About OMICS Group

OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

About OMICS Group Conferences

OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Phrama scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

3rd International Conference and Exhibition on Pathology

Molecular impacts of virus infections and genetic variants on the course of HBV-related liver diseases in Vietnam

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Vietnam Military Medical University
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HBV infection

❑ Worldwide

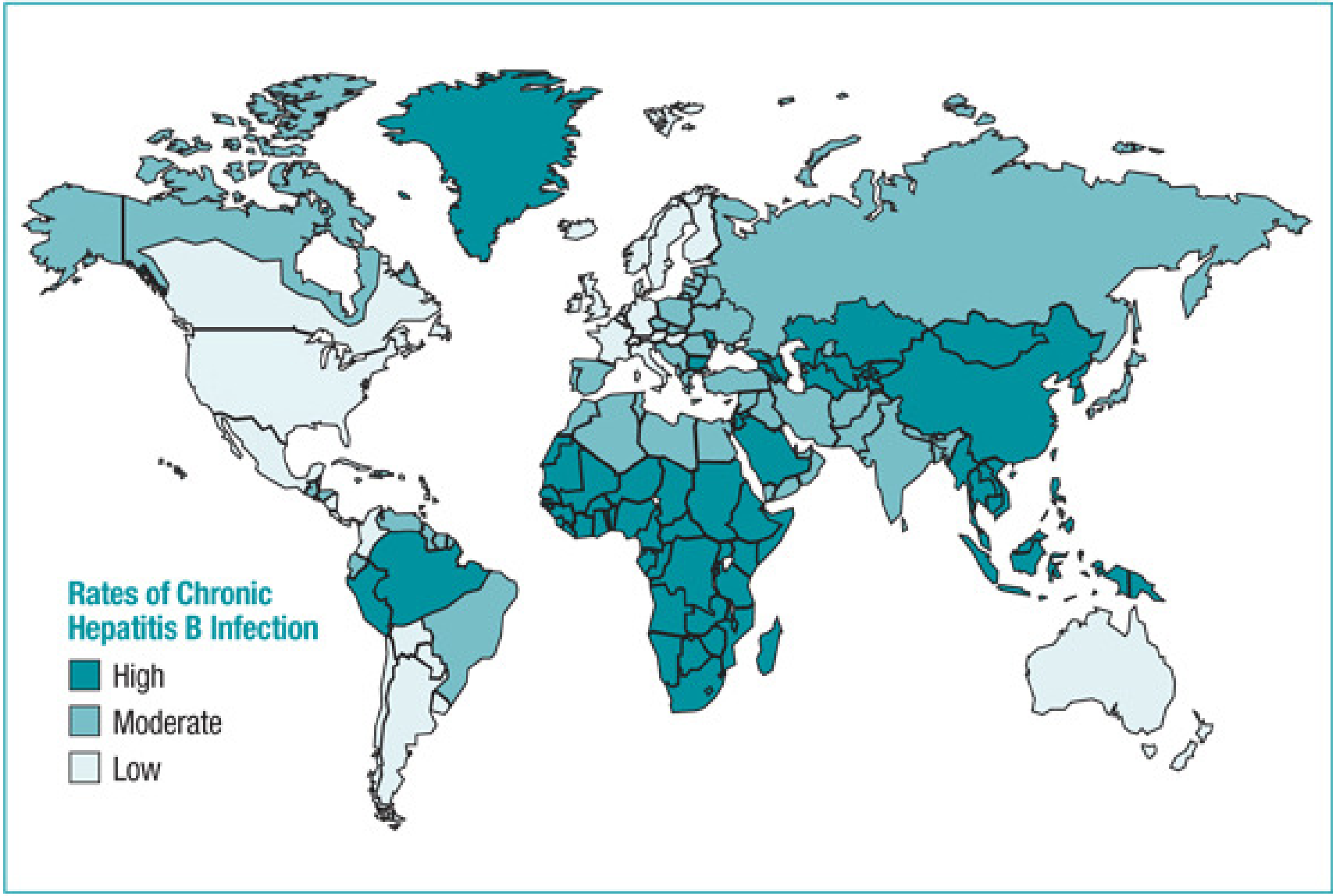
- HBV is leading cause of HBV-related liver diseases (AHB, CHB, LC and HCC)
- Approximately 240 million people are living with chronic hepatitis B
- 600,000 people die each year from HBV-related chronic liver diseases
- 500,000 new cases of HCC are diagnosed each year

