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Polymorphisms of PAI-1 and platelet GP Ia may associate with impairment of renal function and thrombocytopenia in Puumala hantavirus infection

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Abstract

Introduction—Puumala virus (PUUV) infection is a viral hemorrhagic fever with renal syndrome (HFRS) characterized by thrombocytopenia and acute impairment of renal function. We aimed to assess whether genetic polymorphisms of platelet antigens together with those of von Willebrand factor (VWF) and plasminogen activator inhibitor (PAI-1) correlate with disease severity.

Patients and methods—172 consecutive hospital-treated patients with serologically confirmed acute PUUV infection were included. Platelet glycoprotein (GP) IIIa T>C (rs5918), GP Ia T>C (rs1126643), GP Ib C>T (rs6065), GP VI T>C (rs1613662), VWF A>G (rs1063856) and PAI-1 A>G (rs2227631) were genotyped. The associations of the rarer alleles with variables reflecting the severity of the disease were analyzed.

Results—PAI-1 G-carriers had higher maximum creatinine level compared with the non-carriers (median 213 $\mu\text{mol/l}$, range 60–1499 $\mu\text{mol/l}$ vs. median 122 $\mu\text{mol/l}$, range 51–1156 $\mu\text{mol/l}$, $p=0.01$). The GG-genotypes had higher creatinine levels than GA- and AA-genotypes (medians 249 $\mu\text{mol/l}$, 204 $\mu\text{mol/l}$ and 122 $\mu\text{mol/l}$, respectively, $p=0.03$). Polymorphisms of GP VI and VWF associated with lower creatinine levels during PUUV infection. The minor C-allele of GP Ia associated with lower platelet counts (median $44 \times 10^9/\text{l}$, range 20–90 $\times 10^9/\text{l}$ vs median $64 \times 10^9/\text{l}$, range 3–238 $\times 10^9/\text{l}$; $p=0.02$).

Conclusions—Polymorphism of PAI-1, a major regulator of fibrinolysis, has an adverse impact on the outcome of kidney function in PUUV-HFRS. Platelet collagen receptor GP Ia polymorphism associates with lower platelet count.

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Conflict of interest statement

The authors have no conflict of interest.

Keywords

Coagulation; Fibrinolysis; Hantavirus; HPA; Platelet; Polymorphism

Introduction

Puumala hantavirus (PUUV) infection, also known as nephropathia epidemica (NE), is the most common cause of hemorrhagic fever with renal syndrome (HFRS) in Europe. [1] In Finland the average annual incidence is 31/100 000 population and the incidence tends to increase. [2] The course of the disease may be divided into febrile, hypotensive, oliguric, diuretic and convalescent phases, but these phases may overlap, and the clinical severity varies. Renal involvement results in the need for transient hemodialysis treatment in 5% of hospital-treated NE patients. [1] Thrombocytopenia is a characteristic finding but bleeding manifestations usually remain mild. [3] The case fatality rate is 0.08%. [2]

The pathogenesis of NE is complex and multifactorial with host genetic properties having an impact on disease severity. Human leukocyte alloantigen (HLA) B8 and DRB1*0301 alleles are associated with severe clinical disease, and genetic polymorphisms of the cytokines tumor necrosis factor alpha, interleukin-1, and interleukin-1 receptor antagonist also influence the outcome of infection. [4,5] The surface density of platelet β_3 integrin, the cellular receptor of hantaviruses, is associated with disease severity in patients with Hantaan virus. [6] However, a human platelet alloantigen (HPA) -1 polymorphism which affects platelet glycoprotein (GP) IIIa carrying subunit β_3 was not associated with clinical disease severity. Instead, an association of HPA-3 with the severe form of Hantaan virus infection was reported. [7]

