 (15.9%)	5 (26.3%)	0.49 [b]
ALL	5 (11.4%)	5 (26.3%)	0.15 [b]
CML	1 (2.3%)	0 (0%)	>.99 [b]
MDS	18 (18.4%)	8 (20.5%)	0.45[b]
Malignant lymphoma	23 (52.3%)	8 (42.1%)	0.59 [b]
MM	6 (13.6%)	1 (5.3%)	0.66[b]
Laboratory data			
Leukocyte count (uL)	3730 (30-46780)	3570 (190-11760)	0.82 [a]
Hemoglobin (mg/dL)	8.9 (6.5-15.2)	10.1 (6.6-14.5)	0.07 [a]
Serum CRP (mg/L)	34.19 (0.42-342.98)	20.1 (1-333.4)	0.86 [a]
Serum ESR (mm/h)	28.5 (2-120)	17.5 (2-79)	0.58 [a]
Serum Albumin (mg/dL)	2.95 (1.3-4.5)	3.3 (2.4-4.2)	0.07 [a]
Viral loads (log copies/mL)	3.62 (2.65-6.21)	2.65 (0-4.71)	0.03 [a]
Quantitative HCMV IgG (AU/mL)	46 (11-112)	42 (35-81)	0.7 [a]
Acute kidney injury	4 (9.1%)	4 (21.1%)	0.23 [c]
Shock	3 (6.8%)	1 (5.3%)	>0.99[c]
ICU stay	4 (9.1%)	3 (15.8%)	0.42 [c]
Evidence of previous viremia	22 (50%)	10 (52.6%)	0.85[b]
Previous ganciclovir use	7 (15.9%)	5 (26.3%)	0.49[b]
HSCT	15 (34.1%)	9 (47.4%)	0.5[b]

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Duration from HSCT to HCMV viremia, days	219 (5-912)	57 (20-2009)	0.04 [a]
ECOG performance status			0.26[b]
<2	21 (47.7%)	12 (63.2%)	
>=2	23 (52.3%)	7 (36.8%)	
Evidence of HCMV disease	20 (45.5%)	7 (36.8%)	0.53[b]
GVHD	9 (20.5%)	6 (31.6%)	0.65[c]
All-causes of in-hospital mortality	22 (50%)	7 (36.8%)	0.39[d]

Abbreviations: AML= acute myeloid leukemia; ALL= acute lymphoblastic leukemia; CML= chronic myeloid leukemia; MDS= myelodysplastic syndrome; MM= multiple myeloma; CRP=C-reactive protein; ESR= erythrocyte sedimentation rate; HCMV= human cytomegalovirus; ICU= intensive care unit; HSCT= hematopoietic stem transplantation; ECOG= Eastern cooperative oncology group; GVHD= graft-versus-host-disease. Data are expressed as the median (interquartile range) and number (percent). [a]Mann-Whitney *U* test, [b]chi-square test, [c]Fisher's exact test, [d]Kaplan-Meier estimator

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Table 3. Baseline characteristics of patients with solid organ malignancies according to glycoprotein B genotypes.

	gB1 (n=20)	gB3(n=10)	p- value
Age, median	58 (36-84)	48 (36-77)	0.47 [a]
Sex, male	13 (65%)	6 (60%)	0.54 [b]
Co-morbidities			
Diabetes mellitus	10 (50%)	5 (50%)	0.57[c]
Hypertension	12 (60%)	6 (60%)	0.34 [b]
Chronic renal disease	6 (30%)	4 (40%)	0.77[c]
Chronic liver disease	4 (20%)	2 (20%)	0.74[b]
Chronic lung disease	8 (40%)	2 (20%)	0.53 [c]
Cardiovascular disease	8 (40%)	3 (30%)	0.43 [c]
Types of solid organ cancer			
Head and neck cancer	3 (15%)	1 (10%)	0.34[b]
Breast cancer	2 (10%)	2 (20%)	0.23 [b]
Lung cancer	7 (35%)	3 (30%)	0.73 [b]
Gastric cancer	2 (10%)	0 (0%)	0.64 [b]
Colon cancer	2 (10%)	1 (10%)	0.87 [b]
Renal cell cancer	1 (5%)	1 (10%)	>.99 [b]
Liver cancer	2 (10%)	2 (20%)	0.67[b]
Ovarian cancer	1 (5%)	0 (0%)	0.87[b]
Laboratory data			
Leukocyte count (uL)	7715 (160-32640)	9620 (3550-19900)	0.43[a]
Hemoglobin (mg/dL)	8.8 (6.6-13.7)	9.2 (8.5-11.8)	0.64 [a]
Serum CRP (mg/L)	87.9 (11.6-352.2)	157.3 (64.8-294.4)	0.04[a]
Serum ESR (mm/h)	57 (2-120)	86 (60-120)	0.43 [a]
Serum Albumin (mg/dL)	2.6 (1.9-3.8)	2.5 (1.9-3)	0.47 [a]
Viral loads (log copies/mL)	3.6 (2.7-5)	3.1 (2.7-3.3)	0.49 [a]
Quantitative HCMV IgG (AU/mL)	42 (14-112)	44 (37-78)	0.53 [a]
Acute kidney injury	8 (40%)	4 (40%)	0.23 [c]
Shock	6 (30%)	4 (40%)	0.67[c]