❑ Vietnam

- Prevalence of current HBV infection (HBsAg+), >10% in the population
- 10 million Vietnames people with chronic HBV resulting in 23,300 deaths (2005)

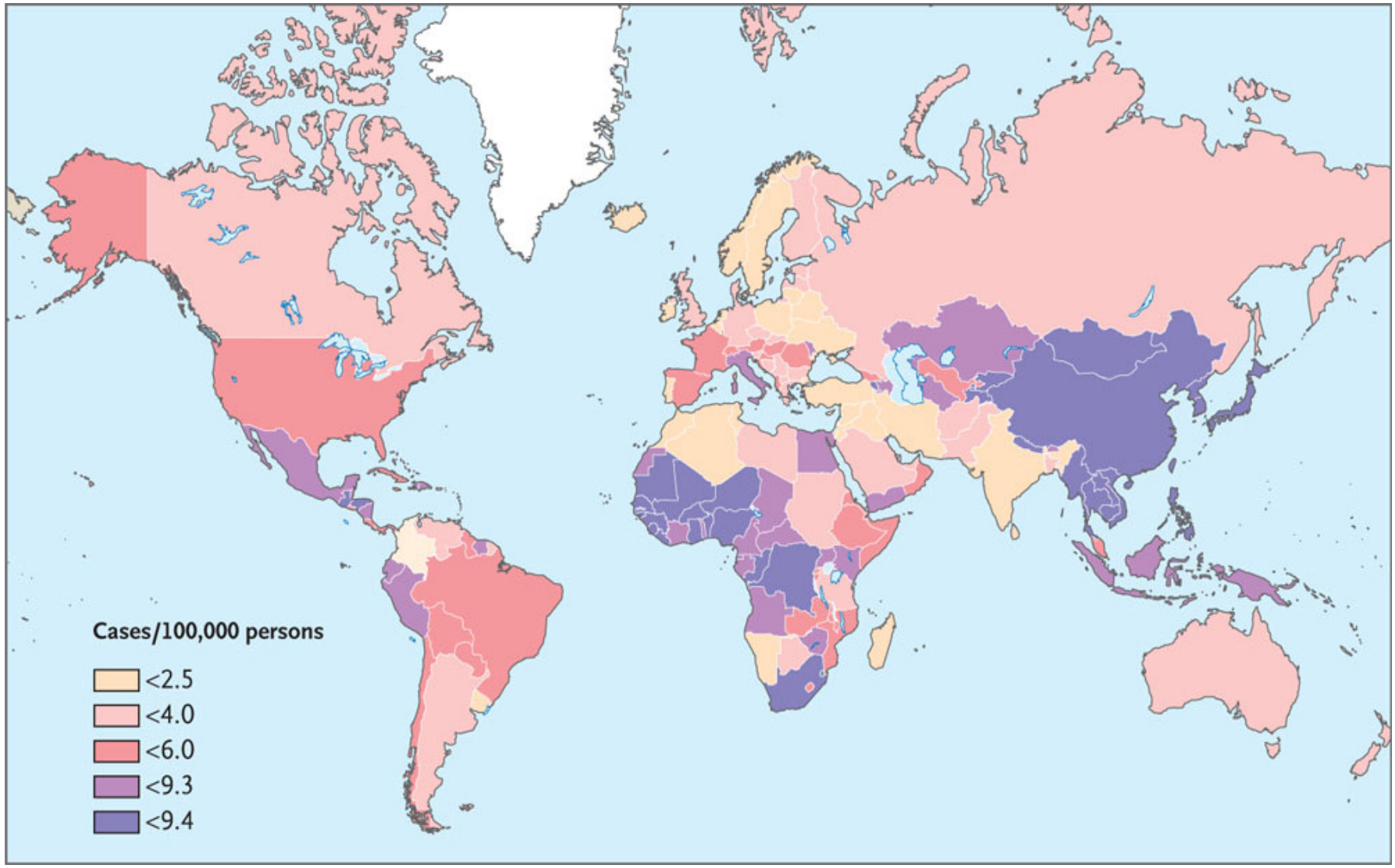
WHO 2012. Fact sheet N°204 *Nguyen VT et al. 2008 Liver Int*