In the acute phase of NE, hemorrhagic problems together with thrombocytopenia and altered coagulation are known to occur, but the data published on platelet function and coagulation activity remain limited. We recently reported decreased levels of natural anticoagulants, shortened thrombin time and enhanced fibrinolysis in patients with acute NE. [8] We also found that the levels of three main platelet ligands for $\alpha II\beta_3$ (subunit β_3 integrin) were altered, i.e. von Willebrand factor (VWF) and fibrinogen were increased and plasma fibronectin was decreased during acute NE. [9]

Here we studied the genetic polymorphisms of the main antigens involved in platelet activation and aggregation, namely glycoprotein (GP) IIIa (HPA-1), GP Ib (HPA-2), GP Ia (HPA-5) and GP VI, together with VWF and plasminogen activator inhibitor (PAI) -1 in patients with acute NE. Our aim was to establish a possible association between these polymorphisms and the clinical course of NE.

Methods

Patients

The study was carried out at Tampere University Hospital and University of Tampere School of Medicine. All patients came from the Pirkanmaa region and were hospitalized at Tampere University Hospital due to serologically confirmed acute PUUV infection during the period from September 1997 to February 2009. [10] Written informed consent was obtained from all patients, and The Ethics Committee of Tampere University Hospital approved the study protocol.

The study group was comprised of 172 prospectively collected, consecutive patients (118 males), median age 40 years (ranging from 15 to 74 years). Concomitant diseases included

arterial hypertension (n=12), dyslipidemia (n=7), coronary heart disease (n=5), bronchial asthma (n=6), atrial fibrillation (n=3) and rheumatoid arthritis (n=3). There were patients with celiac disease, inflammatory bowel disease, valvular heart disease or neurological disease (n=2 for each).

Clinical and basic laboratory data

The following variables were recorded: the number of days from the onset of illness (i.e. fever) before the first blood test was taken, the length of hospital stay (days), clinical diagnosis of shock (yes/no), need for transient hemodialysis treatment (yes/no), the lowest systolic and diastolic blood pressure (mmHg), the lowest daily urinary output (ml) and maximum change in weight (kg). The last variable reflects fluid retention during the hospital stay in the oliguric phase of the disease. Complete blood count (CBC), plasma C-reactive protein (CRP) and plasma creatinine were measured according to the clinical indications, and were determined at the Laboratory Centre of the Pirkanmaa Hospital District using standard methods. Plasma interleukin-6 (IL-6) samples were collected up to five consecutive days after hospitalization and determined from 118 patients using a commercially available ELISA kit following the manufacturer's instructions (PeliKine Compact™ human IL-6 kit, Central Laboratory of The Netherlands, Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). The detection limit for the IL-6 assay was 0.4 pg/ml. The highest or the lowest individual value of the various variables measured during hospitalization was designated as the maximum or the minimum value.

Genotyping

DNA was extracted from whole blood using a commercially available kit (QIAGEN Inc., Hilden, Germany). The gene polymorphisms of GP IIIa (HPA-1) T>C (rs5918) and GP Ib (HPA-2) C>T (rs6065) were genotyped with Assay-By-Design from Applied Biosystems under standard conditions using the ABI Prism 7900HT Sequence Detection System (Taqman, Applied Biosystems, Foster City, CA, USA). The GP Ia (HPA-5) T>C (rs1126643) and the VWF A>G (rs1063856) were genotyped as previously described. [11,12] Genotyping of the GP VI T>C (rs1613662) was performed with minor modifications. [13] For PAI-1 A>G (rs2227631) a commercial kit from Applied Biosystems was used. The distributions of all single nucleotide polymorphisms (SNP) did not deviate from the Hardy–Weinberg equation. Genotyping was successful in 162 of 172 patients (94%) for GP IIIa, in all 172 patients for GP Ia, in 169 patients (98%) for GP Ib, in 171 patients (99%) for GP VI, in 162 patients (94%) for VWF and in 169 patients (98%) for PAI-1.