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ICU stay	4 (20%)	2 (20%)	>.99[c]
Evidence of previous viremia	10 (50%)	5 (50%)	0.85[b]
Previous ganciclovir use	2 (10%)	1(10%)	0.48[b]
ECOG performance status			0.44[b]
<2	4 (22.2%)	2 (20%)	
>=2	14 (77.8%)	6 (60%)	
Evidence of HCMV disease	6 (33.3%)	2 (20%)	0.04[b]
All-causes of in-hospital mortality	12 (60%)	6 (60%)	0.07[d]

Abbreviations: CRP=C-reactive protein; ESR= erythrocyte sedimentation rate; HCMV= human cytomegalovirus; ICU= intensive care unit; ECOG= Eastern cooperative oncology group. Data are expressed as the median (interquartile range) and number (percent). [a]Mann-Whitney *U* test, [b]chi-square test, [c]Fisher's exact test, [d]Kaplan-Meier estimator

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Table 4. Baseline characteristics of patients with infectious diseases with severe sepsis according to glycoprotein B genotypes.

	gB1 (n=18)	gB3(n=8)	p- value
Age, median	58 (36-84)	48 (36-77)	0.53[a]
Sex, male	12 (66.7%)	6(75%)	0.43[b]
Co-morbidities			
Diabetes mellitus	10 (55.6%)	5 (62.5%)	0.66[c]
Hypertension	12 (66.7%)	6 (75%)	0.23[b]
Chronic renal disease	8 (44.4%)	4 (50%)	0.42 [c]
Chronic liver disease	6 (33.3%)	2 (25%)	>.99[b]
Chronic lung disease	8 (44.4%)	2 (25%)	0.47 [c]
Cardiovascular disease	8 (44.4%)	3 (37.5%)	0.53 [c]
Site of infection			
Pneumonia	10 (55.6%)	5 (62.5%)	0.34[b]
Urinary tract infection	4 (22.2%)	2 (25%)	0.23 [b]
Biliary tract infection	2 (11.1%)	0 (0%)	>.99[b]
Bone and soft tissue infection	2 (11.1%)	1 (12.5%)	>.99 [b]
Laboratory data			
Leukocyte count (uL)	14320 (5670-46780)	13780 (6480-11760)	0.78[a]
Hemoglobin (mg/dL)	11.2 (8.5-15.2)	12.1 (6.6-12.4)	0.76 [a]
Serum CRP (mg/L)	53.4 (43.2-342.98)	64.5 (34.5-333.4)	0.58[a]
Serum ESR (mm/h)	48.4 (12-79)	47.3 (28.5-120)	0.74 [a]
Serum Albumin (mg/dL)	3.2 (2.8-4.5)	2.8 (2.4-4.2)	0.07 [a]
Viral loads (log copies/mL)	3.6 (2.4-6.2)	3.1 (2.7-5)	0.49 [a]
Quantitative HCMV IgG (AU/mL)	40 (24-98)	38 (35-74)	0.48 [a]
Acute kidney injury	11 (61.1%)	6 (75%)	0.02[c]
Shock	10 (55.6%)	6 (75%)	0.02[c]
ICU stay	8 (44.4%)	4 (50%)	0.03 [c]
ECOG performance status			0.44[b]
<2	4 (22.2%)	2 (25%)	

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>=2	14 (69.4%)	6 (69.2%)	
Evidence of HCMV disease	6 (33.3%)	2 (25%)	0.04[b]
All-causes of in-hospital mortality	12 (66.7%)	6 (75%)	0.04[d]

Abbreviations: CRP=C-reactive protein; ESR= erythrocyte sedimentation rate; HCMV= human cytomegalovirus; ICU= intensive care unit. Data are expressed as the median (interquartile range) and number (percent). [a]Mann-Whitney *U* test, [b]chi-square test, [c]Fisher's exact test, [d]Kaplan-Meier estimator

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Frequency distributions of gB genotypes between high-risk and non-high-risk groups of hematologic malignancies

Table 5 shows the frequency distributions of gB genotypes between high-risk and non-high-risk groups of hematologic malignancies. The result was not significantly different between gB1 and gB3 in the high-risk group or the non-high-risk group with AML, ALL, CML, and MDS ($p > 0.05$).

