

Genetic determinants
of rodent species susceptibility to hantaviruses
Insights into integrins ...



M. Pagès and N. Charbonnel

Introduction

Importance of risks associated with hantaviruses

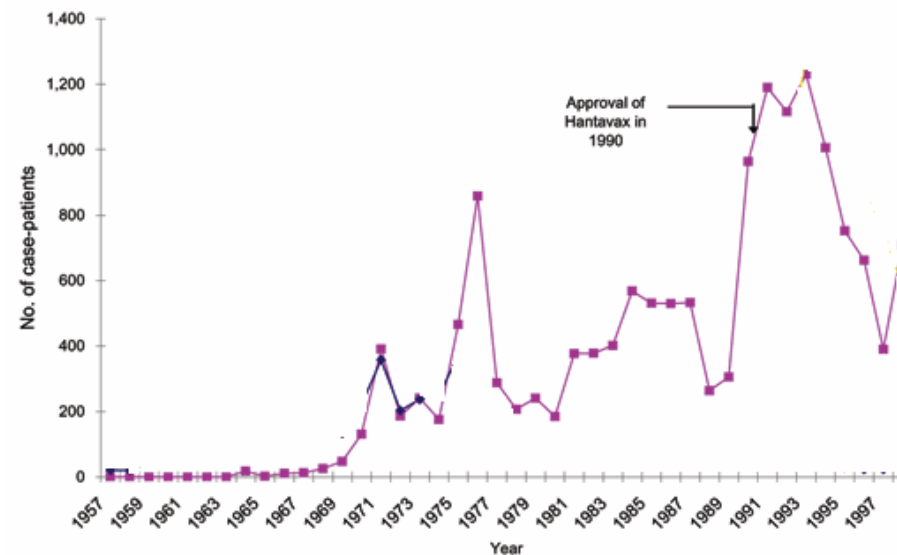
Hantavirus infections affect humans and rodents throughout Asia

Hantavirus infection in East Asia
Hiroaki Kariwa^a, Kumiko Yoshimatsu^b, Jiro Arikawa^{b,*}
Comparative Immunology, Microbiology
& Infectious Diseases 30 (2007) 341–356

Hantaviruses: An Emerging Disease

Ho Wang Lee, M.D., Ph.D.*

Country	No. of Patients
China	60,000-150,000/year
Russia	hundreds to thousands/year
S. Korea	500-2000/year
Japan	hundreds/year
N. Korea	316 (1961-1997)
HongKong	7 (1985-1987)
Malaysia	6 (1985)
Sri Lanka	4 (1987)
Singapore	1 (1991)



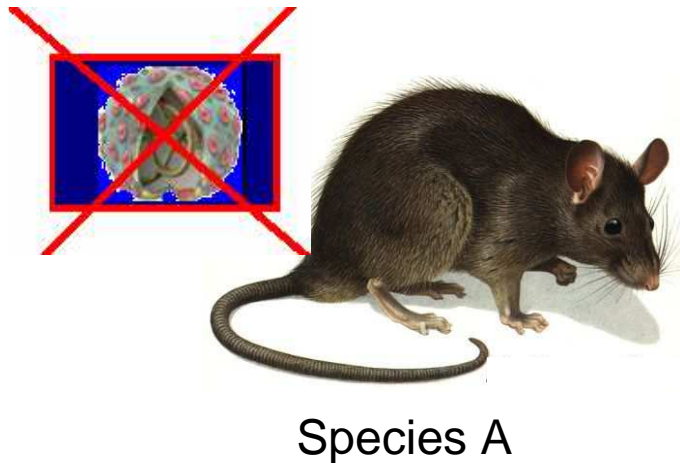
Number of hemorrhagic fever with renal syndrome cases in Korea

Introduction

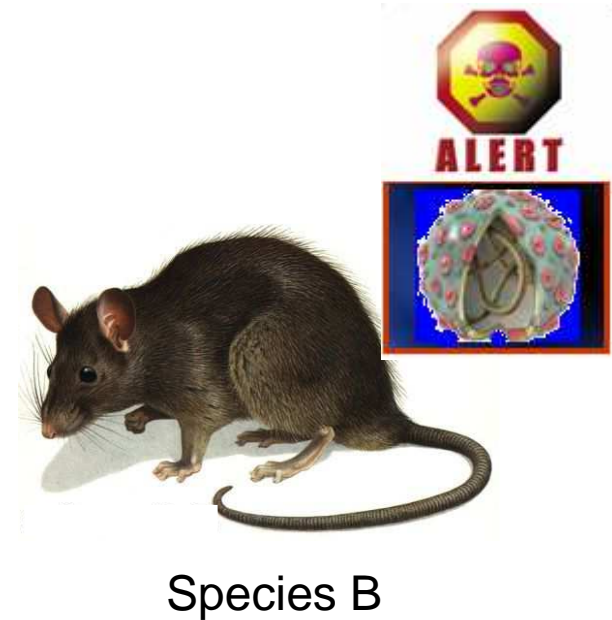
There is a close relationship between hantavirus and rodents,
which act as main reservoirs for these viruses

➡ It is important to identify the hantaviruses / rodents that might cause health problems

These studies may aid the development of new strategies for the prevention and control of such emerging infections ...