Chronic heaptitis B prevalence



Source: http://www.cdc.gov

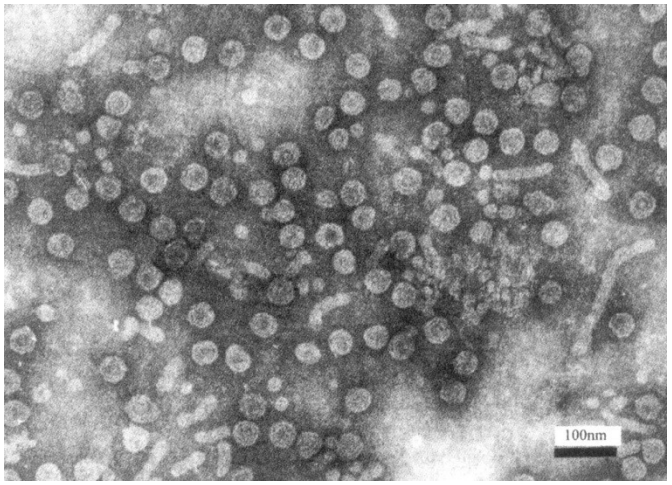
Estimated incidence of liver cancer

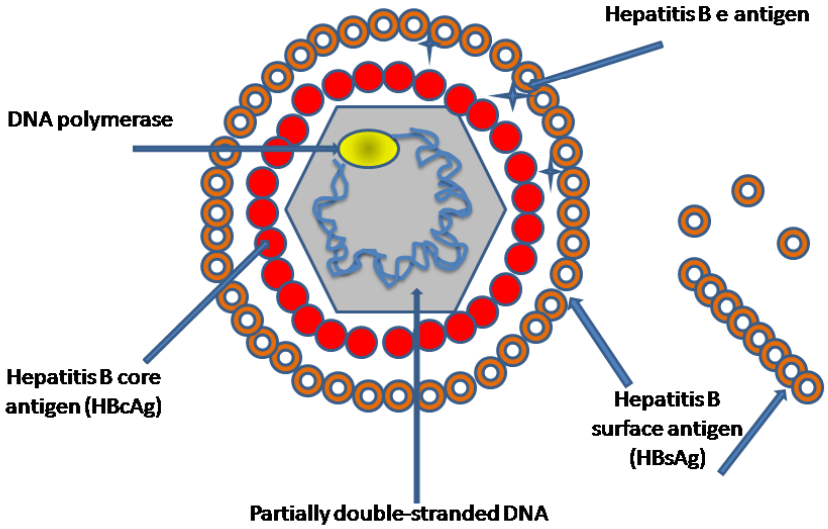


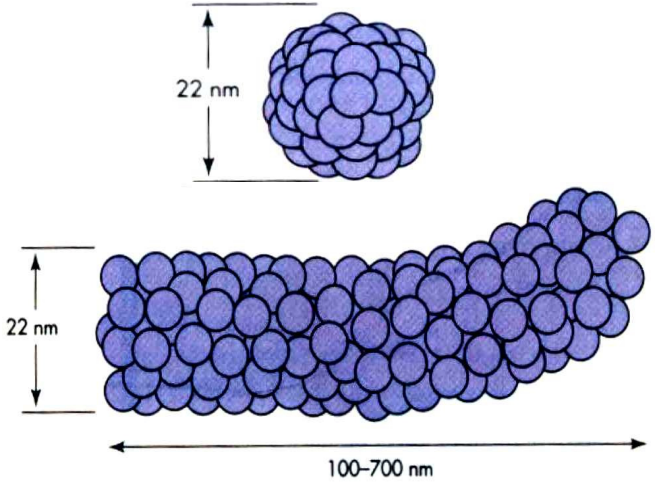
El-Serag HB. 2011, N Engl J Med

Hepatitis B virus

- Circular partially double-stranded DNA virus
- Genus: *Orthohepadnavirus*
- Family: *Hepadnaviridae*