Association factor for gB1 genotype

In multivariate analysis, higher viral load copies (odds ratio [OR], 3.03; 95% confidence interval [CI], 1.17–7.81; $p = 0.02$) and longer duration from HSCT to HCMV viremia (OR, 2.24; 95% CI, 1.56–34.31; $p = 0.04$) were associated with gB1 among hematologic malignancies (Table 6). In multivariate analysis, gB1 was not associated with solid organ malignancies (Table 7). The presence of AKI (OR, 0.92; 95% CI, 0.28–0.96; $p = 0.04$) and higher viral load copies (OR, 0.6; 95% CI, 0.24–0.92; $p = 0.03$) were not associated with gB1 among infectious diseases with severe sepsis (Table 8).

Overall survival and HCMV gB genotype

Among the 138 patients with active HCMV infection, 40 (29%) died during a median follow-up of 20 months. All-cause in-hospital mortality was not significantly different between gB1 and gB3 among hematologic (gB1 vs. gB3, 22 (50%) vs. 7 (36.8%), $p = 0.39$) and solid organ malignancies (gB1 vs. gB3, 12 (60%) vs. 6 (60%), $p = 0.07$). However, all-cause in-hospital mortality was significantly different between gB1 and gB3 among infectious diseases with severe sepsis (gB1 vs. gB3, 12 (66.7%) vs. 6 (75%), $p = 0.04$).

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Table 5. Frequency distributions of glycoprotein B genotypes between high-risk and non-high risk groups of hematologic malignancies.

	gB1	gB3	p- value
AML	(n=7)	(n=5)	0.58[b]
High risk, yes	3 (42.9%)	1 (20%)	
High risk, no	4 (57.1%)	4 (80%)	
ALL	(n=5)	(n=5)	>0.99[b]
High risk, yes	4 (80%)	3 (60%)	
High risk, no	1 (20%)	2 (40%)	
CML	(n=2)	(n=0)	>.99[b]
High risk, yes	2 (100%)	0 (0%)	
High risk, no	0 (0%)	0 (0%)	
MDS	(n=1)	(n=0)	>.99[b]
High risk, yes	1 (100%)	0 (0%)	
High risk, no	0 (0%)	0 (0%)	
Malignant lymphoma	(n=23)	(n=8)	0.34[b]
High risk, yes	19 (82.6%)	5 (62.5%)	
High risk, no	4 (17.4%)	3 (37.5%)	
MM	(n=6)	(n=1)	>.99[b]
High risk, yes	3 (50%)	1 (100%)	
High risk, no	3 (50%)	0 (0%)	
HSCT	(n=15)	(n=10)	>.99[b]
High risk, yes	3 (20%)	2 (20%)	
High risk, no	12 (80%)	8 (80%)	

Abbreviations: AML= acute myeloid leukemia; ALL= acute lymphoblastic leukemia; CML= chronic myelogenous leukemia; MDS= myelodysplastic syndrome; MM= multiple myeloma; HSCT= hematopoietic stem transplantation. Data are expressed as the number (percent). [a]Mann-Whitney *U* test, [b]chi-square test, [c]Fisher's exact test, [d]Kaplan-Meier estimator

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Table 6. Factors associated with glycoprotein B1 genotype among patients with hematologic malignancies.

Variables	OR	95% CI	p-value
Serum Hb	0.71	0.50-1.01	0.05
Serum Albumin	0.74	0.25-2.16	0.58
Viral load copies	3.03	1.17-7.81	0.02
Duration from HSCT to HCMV viremia, days	2.24	1.56-34.31	0.04

Abbreviations: Hb= hemoglobin; HCMV= human cytomegalovirus; OR= odds ratio; CI= confidential interval. Multivariate logistic regression analyses were performed with all statistically significant variables of less than 0.05 of *P*-value obtained from univariate analyses.

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Table 7. Factors associated with glycoprotein B1 genotype among patients with solid organ malignancies.

Variables	OR	95% CI	p-value
Evidence of HCMV disease	1.14	0.503-1.48	0.74
All-causes of in-hospital mortality	2.12	0.29-3.89	0.58
Viral load copies	2.74	0.29-4.3	0.68

Abbreviations: HCMV= human cytomegalovirus; OR= odds ratio; CI= confidential interval. Multivariate logistic regression analyses were performed with all statistically significant variables of less than 0.05 of *P*-value obtained from univariate analyses.