Statistical analysis

Since all continuous variables were skewed, medians and ranges were calculated to describe the data. Percentages were used for categorical variables. The allele frequencies were counted and the patients were divided in to the groups of carriers and non-carriers of the rarer (minor) allele. Comparisons between the groups are based on Mann–Whitney U or Kruskal-Wallis test for numerical and χ^2 or Fisher's exact test for categorical data. The limit of significance was set at 0.05 (2-tailed). If Bonferroni adjustments for multiple tests were applied, the limit of significance would be set at 0.01. SPSS version 14.0 was used for computation.

Results

Clinical and laboratory findings

All patients suffered from clinically typical PUUV infection. All patients were examined and hospitalized in the acute phase of the illness. The clinical findings and the basic laboratory values are shown in Table 1. Of all patients, 97% had their platelet count minimum below $150 \times 10^9/l$, the lower limit of normal range. There was no correlation between the minimum platelet count and the maximum plasma creatinine level. Three out of 172 patients (2%) were in clinical shock at the time of admission. Seven patients (4%) needed transient hemodialysis treatment. All patients recovered.

Polymorphisms and their associations with the clinical and laboratory findings

Table 2 presents the genotype distributions and allele frequencies of GP IIIa (HPA-1), GP Ib (HPA-2), GP Ia (HPA-5), GP VI, VWF and PAI-1. GP Ib (HPA-2) genotypes did not correlate with any of the variables measured (data not shown).

Renal Function

Renal function impairment in the acute phase of the disease associated with the rare G-allele of PAI-1 gene. The carriers of PAI-1 gene G-allele had 1.7 times higher maximum level of creatinine than the non-carriers (median $213 \mu\text{mol/l}$, range $60\text{--}1499 \mu\text{mol/l}$ vs. median $122 \mu\text{mol/l}$, range $51\text{--}1156$; $p=0.01$; Fig. 1A). Also, the level of creatinine exceeded $300 \mu\text{mol/l}$ 1.7 times more often in the G-carriers compared with the non-carriers (38% vs. 22%, $p=0.04$). As shown in Table 3, GG-homozygotes had the highest maximum level of creatinine of all PAI-1 carrier genotypes, followed by AG-heterozygotes and AA-homozygotes (median $249 \mu\text{mol/l}$, median $204 \mu\text{mol/l}$ and median $122 \mu\text{mol/l}$, respectively, $p=0.03$). Six out of seven hemodialysis patients were G-allele carriers, and one of them was a GG-homozygote.

The rare G-allele of VWF A>G was associated with a lower level of maximum creatinine (median $131 \mu\text{mol/l}$, range $52\text{--}1285 \mu\text{mol/l}$ vs. median $241 \mu\text{mol/l}$, range $51\text{--}1499 \mu\text{mol/l}$; $p=0.02$). Accordingly, the level of creatinine exceeded $300 \mu\text{mol/l}$ less often in the carriers of the VWF G-allele than in the non-carriers (21% vs. 38%, $p=0.03$).

Analyses of genotype combinations revealed that the patients ($n=18$, 11% of all patients) carrying the favorable VWF G-allele and not carrying the unfavorable PAI-1 G-allele had lower levels of creatinine compared with the patients not carrying the VWF G-allele and carrying the PAI-1 G-allele ($n=73$, 46% of all patients; $99 \mu\text{mol/l}$ vs. $250 \mu\text{mol/l}$, respectively, $p=0.002$).

The carriers of the GP VI gene rare C-allele had maximum creatinine levels $>300 \mu\text{mol/l}$ less often than non-carriers (17% vs. 36%, $p=0.03$).

Platelet count

The platelet count nadir was lower in carriers of the GP Ia (HPA-5) gene rare C-allele compared to non-carriers (median $44 \times 10^9/l$, range $20\text{--}90 \times 10^9/l$ vs. median $64 \times 10^9/l$, range $3\text{--}238 \times 10^9/l$; $p=0.02$). The minor G-allele carriers of PAI-1 gene turned out to have a higher platelet count nadir compared with non-carriers (median $65 \times 10^9/l$, range $9\text{--}238 \times 10^9/l$ vs. median $54 \times 10^9/l$, range $3\text{--}187 \times 10^9/l$; $p=0.04$; Fig. 1B). Minimum platelet counts by genotypes of GP Ia (HPA-5) and PAI-1 are presented in Table 4.