↪ Not a priority for control policies
Time consuming
Waste of money



↪ **This is the target !!!**

Introduction

Problems :

Determine which animal species carry pathogenic hantaviruses for humans ?



⇒ First solution = Screening of thousands of rodents, sequencing of hantaviruses...
• but in Asia, 7 genera, 35 species of murine rodents



⇒ Other solution = Provide keys that determine *a priori*
• the possibility for a rodent to carry a hantavirus
• the level of pathogenesis of a new hantavirus



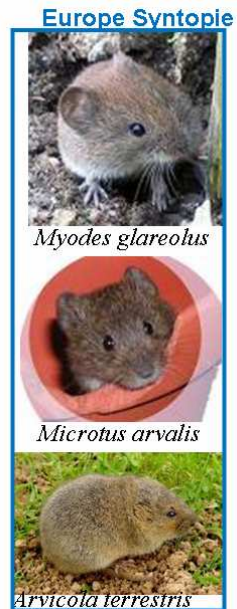
Introduction

What determines the possibility for a rodent to carry a hantavirus ?

It does not depend on the environment :

Sympatric species carry different types of hantaviruses

Ex in Europe



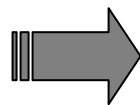
Puumala hantavirus



Tula hantavirus



No hantavirus



Molecular differences between rodent species
for proteins involved in hantavirus entry ?

Introduction

Part 1-
Rodent
integrins

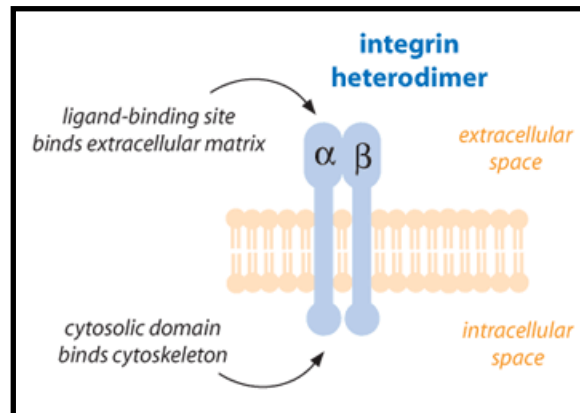


Integrins are heterodimeric receptors that could mediate hantavirus cellular entry

Hantaviruses replicate in platelets and endothelial cells

Hemorrhagic diseases are caused by permeability changes within the vascular endothelium. $\beta 3$ integrins on endothelial cells (v3) regulate vascular permeability.

There are similarities between integrin disorders and hantavirus pathogenesis (e.g. Glanzmann's disease Goodpasture's syndrome)



Introduction

Part 1-
Rodent
integrins



The mechanisms of hantavirus cellular entry differ between pathogenic and non pathogenic ones

Pathogenic hantaviruses (Hantaan, Seoul, Puumala, Sin nombre) use $\alpha_v\beta_3$ integrin
Non pathogenic hantaviruses (Tula, Prospect Hill) use β_1 integrin

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 7074–7079, June 1998
Microbiology

β_3 integrins mediate the cellular entry of hantaviruses that cause respiratory failure

IRINA N. GAVRILOVSKAYA*[†], MICHAEL SHEPLEY[†], ROBERT SHAW*[‡], MARK H. GINSBERG[§], AND ERICH R. MACKOW*^{†‡¶}

JOURNAL OF VIROLOGY, May 1999, p. 3951–3959
0022-538X/99/\$04.00+0

Vol. 73, No. 5

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Cellular Entry of Hantaviruses Which Cause Hemorrhagic
Fever with Renal Syndrome Is Mediated by β_3 Integrins

IRINA N. GAVRILOVSKAYA,^{1,2} ERIC J. BROWN,³ MARK H. GINSBERG,⁴
AND ERICH R. MACKOW^{1,2,5*}



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Biochemical and Biophysical Research Communications 339 (2006) 611–617

BBRC

www.elsevier.com/locate/ybbrc

Cellular entry of Hantaan virus A9 strain: Specific interactions
with β_3 integrins and a novel 70 kDa protein