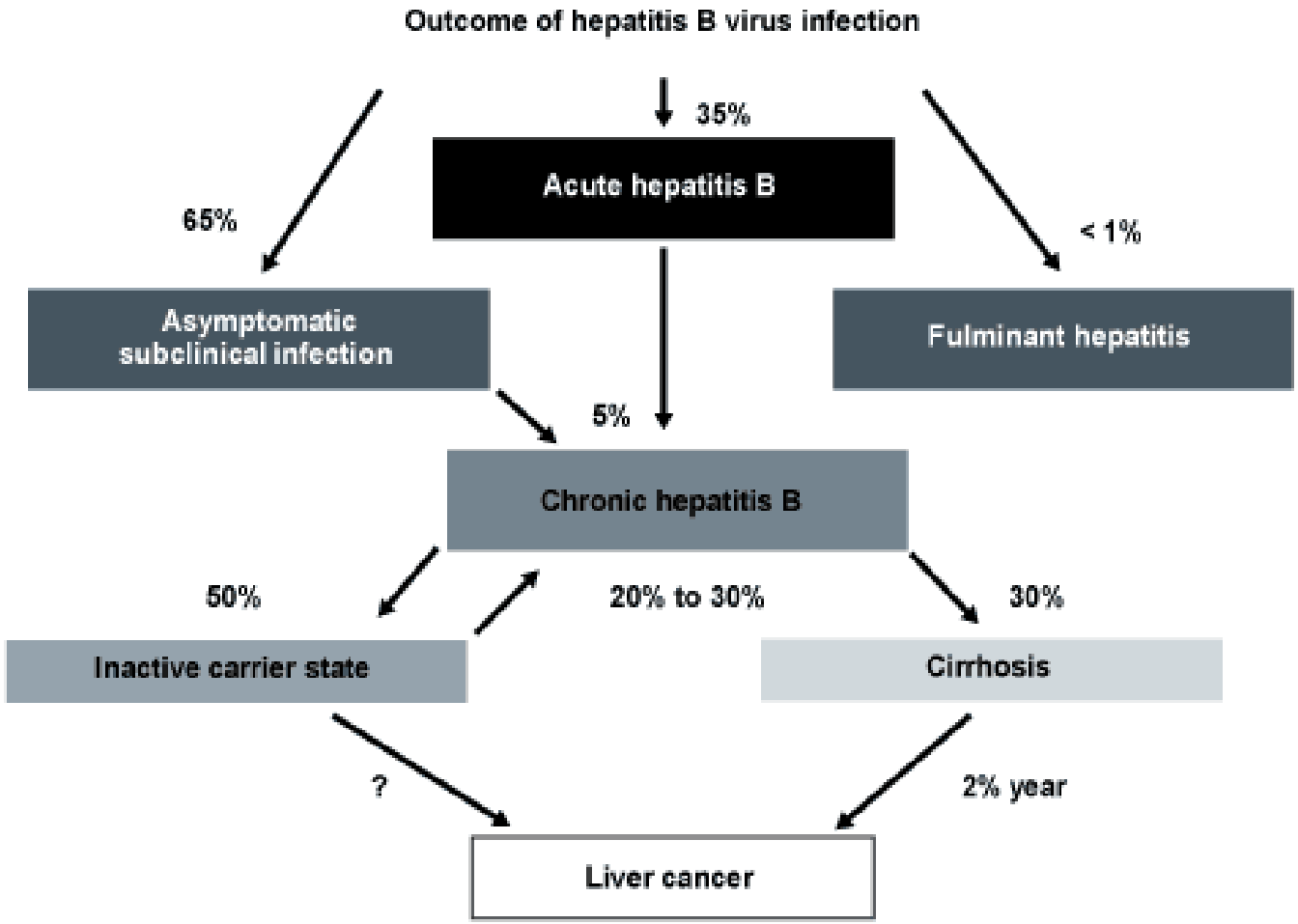






http://en.wikipedia.org/wiki/File:Hepatitis_B_virus.png

Natural history of hepatitis B infection



Clin Infect Dis. (1995) 20(4): 992-1000

HBV genotype and liver diseases

Table 4. Distribution of HBV Genotypes in Vietnamese Patients With HBV Infection

Genotype	ASYM n = 86 (%)	AHB n = 43 (%)	CHB n = 70 (%)	LC n = 92 (%)	HCC n = 84 (%)	Total n = 375 (%)
A	29 (33.7)	6 (13.9)	15 (21.4)	10 (10.8)	8 (9.5)	68 (18.13)
B	4 (4.6)	8 (18.6)	7 (10.0)	8 (8.7)	10 (11.9)	37 (9.86)
C	29 (33.7)	10 (23.3)	13 (18.6)	13 (14.1)	29 (34.5)	94 (25.06)
D	5 (5.8)	6 (13.9)	8 (11.4)	41 (44.6)	16 (19.0)	76 (20.26)
E	2 (2.3)	3 (6.9)	2 (2.8)	2 (2.2)	4 (4.8)	13 (3.47)
F	2 (2.3)	2 (4.6)	0	3(3.3)	2 (2.4)	9 (2.40)
G	0	4 (9.3)	5 (7.1)	3 (3.3)	7 (8.3)	19 (5.06)
A/C	2 (2.3)	2 (4.6)	9 (12.8)	7 (7.6)	5 (6.0)	25 (6.66)
A/D	1 (1.2)	2 (4.6)	0	3 (3.3)	0	6 (1.6)