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Table 8. Factors associated with glycoprotein B1 genotype among patients with infectious diseases on severe sepsis.

Variables	OR	95% CI	p-value
Acute kidney injury	0.92	0.28-0.96	0.04
Shock	0.87	0.34-3.27	0.58
ICU stay	0.76	0.52-1.48	0.84
Evidence of HCMV disease	0.28	0.48-2.64	0.64
Viral load copies	0.6	0.24-0.92	0.03

Abbreviations: ICU= intensive care unit; HCMV= human cytomegalovirus; OR= odds ratio; CI= confidential interval. Multivariate logistic regression analyses were performed with all statistically significant variables of less than 0.05 of *P*-value obtained from univariate analyses.

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IV. DISCUSSION

Host immunity to HCMV infection is associated with numerous viral proteins and glycoproteins.[45] gB is highly immunologic and essential for both *in vivo* and *in vitro* viral entry and replication, and plays a role in viral–host interaction.[52] Together with gB, the gH/gL dimer compose the "core membrane fusion machinery" conserved among all herpes viruses. The dominant concept is that gH/gL complexes regulate the fusogenic activity of gB.[78] HCMV is an important opportunistic pathogen in severely immunocompromised patients that causes life-threatening conditions, such as tissue-invasive diseases and graft failure, and increases mortality/morbidities.[33]However, previous studies reported inconsistent results concerning various gB genotypes and their distributions in different groups of immunocompromised patients.[52,61,79-82] For instance, gB1 was highly prevalent in congenital infections in some studies;[52,80] one study reported that gB1 was associated with acute rejection in transplant recipients.[81] However, in another study, gB3 was predominant in congenital infection,[82] and with high incidence of HCMV pneumonitis[83] and gastrointestinal HCMV disease.[84] Acute GVHD and HCMV replication are pathogenetically related. One study demonstrated that most patients had GVHD before the onset of HCMV infection, confirming what several reports have already shown that acute GVHD and its treatment put patients at risk for HCMV replication.[85-88] In contrast, the role of HCMV replication as a cause of acute GVHD is still on debate. One small study showed no effect of HCMV replication on subsequent development of acute GVHD.[89] Others found the reciprocal results that patients are at considerable risk of developing acute GVHD during HCMV replication.[85,90] HCMV gB3 and gB4 were demonstrated to be related to myelosuppression in HSCT patients.[91] Some studies reported that HCMV gB3 was related to GVHD in HSCT patients,[75,84] but other study failed to show an association of gB genotypes with GVHD.[83] One study involving acquired immune deficiency syndrome (AIDS) patients reported that gB2 genotype was related to worse prognosis.[85] Similarly, gB3 showed a

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different behavior when compared with other genotypes, leading to the belief that this might be related to a more severe and uncontrolled infection that caused all cases of gB3 gastrointestinal HCMV disease and a worse survival.[84] In contrast, mixed infections with multiple gB genotypes were reportedly related to severe clinical manifestations.[61,79]

Many reports have attempted to find a relationship between gB genotype and the occurrence of HCMV associated disease in immunocompromised patients; however, it remains unclear whether certain gB genotypes are related to an increased frequency of disease.[92] There are few studies about functional differences that may exist among various HCMV strains. It was demonstrated that the existence of HCMV variants played an important role in the pathogenesis of diseases, as these variants affected several genes that might be responsible for different diseases related to active HCMV infection.[59,93-95]

Various studies have shown that the local incidence of individual genotypes may differ,[60,63,82] and most genotypes determined to date are probably distributed worldwide.[64-67] The current hypothesis to explain this wide variety of HCMV strains is that different HCMV genotypes have evolved over very long periods of time along with human populations, and that they have developed owing to population founder or bottleneck effects.[67,96] In more recent times, different virus strains have probably spread further between populations worldwide, and new strains have evolved due to recombination events, which may occur when more than one HCMV strain infects a host. Such recombination events are probably more common in immunosuppressed hosts, where different virus strains may replicate simultaneously to substantial levels.[97-100]

In this cohort of Korean patients with active HCMV infection, the most common genotypes were gB1 (71%, 98/138), gB3 (28.3%, 39/138), and gB2 (0.72%, 1/138). The distribution of gB1 and gB3 was not significantly different between age, sex, and underlying comorbidities. There was not statistically different between gB1 and gB3 among hematologic malignancies, solid organ

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malignancies, and infectious diseases with severe sepsis ($p > 0.05$). Our present results suggest that a higher viral load and a longer duration from HSCT to HCMV viremia were associated with gB1 among hematologic malignancies. Conversely, the presence of AKI and higher viral load were not associated with gB1 among infectious diseases with severe sepsis. For gB2, the patient was an 80-year-old man with hypertension, diabetes, chronic renal disease, and lung cancer. His ECOG performance scale was over 2. The HCMV viral load was 2.65 log copies/mL.