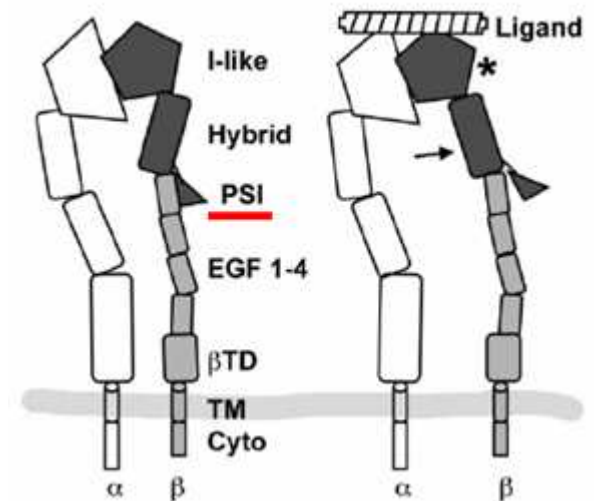
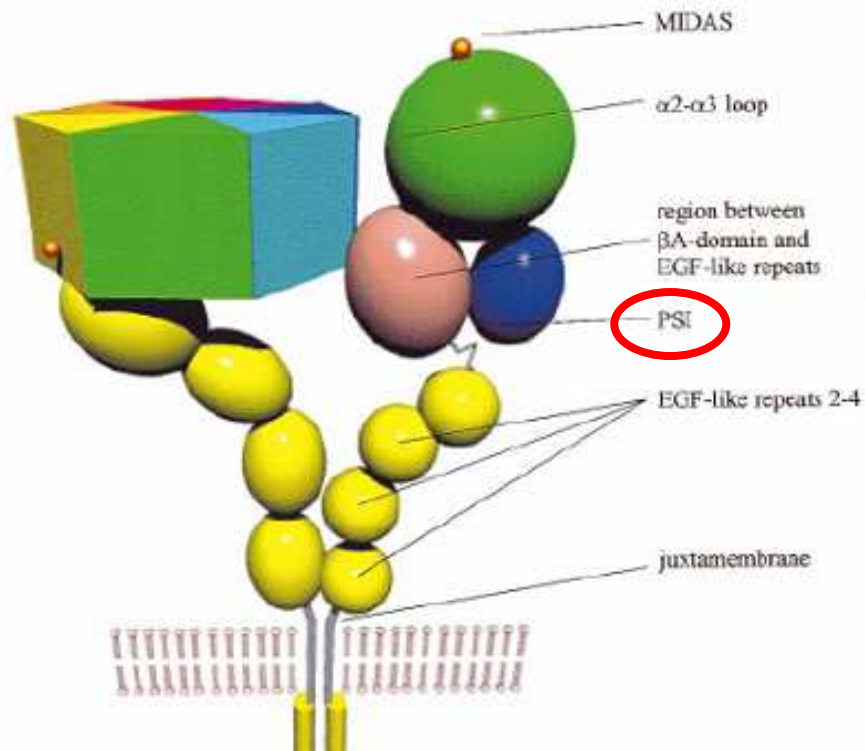
Dan Lei Mou, Ying Peng Wang, Chang Xing Huang, Guang Yu Li, Lei Pan,
Wei Song Yang, Xue Fan Bai*

Introduction

Part 1-
Rodent
integrins

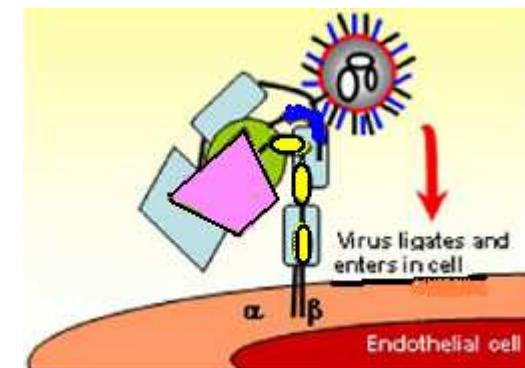


Pathogenic hantaviruses bind on the PSI domain of $\alpha\beta 3$ integrin



Pathogenic hantaviruses bind plexin–semaphorin–integrin domains present at the apex of inactive, bent $\alpha v \beta 3$ integrin conformers

Tracy Raymond^{1†}, Elena Gorbunova^{1†}, Irina N. Gavrilovskaya^{1‡}, and Erich R. Mackow^{1*1§}



Introduction

Part 1-
Rodent
integrins



Objectives :

Analysis of the polymorphism of the beta 3 integrin PSI domain between Asian rodent sp.

Hypothesis 1:

Differences in amino acid sequences ⇒ Differences in protein structure
⇒ Differences in possibility for hantavirus to entry

Ex: Mutation Asn → Asp on the 39^e amino acid allowed infection of murine cells by hantaviruses

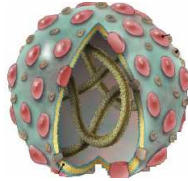
Hypothesis 2:

Differences in selective pressure acting on PSI domain
⇒ Differences in possibility for hantavirus to entry

Ex: If only pathogenic hantaviruses induce coevolution with B3 integrin, we should observe positive selection on rodent B3 integrin sequences only for rodents carrying pathogenic hantaviruses

Introduction

Part 2- Hantavirus



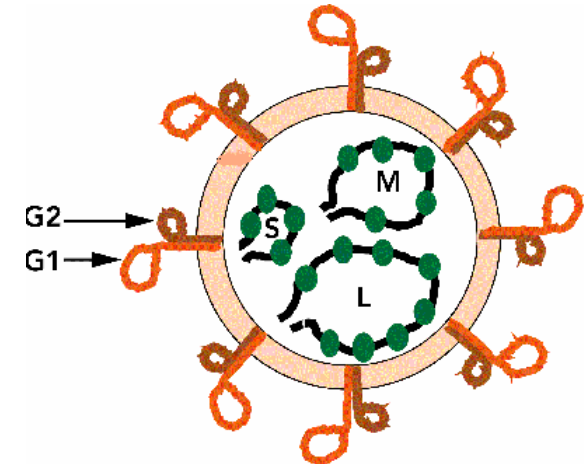
The binding site of hantaviruses on integrins is a
CNP motif on G2 glycoprotein