Inflammation

The maximum level of plasma IL-6 was lower in carriers of the rare GP IIIa (HPA-1) C-allele than in non-carriers (median 13.0 pg/ml, range 1.3–60 pg/ml vs. median 16.5 pg/ml, range 2.6–107 pg/ml; $p=0.05$). There were no significant associations with GP IIIa (HPA-1) genotype and CRP or leukocyte count (data not shown).

We found no statistically significant differences in the length of hospital stay, lowest blood pressure, maximum weight change, need for transient hemodialysis treatment or occurrence of shock compatible with polymorphisms studied here (data not shown).

Discussion

In our study, carriers of the PAI-1 gene minor G-allele suffered from more severe renal impairment than non-carriers. Both the maximum level of creatinine and the proportion of patients having their creatinine value $>300 \mu\text{mol/l}$ were higher in the carriers of G-allele compared with non-carriers. GG-homozygotes had the highest creatinine levels, and 6/7 patients who needed transient hemodialysis treatment were G-allele carriers. The platelet count nadir of the PAI-1 G-allele carriers was higher than that of non-carriers, and was concordant with our observation that the severity of renal impairment does not associate with the depth of thrombocytopenia in acute PUUV infection.

PAI-1 is secreted by a variety of cells including platelets and endothelial cells in response to inflammatory stimuli, and PAI-1 is the primary physiological regulator of tissue- and urokinase-type plasminogen activators and fibrinolysis. [14] PAI-1 polypeptide is encoded by the SERPINE1 (SERine Proteinase INhibitor, clade E, member 1) gene on chromosome 7q21.3-q2, and association of the 4 G/5 G polymorphism (rs1799889) with increased PAI-1 levels of 4 G-allele carriers has been suggested by several studies. Elevated levels of PAI-1 have been related to poor outcome in several infections such as pneumonia, sepsis, meningococcal disease, and dengue virus infection. [15–21] The 4 G-allele also seems to associate with atherosclerotic diseases of coronary arteries and reduced kidney graft survival. [14,22] We studied the SNP rs2227631 of the PAI-1 gene which is very close to 4 G/5 G polymorphism (rs1799889) and has a relatively high correlation with 4 G/5 G. [14,23] We found that the carriers of the rare G-allele suffered from more severe renal failure during the acute phase of NE compared with non-carriers. Unfortunately, we could not measure the plasma levels of PAI-1, and to our knowledge, there are no data on the plasma level and role of PAI-1 in hantavirus infections. Thus the plausible increase of PAI-1 level in response to infection and acute phase reaction and its modification by polymorphisms of PAI-1 gene in hantavirus infection remain the subjects of further studies.

The hemostatically active ligand VWF plays a critical role in platelet aggregation and adhesion to injured endothelium during primary hemostasis and pathologic thrombus formation. The VWF gene is highly polymorphic, and genetic factors are responsible for up to 66% of the variation in plasma VWF levels. [24] We recently observed high plasma VWF concentrations during acute PUUV infection. [9] Here we studied the A>G polymorphism of the VWF gene (rs 1063856) in exon 18 of chromosome 12. This polymorphism has been associated with high VWF levels, and is close to SNP rs10638857 which is known to associate with VWF levels in healthy subjects. [24–26] We found the minor G-allele (rs 1063856) of the VWF gene to have some protective role in kidney function, but we were unable to conclude whether this favorable effect depends on modulation of plasma VWF levels.