Moreover, a substantial number of HCMV vaccine strategies have been evaluated in preclinical and clinical trials over the years.[101-106] The major categories of HCMV vaccines that have made significant progress in clinical trials are replication-impaired or replication-defective HCMV (attenuated vaccines or disabled single-cycle [DISC] vaccines); adjuvanted recombinant protein vaccines based on the immunodominant envelope glycoprotein, HCMV gB; other expression strategies for HCMV gene products important in protective humoral and/or cellular immune responses, such as DNA and peptide vaccines; and vectored vaccines expressing gB and other HCMV antigens using a variety of live virus systems. In addition to gB, other viral antigens that have undergone evaluation as subunit or vectored vaccines in humans are the immunodominant T-cell target, ppUL83 (pp65), and the major immediate early protein 1.[107] The earliest attempts at developing a vaccine against HCMV infection utilized live, attenuated viruses. Initial studies focused on the attenuated HCMV strains AD169 and Towne. In addition, human challenge studies in vaccinated subjects have been performed with a less-attenuated HCMV strain, referred to as the Toledo strain. These strains have varying modifications in an area of the genome referred to the *ULb*' region. This region consists of sequences spanning HCMV ORFs UL128–151, sequences that are present in all low-passage primary clinical isolates but undergo extensive deletion, rearrangement and mutation after serial passage of the virus in cell culture, particularly in fibroblast cells.[108,109]

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Among them, gB is one of the most extensively studied HCMV antigens and is a potent inducer of HCMV-specific neutralizing antibodies and $CD4^+$ and $CD8^+$ T-cell responses in natural infection. While attenuated viruses were the first strategy investigated in HCMV vaccine development, subunit vaccines utilizing recombinant gB have advanced the furthest in clinical trials. gB works in concert with the gH/gL complex to facilitate viral entry into human fibroblasts, and with the pentameric complex to enter epithelial and endothelial cells.[47] Antibodies to gB are invariantly present in HCMV-seropositive individuals, and are capable of neutralizing virus infectivity.[7,110,111] Studies in both the murine and guinea pig models of HCMV infection identified that gB vaccines, expressed using a number of recombinant technologies, elicited immune responses that were protective in the context of subsequent viral challenge studies and, in the case of the guinea pig model, against the transplacental transmission of the virus.[112-114] Several phase II clinical trials which were double-blind, randomized and placebo-controlled trials, utilizing a recombinant HCMV gB in microfluoridized adjuvant 59 (MF59), a proprietary oil-in-water emulsion from Novartis first used in influenza vaccines, have been completed.[115-121] Most studies have adapted a three-dose series of vaccine. An initial phase I trial studying Sanofi's iteration of the gB subunit vaccine in an MF59 adjuvanted system found peak levels of antibody to gB to be higher in vaccinated individuals versus those who received the placebo.[116,121] The gB construct used in this trial was derived from the HCMV Towne strain gB sequence, and was modified such that the transmembrane domain and the furin cleavage site had been removed.[107] A phase II double-blinded study (NCT00299260) detected antibody titers and HCMV viremia in kidney or liver transplant patients after administration of gB/MF59 vaccine and demonstrated a significant increase in the gB-binding antibody titer one month after the second vaccine dose, regardless of initial HCMV serostatus.[122] Neutralizing antibody titers detected at the same time point yielded a significant increase in titer levels only in seropositive vaccine recipients. Seronegative organ