JOURNAL OF VIROLOGY, Oct. 2004, p. 10839-10847
0022-538X/04/\$08.00+0 DOI: 10.1128/JVI.78.20.10839-10847.2004
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Vol. 78, No. 20

VP7 Mediates the Interaction of Rotaviruses with Integrin $\alpha\beta 3$ through a Novel Integrin-Binding Site

Selene Zárate, Pedro Romero, Rafaela Espinosa, Carlos F. Arias, and Susana López*

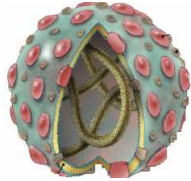


```
nt
161 NEWLCNPMD VP7 Rotavirus RRV
   * * *** *
759 NSWACNPPD G1G2 Hantavirus L99
```

FIG. 4. Alignment of rotavirus RRV protein VP7 (aa 161 to 169) with the G1G2 protein of hantavirus L99 (aa 759 to 767).

Introduction

Part 2- Hantavirus



Objectives :

Analysis of the polymorphism around the CNP motif between Asian hantaviruses

Hypothesis 1:

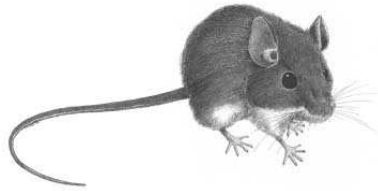
Differences in amino acid sequences ⇒ Differences in glycoprotein structure
⇒ Differences in possibility for hantavirus to entry

Hypothesis 2:

Differences in selective pressure acting on the region around the CNP motif
⇒ Differences in possibility for hantavirus to entry

Ex: If only pathogenic hantaviruses use the CNP motif to enter cells via B3 integrin, we should observe positive selection around this hantavirus sequence only for pathogenic hantaviruses

Beta3-Integrin

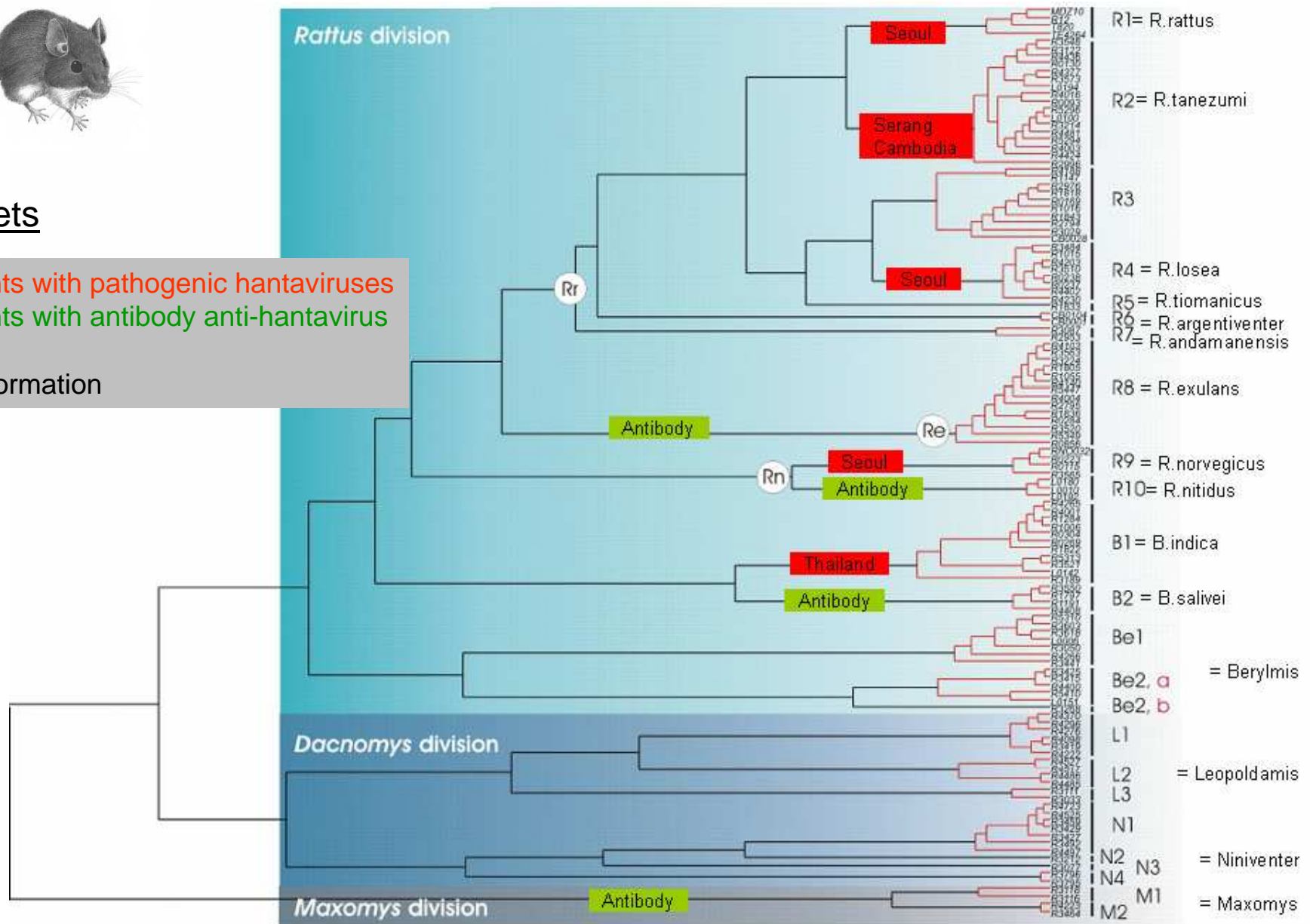


Datasets

Rodents with pathogenic hantaviruses

Rodents with antibody anti-hantavirus

No information



ML analysis (GTR+G), (RAxML software, Stamatakis, 2006)

based on the combined analysis of cytb, COI and IRBP genes. See Pagès et al., submitted