Binding of collagen to GP VI leads to platelet activation and pro-coagulant activity contributing to the formation of the hemostatic plug. Low frequency allele homozygotes of

GP VI, such as the CC genotype of T>C rs 1613662, are associated with reduced activation and platelet responses to collagen when compared with high frequency allele homozygotes. [27] In this study, the rare C-allele of GP VI offered some protection of renal function, but did not correlate with the platelet count or other variables of disease severity. A lower platelet count nadir was associated with the low frequency C-allele of GP Ia (HPA-5), the other major platelet receptor for collagen. [28] However, the associations between genetic polymorphisms and platelet function could not be studied here.

GP IIbIIIa complex mediates platelet aggregation by binding the adhesive proteins VWF, fibrinogen, and thrombus stabilizing fibronectin. [29] GP IIbIIIa carries the HPA-1 polymorphism site and contains subunit β_3 integrin (IIIa), the receptor for hantaviruses. [30,31] We found that carriers of the low frequency C-allele of GP IIIa (HPA-1) had slightly lower levels of plasma IL-6, a variable known to positively correlate with the severity of PUUV infection.[32] Our findings are similar to the only previous study on hantavirus infection and GP IIIa polymorphism which found that the HPA-1 polymorphism does not associate with disease severity.[7]

Due to the design of the study, we were not able to obtain reliable information about bleeding symptoms, plasma levels of PAI-1 and VWF, or platelet functions. However, for the first time, genetic polymorphisms related to platelet activation, blood coagulation and fibrinolysis were shown to have an impact on kidney function and platelet count in HFRS. Future studies could clarify the role of each of these genetic polymorphisms, their synergistic effects, and their functional relevances in PUUV-induced HFRS.

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Abbreviations

PUUV	Puumala virus
HFRS	hemorrhagic fever with renal syndrome
VWF	von Willebrand factor
PAI	plasminogen activator inhibitor
GP	glycoprotein
NE	nephropathia epidemica
HLA	human leukocyte antigen
HPA	human platelet alloantigen
CBC	complete blood count
CRP	C-reactive protein