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recipients who received the experimental vaccine and had seropositive organ donors demonstrated reduced viremia and underwent days of ganciclovir treatment compared to those who received the placebo.[122] In addition, the duration of viremia that manifested post-transplantation was inversely correlated to gB antibody titers. The study proposed that antibodies induced by gB/MF59 vaccination may have limited the infectivity of HCMV virions released by the donated organs via an antibody-dependent cellular cytotoxicity mechanism,[122] in the process preventing the transmission of the virus to the susceptible host. The gB/MF59 vaccine has also been studied in postpartum women. A phase II randomized study (NCT00125502) demonstrated gB/MF59 to have 50% efficacy against primary HCMV infection in seronegative women vaccinated within one year of giving birth compared to women in the same trial who received the placebo.[115] Women who enrolled in this study but were identified to be HCMV-immune were also vaccinated with either the gB/MF59 vaccine or a placebo, in a parallel study aiming to investigate whether vaccination could augment the antibody response in seropositive individuals.[123] In detecting neutralizing antibody titers, gB specific responses were found to be boosted in vaccinated seropositive women compared to those who received the placebo.[123] Antibody titers remained higher in seropositive vaccine recipients at 6 months after the final vaccine dose than at day 0 of the vaccination series. The CD4+ T cell response to gB was higher on day 14 in vaccine recipients compared to their response at day 0 and to placebo recipients, and levels of interferon-γ producing T cells were higher in vaccine recipients at the majority of time points, including 6 months after the final vaccination.[123] A clinical trial was performed to investigate the efficacy and immunologic response to gB/MF59 in healthy, seronegative adolescent girls (NCT00133497). The incidence of HCMV infection after three vaccinations was decreased in the vaccine recipients as compared to the placebo recipients, although this difference was not significant ($p = 0.2$).[124]

Therefore, confirming the gB distribution among Koreans provides helpful

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information to develop a specific HCMV vaccine for Asian as well as Korean populations. Based on economic costs saved and the improvement in the quality of life that could potentially be conferred by a successful vaccine to prevent HCMV infection, the Institute of Medicine has identified HCMV vaccine development as a major public health priority. Moreover, numerous researchers have tried to investigate how individual HCMV genotypes might influence viral infections and diseases, motivated in part to intentionally determine possible prognostic factors that could predict the likelihood and/or severity of disease in immunocompromised individuals. Until recently, many studies have reported that gB distribution and clinical manifestations were not associated with each other.[54-58,79,80] Similar to previous studies in Asian regions, our study showed that the most common genotypes were gB1 (71%, 98/138) and gB3 (28.3%, 39/138). However, interestingly, the duration from HSCT to HCMV viremia was significantly longer for gB1 than for gB3 (gB1 vs. gB3, 219 (5–912) vs. 57 (20–2099), p = 0.04). Regarding AKI, shock, and ICU stay, gB3 was significantly more common than gB1 among infectious diseases with severe sepsis (gB1 vs. gB3, 61.1% (11/18) vs. 75% (6/8), p = 0.02; gB1 vs. gB3, 55.6% (10/18) vs. 75% (6/8), p = 0.02; gB1 vs. gB3, 44.4% (8/18) vs. 50% (4/8), p = 0.03). The frequency of HCMV disease was approximately 39.1% (54/138), the frequency of the gB1 genotype was 40.8% (40/98), and that of gB3 was 35.9% (14/39). gB distribution was not associated with HCMV disease, which is similar to the findings of previous studies.[54-58,79,80] In addition, gB distribution was not associated with GVHD.

In multivariate analysis, higher viral load copies (OR, 3.03; 95% CI, 1.17–7.81; p = 0.02) and longer duration from HSCT to HCMV viremia (OR, 2.24; 95% CI, 1.56–34.31; p = 0.04) were associated with gB1 among hematologic malignancies, and the presence of AKI (OR, 0.92; 95% CI, 0.28–0.96; p = 0.04), and higher viral load copies (OR, 0.6; 95% CI, 0.24–0.92; p = 0.03) were not associated with gB1 among infectious diseases with severe sepsis. All-cause in-

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hospital mortality was not significantly different between gB1 and gB3 among hematologic (gB1 vs. gB3, 22(50%) vs. 7(36.8%), p = 0.39) and solid organ malignancies (gB1 vs. gB3, 12(60%) vs. 6(60%), p = 0.07); however, it was significantly different between gB1 and gB3 among infectious diseases with severe sepsis (gB1 vs. gB3, 12 (66.7%) vs.6(75%), p = 0.04). Sepsis, defined as life-threatening organ dysfunction caused by dysregulated host responses to infection according to the Third International Consensus Definition for Sepsis and Septic Shock, is estimated to cause over 5.3 million deaths worldwide annually.[125,126] Classically, the time course of sepsis is characterized by pro-inflammatory and anti-inflammatory phases that occur during variable time sets after sepsis. Bacterial sepsis is an associated trigger of HCMV reactivation that was first recognized in the 1990s. One study first reported that a significant number of immunocompetent patients with mediastinitis following cardiac surgery had HCMV reactivation.[127] Subsequent work by Prosch and Volk and the Berlin group reported in a trio of manuscripts that HCMV reactivation occurs at a high rate in septic patients, and proposed that this reactivation might be a consequence of stimulation of the major immediate early promoter by tumor necrosis factor and nuclear factor-κB.[128-130] This clinical relationship was later experimentally identified by combining murine models of HCMV latency and polymicrobial sepsis.[131,132] It has been recently suggested that reactivation events related to sepsis are a consequence of inflammatory stimulation of the major immediate early promoter, transient immune compromise, and likely some part of epigenetic regulation of viral DNA.[133] It has been reported that pulmonary inflammatory responses induced by polymicrobial sepsis are exaggerated in mice with latent HCMV.[134] This exaggerated inflammatory response, something that is termed HCMV-associated lung injury,[135] is related to enhanced pulmonary fibrosis in latently infected mice after sepsis.[134] One study identified subsequently that previous infection with HCMV or the Epstein Barr Virus homolog γ-herpes virus-68 may offer protection against subsequent bacterial challenges.[136] The