Beta3-Integrin

Part 1-
Rodent
integrins



Methods



Sequencing of b3 integrins (exon 2 to exon 4) in 4/5 rodents of each species (after molecular species-specific identification of rodents)



Phylogenetic analyses of these sequences (ML, RAxML software, Stamatakis, 2006)

Determination of amino acid discriminating groups of rodent species



Detection of selection over these sequences (3 methods implemented in Datamonkey, Kosakovsky Pond and Frost, 2005)

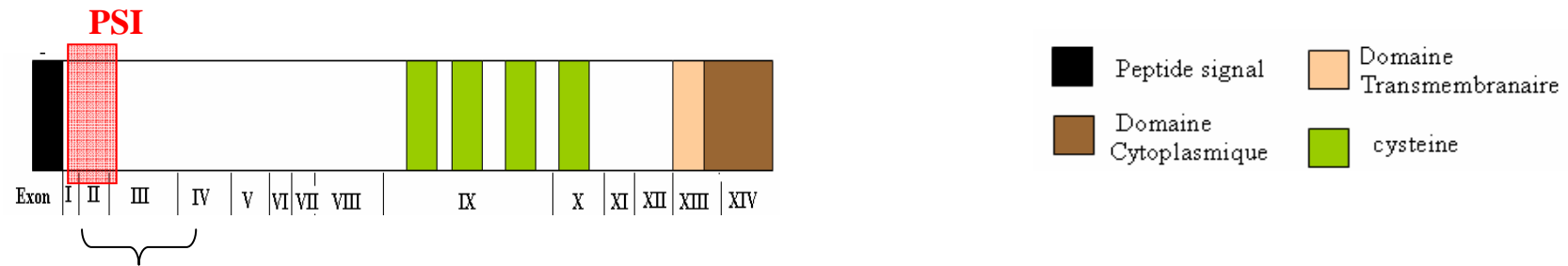
Determination of sites under selection

Beta3-Integrin

Part 1-
Rodent
integrins



Sequences



We obtained sequences of 330 bp, for 15 Asian species and 62 individuals

60 variable sites (15 among the *Rattus* genus)

% divergence between species (p-distance) : 0 – 10%

(max 3% inside the *Rattus* genus)