IL-6 interleukin-6
SNP single nucleotide polymorphism

References

1. Vapalahti O, Mustonen J, Lundkvist Å, Henttonen H, Plyusnin A, Vaheri A. Hantavirus infections in Europe. *Lancet Infect Dis.* 2003; 3:653–61. [PubMed: 14522264]
2. Makary P, Kanerva M, Ollgren J, Virtanen MJ, Vapalahti O, Lyytikäinen O. Disease burden of Puumala virus infections, 1995–2008. *Epidemiol Infect.* 2010; 138:1484–92. [PubMed: 20109263]
3. Settergren B. Nephropathia epidemica (hemorrhagic fever with renal syndrome in Scandinavia). *Rev Infect Dis.* 1991; 13:736–44. [PubMed: 1681581]
4. Mustonen J, Partanen J, Kanerva M, Pietila K, Vapalahti O, Pasternack A, et al. Genetic susceptibility to severe course of nephropathia epidemica caused by Puumala hantavirus. *Kidney Int.* 1996; 49:217–21. [PubMed: 8770970]
5. Makela S, Hurme M, Ala-Houhala I, Mustonen J, Koivisto A-M, Partanen J, et al. Polymorphism of the cytokine genes in hospitalized patients with Puumala hantavirus infection. *Nephrol Dial Transplant.* 2001; 16:1368–73. [PubMed: 11427627]
6. Liu Z, Gao M, Han Q, Fang J, Zhao Q, Zhang N. Intensity of platelet B₃ integrin in patients with hemorrhagic fever with renal syndrome and its correlation with disease severity. *Viral Immunol.* 2008; 21:255–61. [PubMed: 18419255]
7. Liu Z, Gao M, Han Q, Lou S, Fang J. Platelet glycoprotein IIb/IIIa (HPA-1 and HPA-3) polymorphisms in patients with hemorrhagic fever with renal syndrome. *Human Immunol.* 2009; 70:452–6. [PubMed: 19286442]
8. Laine O, Makela S, Mustonen J, Huhtala H, Szanto T, Vaheri A, et al. Enhanced thrombin formation and fibrinolysis during acute Puumala hantavirus infection. *Thromb Res.* 126:154–8. [PubMed: 20579693]
9. Laine O, Makela S, Mustonen J, Helminen M, Vaheri A, Lassila R, et al. Platelet ligands and ADAMTS13 during Puumala hantavirus infection and associated thrombocytopenia. *Blood Coagul Fibrinolysis.* 22:468–72. [PubMed: 21508829]
10. Vapalahti O, Lundkvist A, Kallio-Kokko H, Paukku K, Julkunen I, Lankinen H, et al. Antigenic properties and diagnostic potential of puumala virus nucleocapsid protein expressed in insect cells. *J Clin Microbiol.* 1996; 34:119–25. [PubMed: 8748286]
11. Kekomaki S, Partanen J, Kekomaki R. Platelet alloantigens HPA-1, -2, -3, -5 and -6b in Finns. *Transfus Med.* 1995; 5:193–8. [PubMed: 8593523]
12. Kunkel GR, Graham JB, Fowlkes DM, Lord ST. RsaI polymorphism in von Willebrand factor (vWF) at codon 789. *Nucleic Acid Res.* 1990; 18:4961. [PubMed: 1975676]
13. Lepantalo A, Mikkelsson J, Resendiz JC, Viiri L, Backman JT, Kankuri E, et al. Polymorphisms of COX-1 and GPVI associate with the antiplatelet effect of aspirin in coronary artery disease patients. *Thromb Haemost.* 2006; 95:253–9. [PubMed: 16493486]
14. Koch W, Schrepf M, Erl A, Mueller JC, Hoppmann P, Schömig A, et al. 4G/5G polymorphism and haplotypes of SERPINE1 in atherosclerotic diseases of coronary arteries. *Thromb Haemost.* 2010; 103:1170–80. [PubMed: 20352162]
15. Hermans PW, Hazelzet JA. Plasminogen activator inhibitor type 1 gene polymorphism and sepsis. *Clin Infect Dis.* 2005; 41(Suppl 7):453–8.
16. Hermans PW, Hibberd ML, Booy R, Daramola O, Hazelzet JA, de Groot R, et al. 4G/5G promoter polymorphism in the plasminogen-activator-inhibitor-1 gene and outcome of meningococcal disease. Meningococcal Research Group. *Lancet.* 1999; 354:556–60. [PubMed: 10470700]
17. Mairuhu AT, Setiati TE, Koraka P, Hack CE, Leyte A, Faradz SM, et al. Increased PAI-1 plasma levels and risk of death from dengue: no association with the 4G/5G promoter polymorphism. *Thromb J.* 2005; 3:17. [PubMed: 16274483]