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mechanism underlying this resistance seemed to be macrophage activation, and more recent work suggests that for HCMV infection this may be a consequence of enhanced Toll-like receptor (TLR) expression and responsiveness on infected macrophages.[137] This enhanced TLR responsiveness is accompanied by enhanced CD14 expression, thereby increasing macrophage responsiveness to TLR-2, TLR-4, and TLR-5 ligands. This enhanced TLR responsiveness might actually provide a survival advantage for the virus, by ensuring that the major immediate early promoter is stimulated repeatedly by endogenous bacteria throughout an infected hosts lifetime, perhaps causing viral shedding and thus transmission opportunities.[138] If this suggestion is true, then one would expect to see some differences in germ free hosts after HCMV infection. It is known that HCMV infection can induce impressive HCMV-specific T-cell responses, a phenomenon that is popularly referred to as 'memory inflation.' Interestingly, germ free mice do not induce memory inflation after HCMV infection, but do induce memory inflation after bacterial reconstitution.[139] This makes the enhanced TLR/CD14 expression after HCMV infection even more interesting, causing hosts to be even more susceptible to bacterial stimulation and inflammation. Such stimulation might explain the perpetual low level viral transcriptional activity during "latency,"[140,141] and in moments of immune weakness during relative health promote intermittent shedding, providing a survival advantage to the virus. In contrast, such enhancements in TLR might also exaggerate immune responses seen during sepsis, becoming harmful when those same hosts encounter severe bacterial infections.[138] Similarly, the septic response is caused by a deranged host response, and it is equally logical that HCMV preconditioning might contribute to such exaggerated inflammation. Hosts with concomitant HCMV reactivation and bacterial infections experience more inflammation and immune system activation that is accompanied by an increased risk of septic shock, supporting the detrimental proposal.[134,136,142] However, whether such viral preconditioning by HCMV has a beneficial or harmful impact on humans during bacterial septic

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challenges is not certain.[138] A previous study reported on more severe and uncontrolled gB3 gastrointestinal HCMV disease,[69] and our study showed the possibility of detrimental gB3. Therefore, viral toxicity assays for infectious diseases should also be considered.

V. CONCLUSION

Human cytomegalovirus (HCMV) is a large-envelope virus containing 220–240 kbps of double-stranded DNA, and belongs to the *Herpesviridae* family. The spectrum of diseases caused by HCMV varies, and is mostly dependent on host immunity. Host immunity to HCMV infection is associated with numerous viral proteins and glycoproteins. The HCMV gB is a multifunctional envelope glycoprotein and is used for genotyping. gB is highly immnogenic and essential for both *in vivo* and *in vitro* viral entry and replication and plays a role in viral–host interaction. Based on the sequence variations, HCMV is classified into four major gB genotypes (from gB1 to gB4) and additional non-prototypic genotypes.

Many studies have reported the association between different gB genotypes and their virulence, cell tropism, viral pathogenesis, and clinical manifestations. Relying on the functions of gB, any alteration in the gB gene might predispose individuals to HCMV disease, either by promoting viral replication or provoking a immunopathological response, which has been linked with adverse outcomes. It remains unclear whether particular gB genotypes are related to a greater risk of developing HCMV disease. Recently, it has been proposed that HCMV disease and pathogenesis may be related to the genetic diversity of the virus. gB genotypes varies based on geographical distributions of global HCMV strains. In western countries, gB2 and gB3 are predominant, whereas in eastern countries, gB1 is the predominant strain. Considering regional differences in the frequency of gB genotypes would be useful to understand the role of gB genotypes in virulence. However, various studies have shown that the local incidence of

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individual genotypes may differ, and most genotypes determined to date are probably distributed worldwide.

In our Korean cohort, the most common genotypes were gB1 (71%, 98/138), followed by gB3 (28.3%, 39/138), and the duration from HSCT to HCMV viremia was significantly longer for gB1 than for gB3. Regarding AKI, shock, and ICU stay, gB3 was significantly more common than gB1 among infectious diseases with severe sepsis. Based on these results, further studies on viral toxicity assays should be considered for infectious diseases.