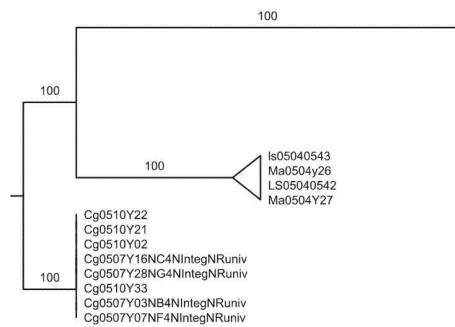
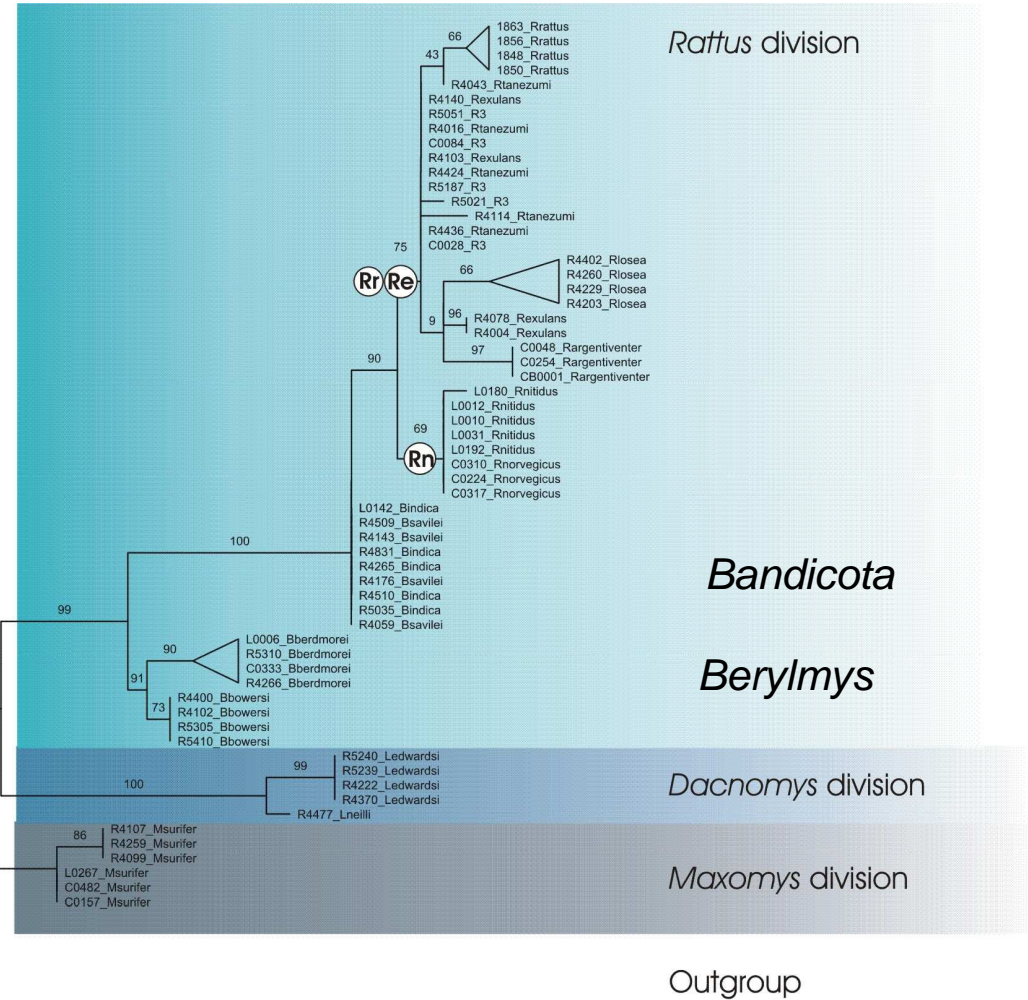
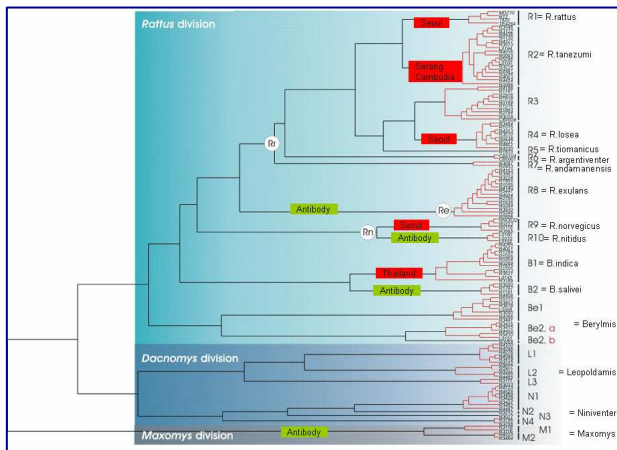
Beta3-Integrin

Part 1- Rodent integrins



Phylogenetic analyses

Perfectly consistent with the species tree



ML analysis (GTR+G), (RAxML software, Stamatakis, 2006)
based on 331 bp Integrin beta 3 / 34 sequences

Beta3-Integrin

Part 1-
Rodent
integrins



Amino acids discriminating groups of rodent species

39^e aa

Seq:1	1	Seq:2	Pos:1 0	[]
1850_Rrattus	E	S	N	I
BC127667_Homo	G	P	.	.
PSI_BC127667_Homo	G	P	.	.
Musmusculus
1848_Rrattus
1856_Rrattus
1863_Rrattus
R4203_Rlosea
R4229_Rlosea
R4260_Rlosea
R4402_Rlosea
R4016_Rtanezumi
R4043_Rtanezumi
R4424_Rtanezumi
R4114_Rtanezumi
R4436_Rtanezumi
C0224_Rnorvegicus
C0310_Rnorvegicus
C0317_Rnorvegicus
R4230_Rnorvegicus
L0142_Bindica
R4265_Bindica
R4510_Bindica
R4831_Bindica
R5035_Bindica
R4222_Ledwardsi
R4370_Ledwardsi
R5239_Ledwardsi
R5240_Ledwardsi
R4477_Lneilli
C0333_Bberdmorei
L0006_Bberdmorei
R4266_Bberdmorei
R5310_Bberdmorei
R4102_Bbowersi
R4400_Bbowersi
R5305_Bbowersi
R5410_Bbowersi

Sequences of PSI domain of the b3 integrin

Rodents known to carry hantaviruses pathogenic for humans



Rodents that were never found to be seropositive for hantaviruses



Amino acids preventing the fixation of hantaviruses pathogenic for humans ?