18. Van Gorp EC, Setiati TE, Mairuhu AT, Suharti C, Cate H, Dolmans WM, et al. Impaired fibrinolysis in the pathogenesis of dengue hemorrhagic fever. *J Med Virol*. 2002; 67:549–54. [PubMed: 12116003]
19. Madach K, Aladzsity I, Szilagyi A, Fust G, Gal J, Penzes I, et al. 4G/5G polymorphism of PAI-1 gene is associated with multiple organ dysfunction and septic shock in pneumonia induced severe sepsis: prospective, observational, genetic study. *Crit Care*. 2010; 14:R79. [PubMed: 20429897]
20. Kornelisse RF, Hazelzet JA, Savelkoul HF, Hop WC, Suur MH, Borsboom AN, et al. The relationship between plasminogen activator inhibitor-1 and proinflammatory and counterinflammatory mediators in children with meningococcal septic shock. *J Infect Dis*. 1996; 173:1148–56. [PubMed: 8627066]
21. Wills BA, Oragui EE, Stephens AC, Daramola OA, Dung NM, Loan HT, et al. Coagulation abnormalities in dengue hemorrhagic Fever: serial investigations in 167 Vietnamese children with Dengue shock syndrome. *Clin Infect Dis*. 2002; 35:277–85. [PubMed: 12115093]
22. Rerolle JP, Munteanu E, Drouet M, Szelaq JC, Champtiaux B, Yagoubi F, et al. PAI-1 donor polymorphism influences long-term kidney graft survival. *Nephrol Dial Transplant*. 2008; 23:3325–32. [PubMed: 18463321]
23. Kathiresan S, Gabriel SB, Yang Q, Lochner AL, Larson MG, Levy D, et al. Comprehensive survey of common genetic variation at the plasminogen activator inhibitor-1 locus and relations to circulating plasminogen activator inhibitor-1 levels. *Circulation*. 2005; 112:1728–35. [PubMed: 16172282]
24. Campos M, Sun W, Yu F, Barbalic M, Tang W, Chambless LE, et al. Genetic determinants of plasma von Willebrand factor antigen levels: a target gene SNP and haplotype analysis of ARIC cohort. *Blood*. 2011; 117:5224–30. [PubMed: 21343614]
25. Lacquemant C, Gaucher C, Delorme C, Chatellier G, Gallois Y, Rodier M, et al. Association between high von willebrand factor levels and the Thr789Ala vWF gene polymorphism but not with nephropathy in type I diabetes. The GENEDIAB Study Group and the DESIR Study Group. *Kidney Int*. 2000; 57:1437–43. [PubMed: 10760079]
26. Klemm T, Mehnert AK, Siegemund A, Wiesner TD, Gelbrich G, Buchner M, et al. Impact of the Thr789Ala variant of the von Willebrand factor levels, on ristocetin co-factor and collagen binding capacity and its association with coronary heart disease in patients with diabetes mellitus type 2. *Exp Clin Endocrinol Diabetes*. 2005; 113:568–72. [PubMed: 16320153]
27. Joutsu-Korhonen L, Smethurst PA, Rankin A, Grey E, IJsseldijk M, Onley CM, et al. The low-frequency allele of the platelet collagen signaling receptor glycoprotein VI is associated with reduced functional responses and expression. *Blood*. 2003; 101:4372–9. [PubMed: 12560230]
28. Meisel C, Lopez JA, Stangl K. Role of platelet glycoprotein polymorphisms in cardiovascular diseases. *Naunyn Schmiedebergs Arch Pharmacol*. 2004; 369:38–54. [PubMed: 14614592]
29. Denis CV, Wagner DD. Platelet adhesion receptors and their ligands in mouse models of thrombosis. *Arterioscler Thromb Vasc Biol*. 2007; 27:728–39. [PubMed: 17272754]
30. Gavrilovskaya IN, Brown EJ, Ginsberg MH, Mackow ER. Cellular entry of Hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by B₃ integrins. *J Virol*. 1999; 73:3951–9. [PubMed: 10196290]
31. Saidi S, Mahjoub T, Slamia LB, Ammou SB, Al-Subaie AM, Almawi WY. Polymorphisms of the human platelet alloantigens HPA-1, HPA-2, HPA-3, and HPA-4 in ischemic stroke. *Am J Hematol*. 2008; 83:570–3. [PubMed: 18383324]
32. Outinen TK, Makela SM, Ala-Houhala IO, Huhtala HS, Hurme M, Paakkala AS, et al. The severity of Puumala hantavirus induced nephropathia epidemica can be better evaluated using plasma interleukin-6 than C-reactive protein determinations. *BMC Infect Dis*. 10:132. [PubMed: 20500875]

Fig. 1.

Box plots of the highest plasma creatinine (A) and the blood platelet count nadir (B) in non-carriers (n=54) and carriers (n=115) of the plasminogen activator inhibitor (PAI-1) G-allele (rs 2227631) in 169 patients with PUUV infection. Box plot illustrates the median, the 25% and 75% percentiles, and the lowest and highest values that are not outliers. Outliers are indicated by open circles and extreme values by asterisks.