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< ABSTRACT(IN KOREAN)>

국내 면역 저하자 환자들에서의 거대 세포 바이러스 당 단백질 B의 임상적 의의

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배경: 인간 거대 세포 바이러스의 당 단백질 B는 높은 면역원성을 가지고 있으며, 바이러스와 숙주 세포간의 상호 작용에 중요한 역할을 하는 것으로 알려져 있다. 당 단백질 B의 유전형은 지역별 분포에 차이가 있는 것으로 알려져 있으나 당 단백질 B의 유전형과 다양한 질병을 가진 환자들 간의 임상적 유용성에 대한 문헌은 많지 않고, 국내에서 이와 관련된 연구는 현재까지 이루어지지 않았다. 따라서 저자는 국내의 면역 저하 환자들에서 인간 거대 세포 바이러스의 당 단백질 B의 유전형을 확인하고, 생존율이나 조직 침습적 질환의 발생 등의 임상적 요인과의 연관성을 살펴보았다.

방법: 2013년 1월부터 2014년 3월 사이에 서울의 한 대학 병원에 내원하여 시행한 인간 거대 세포 바이러스 유전자 검사 양성 환자 가운데, 면역 저하 환자 총 138명을 대상으로 후향적 연구를 시행하였다. 중합 효소 연쇄반응-제한 절편 길이

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다형성 분석법과 염기서열 분석법을 통해 당 단백질 B의 유전자형을 확인하였고, 동반 질환과 치료 및 환자의 예후와의 관계를 분석하였다.

결과: 다양한 질환군의 면역 저하 환자들을 대상으로 연구가 진행되었으며, 혈액 종양 환자가 45.7% (63/138)로 가장 많았으며, 다음으로 고형암 환자 22.5% (31/138), 중증 패혈증으로 이환된 감염병 환자 18.8% (26/138), 인간 면역결핍 바이러스 환자 10.1% (14/138), 마지막으로 자가 면역 질환 환자 3.6% (5/138) 순의 빈도로 나타났다. 당 단백질 B의 유전형 분석 결과, 가장 흔한 것은 gB1형 (71%, 98/138)이었고, 그 다음으로 gB3형 (28.3%, 39/138)과 1명의 gB2형을 검출할 수 있었다 (0.7%, 1/138). 조혈모세포 이식을 받은 날부터 거대 세포 바이러스혈증이 발생하기까지의 기간이 gB1형에서 gB3형보다 더 길었다 (gB1 vs. gB3, 219일 (5–912) vs. 57일 (20–2,099), $p = 0.04$). 중증 패혈증으로 이환된 감염병 환자에 있어서는 급성 신부전, 저혈압, 중환자실 입원 유무가 gB3형에서 더 높은 빈도를 나타냈다 (gB1 vs. gB3, 61.1% (11/18) vs. 75% (6/8), $p = 0.02$; gB1 vs. gB3, 55.6% (10/18) vs. 75% (6/8), $p = 0.02$; and gB1 vs. gB3, 44.4% (8/18) vs. 50% (4/8), $p = 0.03$). 하지만, 면역 저하 상태에 있는 다양한 질병군 간에 거대 세포 바이러스병과 당 단백질 B의 유전형 간의 유의한 차이는 없었다. 또한 전체 질환군에서 원내 사망과 당 단백질 B의 유전형 간의 유의한 차이는 없었으나, 중증 패혈증으로 이환된 감염병 환자에 있어서는 gB1형과 gB3 형 간의 유의한 차이가 확인할 수 있었다 (gB1 vs. gB3, 12 (66.7%) vs. 6 (75%), $p = 0.04$).

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결론: 한국에서 인간 거대 세포 바이러스의 당 단백질 B의 유전형은 gB1 (71%, 98/138), gB3 (28.3%, 39/138) 순서로 흔하였으며, 조혈모세포 이식을 받은 날로부터 거대 세포 바이러스혈증이 발생하기까지의 기간이 gB1형에서 gB3형보다 더 길었다. 중증 패혈증으로 이환 된 감염병 환자에 있어서는 급성 신부전, 저혈압, 중환자실 입원 유무가 gB3형에서 더 높은 빈도를 나타내는 것으로 확인되었다.추후 거대 세포 바이러스의 유전형과 독성 분석 연구를 동시에 분석하는 것이 병리 기전을 이해하는 데에 도움이 될 것으로 생각된다.

핵심되는 말 : 인간 거대 세포 바이러스; 당 단백질 B; 유전형; 중증도; 연관 인자