Beta3-Integrin

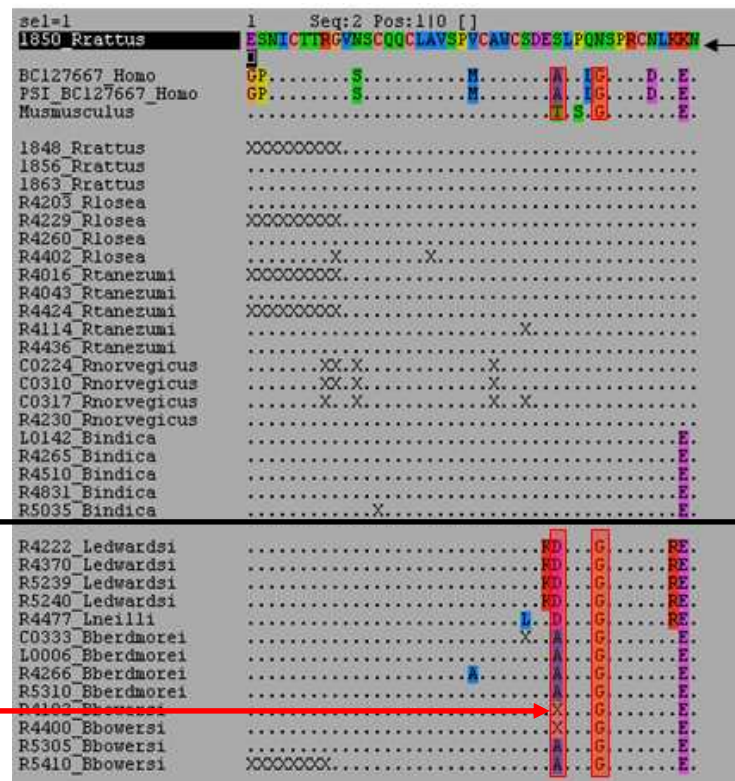
Part 1-
Rodent
integrins



Detection of selection

No selection detected when considering rodents that carry pathogenic hantaviruses

Positive selection detected when considering rodents that were never found to be seropositive for hantaviruses



Sequences of PSI domain of the b3 integrin

Rodents known to carry hantaviruses pathogenic for humans



Rodents that were never found to be seropositive for hantaviruses



Only one amino-acid is under positive selection (evidenced by 3 different methods) 30^e aa

Beta3-Integrin

Part 1-
Rodent
integrins



Perspectives

⇒ Verify that these 30th and 34th amino-acid changes induce changes in integrin functionality
Collaboration with Pr Law Sai-Kit (Singapore)

⇒ Infer the status of other rodent species

Ex

C0028_R3		
C0084_R3X.....X.....		
R5021_R3X.....		
R5051_R3X.....X.....X.....		
R5187_R3X.....		
CB0001_Rargentivente		
C0048_RargentiventerX.....		
C0254_Rargentiventer		
L0010_RnitidusX.....		
L0012_RnitidusXX.....X.....		
L0031_RnitidusX.....X.....		
L0180_RnitidusX.....X.....		
L0192_Rnitidus		
R4004_Rexulans		
R4078_Rexulans		
R4103_Rexulans	XXXXXXXXXX.....		
R4140_Rexulans	XXXXXXXXXX.....		
R4059_Bsavilei	XXXXXXXXXX.....		
R4143_Bsavilei		
R4176_Bsavilei		
R4509_Bsavilei		
C0482_Msurifer		
L0267_Msurifer		
R4107_Msurifer		
R4259_Msurifer		
C0157_MsuriferX.....		
R4099_Msurifer		

Predictions



ALERT

These rodent species may carry hantaviruses pathogenic for humans



These rodent species may carry non pathogenic hantaviruses (antibodies have been found)

Amino acids supposed to interfere with the fixation of hantaviruses pathogenic for humans

G2 glycoprotein

Part 2- Hantavirus



Unknown ?

Protein Sequences		*																				
HantaanH8205	YGACTYQYFWHTA...CHY...DYQYF...SWG...CNP...DCG...GVG...GCTACGLYL...QL																					
HantaanA9	YGACTYQYFWHTA...CHY...DYQYF...SWG...CNP...DCG...GVG...GCTACGLYL...QL																					
HantaanHV114	YGACTYQYFWHTA...CHY...DYQYF...SWG...CNP...DCG...GVG...GCTACGLYL...QL																					
SeoulNC005237	YGACTYQYFWHTA...CHF...DYQYF...SWG...CNP...DCG...GVG...GCTACGLYL...QL																					
PuumalaNC005223	YGACEYAYFWHTA...CFV...DYQYF...HWG...CNP...DCG...GVG...GCTACGVYL...L																					
TulaNC005228	YGACIYAYFWHTA...CFL...DYQYF...HWG...CNP...DCG...GVG...GCTACGVYL...L																					
ProspectHillX55129	YGACEYAYFWHTA...CFL...DYQYF...HWG...CNP...DCG...GVG...GCTACGVYL...L																					
Thai749	YGACDYQYFWHTA...CHF...DYQYF...SWG...CNP...DCG...GVG...GCTACGLYL...M																					
Gou3	YGACTYQYFWHTA...CHF...DYQYF...SWG...CNP...DCG...GVG...GCTACGLYL...L																					
Serang	YGACDYQYFWHTA...CHF...DYQYF...SWG...CNP...DCG...GVG...GCTACGLYL...L																					

One amino acid that differs between pathogenic hantaviruses (Y) and non pathogenic hantaviruses (F)

⇒ Prediction 1 : Thai, Gou and Serang have a 'Y'.
They should be pathogenic for humans

⇒ Prediction 2 : Khabarovsk, Fusong and Muju have a 'F'.
They should be non pathogenic for humans

Global conclusions

Part 1-
Rodent
integrins



Carry pathogenic
hantaviruses

Positive selection (30^e aa) + G (34^e aa)