Table 1

The clinical and basic laboratory findings in 172 patients with acute Puumala hantavirus infection.

Clinical or laboratory variable	Median	Range
Days from onset of illness*	4	1–14
Length of hospital stay (days)	6	2–15
Systolic BP min (mmHg)	113	74–170
Diastolic BP min (mmHg)	70	40–100
Daily urinary output min (ml)	1450	50–7000
Change in weight max (kg)**	2.1	0.0–12.0
Hematocrit min	0.36	0.25–0.46
Platelet count min ($\times 10^9/l$)	62	3–238
Leukocyte count max ($\times 10^9/l$)	10	3.9–31.2
CRP max (mg/ml)	75	11–269
Interleukin-6 max (pg/ml)	14.5	1.3–107
Creatinine max ($\mu\text{mol/l}$)	185	51–1499

Abbreviations: min=minimum, max=maximum. References: CRP <10 mg/l, creatinine <105 $\mu\text{mol/l}$ for males and <95 $\mu\text{mol/l}$ for females, platelet count 150–360 and leukocyte count $3.4\text{--}8.2 \times 10^9/l$, hematocrit 0.35–0.50 for males and 0.35–0.46 for females.

* Days from onset of illness equals to the number of days of fever before the first blood test was taken.

** Change in weight during hospital stay reflects the amount of fluid accumulating in the body during the oliguric phase.

The genotype distributions and allele frequencies of platelet GP IIIa, GP Ib, GP Ia, GPVI, VWF and PAI-1 in 172 patients with acute Puumala hantavirus infection.

Table 2

Polymorphism	Genotype %			Allele %	
	homozygote common	heterozygote	homozygote rare	common	rare
GP IIIa (HPA-1)	66 (TT)	32 (TC)	2 (CC)	82 (T)	18 (C)
GP Ib (HPA-2)	78 (CC)	21 (CT)	1 (TT)	88 (C)	12 (T)
GP Ia (HPA-5)	93 (TT)	7 (TC)	0 (CC)	96 (T)	4 (C)
GP VI T>C (rs1613662)	79 (TT)	19 (TC)	2 (CC)	89 (T)	11 (C)
VWF A>G (rs1063856)	68 (AA)	25 (AG)	7 (GG)	81 (A)	19 (G)
PAI-1 A>G (rs227631)	32 (AA)	53 (AG)	15 (GG)	59 (A)	41 (G)

Abbreviations: GP=glycoprotein, HPA=human platelet alloantigen, VWF= von Willebrand factor, PAI-1=plasminogen activator inhibitor.

Table 3

Maximum creatinine levels in patients with acute Puumala hantavirus infection presented by genotypes of GP VI T>C (rs1613662), VWF A>G (rs1063856) and PAI-1 A>G (rs227631) polymorphisms.

Polymorphism	Maximum creatinine $\mu\text{mol/L}$, median (range)			p-value
	Homotsygote common	Heterotsygote	Homotsygote rare	
GPVI T>C (rs1613662)	199 (52–1499)	191 (51–679)	137 (110–196)	0.381
VWF A>G (rs1063856)	241 (51–1499)	125 (52–1285)	137 (60–384)	0.072
PAI A>G (rs227631)	122 (51–1156)	204 (60–1499)	249 (65–906)	0.033

Table 4

Minimum platelet counts in patients with acute Puumala hantavirus infection presented by genotypes of GP Ia T>C (HPA-5) and PAI-1 A>G (rs227631) polymorphisms.

Polymorphism	<u>Minimum platelet count x 10⁹/l, median (range)</u>			p-value
	Homotsygote common	Heterotsygote	Homotsygote rare	
GP Ia T>C (HPA-5)	64 (3–238)	44 (20–90)	-	0.023
PAI-1 A>G (rs227631)	54 (3–187)	65 (9–181)	71 (14–238)	0.108