Can only carry
non pathogenic
hantaviruses

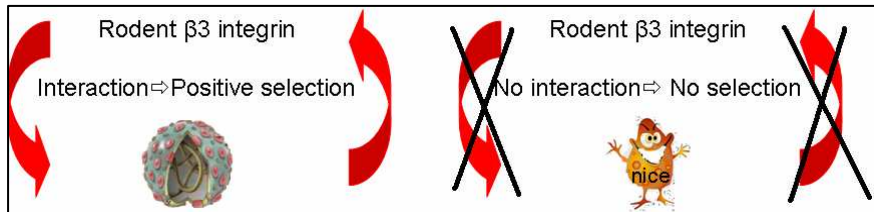
S (30^e aa) + N (34^e aa)



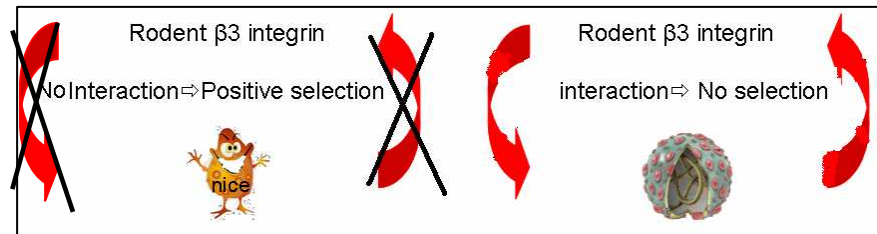
Necessity to validate this hypothesis with *Rattus exulans* or *R. argentiventer*, and with *Maxomys surifer*.

Question: we expected positive selection on rodent B3 integrin sequences only for rodents carrying pathogenic hantaviruses / we observe the opposite pattern...

Expected patterns

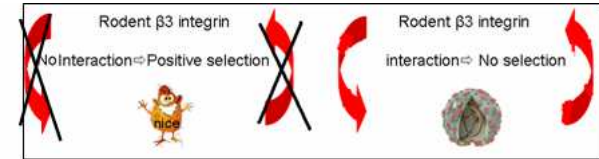
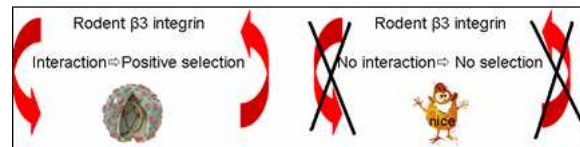


Observed patterns



Global conclusions

Part 1- Rodent integrins



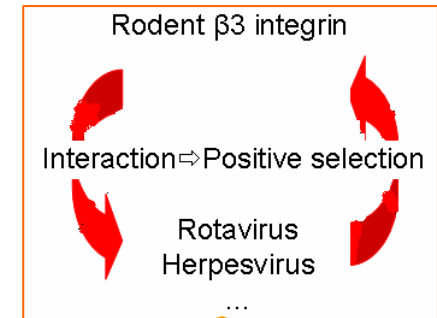
Potential explanations :



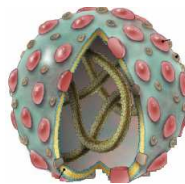
associated with



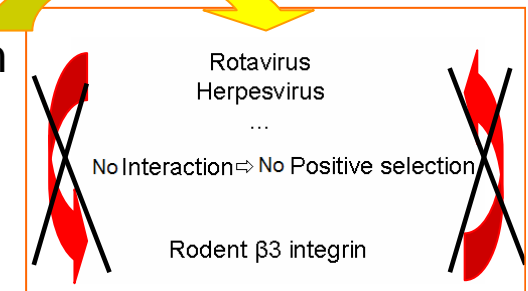
are also associated with



associated with

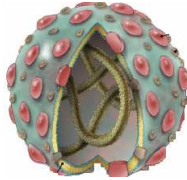


are not associated with



Global conclusions

Part 2-
G2 of
Hantavirus



Are pathogenic
hantaviruses



Are non pathogenic
hantaviruses



Necessity to validate this hypothesis with *Khabarovsk*, *Fusong*, *Muju*

↓
Host=*Microtus* sp. ⇒ non pathogenic

with *Thai*, *Gou* and *Serang*

↓
pathogenic, see Pattamadilok et al. 2006

Am. J. Trop. Med. Hyg., 75(5), 2006, pp. 994-1002

GEOGRAPHICAL DISTRIBUTION OF HANTAVIRUSES IN THAILAND AND
POTENTIAL HUMAN HEALTH SIGNIFICANCE OF *THAILAND VIRUS*

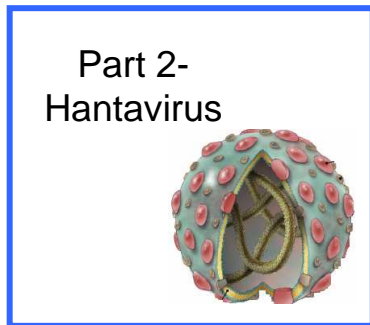
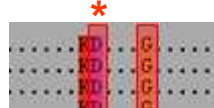
Perspectives



What are the consequences of the different combinations ?

Do they all exist in natura ?



Positive selection



 <p>Carry non pathogenic H</p> <p>Are non pathogenic H</p>	<p>Carry pathogenic H</p> <p>?</p> <p>Are non pathogenic H</p>
<p>Carry non pathogenic</p> <p>?</p> <p>Are pathogenic H</p>	<p>Carry pathogenic H</p>  <p>Are pathogenic H</p>