C/D	4 (4.6)	0	3 (4.3)	1 (1.1)	2 (2.4)	10 (2.66)
Other remaining genotype mixtures	8 (9.3)	0	8 (11.4)	1 (1.1)	1 (1.2)	18 (4.80)

Table 5. Distribution of Genotype Mixtures in HBV-Infected Vietnamese Patients

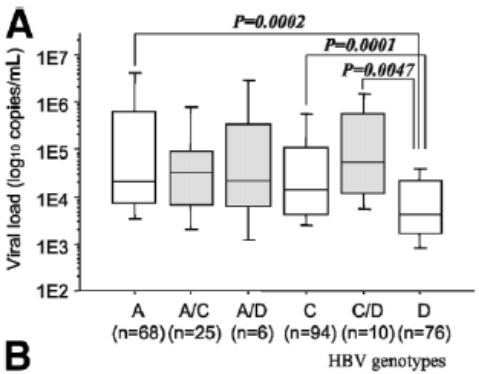
Genotype	ASYM n = 86 (%)	AHB n = 43 (%)	CHB n = 70 (%)	LC n = 92 (%)	HCC n = 84 (%)
Mixtures	15 (17.4)	4 (9.3)	20 (28.6)*	12 (13)	8 (9.5)
Single	71 (82.6)	39 (90.7)	50 (71.4)	80 (87)	76 (90.5)

*$P < .02$ in comparison to AHB, LC, and HCC groups.

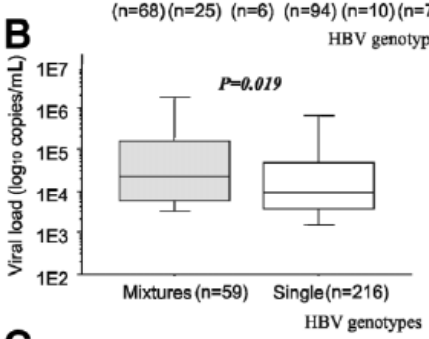
HBV-genotype mixtures were more frequent in Vietnam (59/375; ~16%)

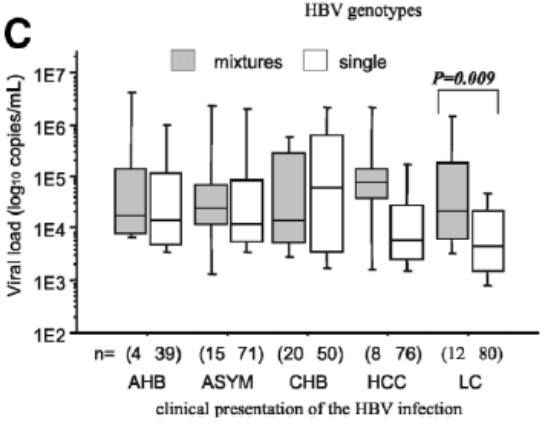
Toan NL et al. (2006) Hepatology

HBV genotype and liver diseases

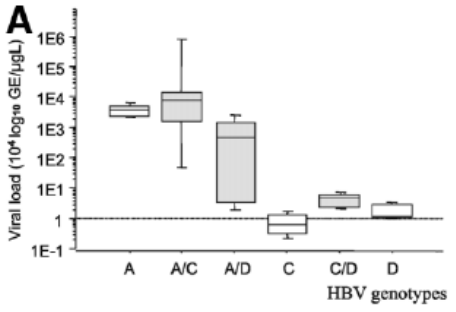


Genotypes	P value
A vs C	0.0159
A vs D	0.0002*
A/C vs D	0.0012
A/D vs D	0.0079
C vs D	0.0001*
C/D vs D	0.0047*

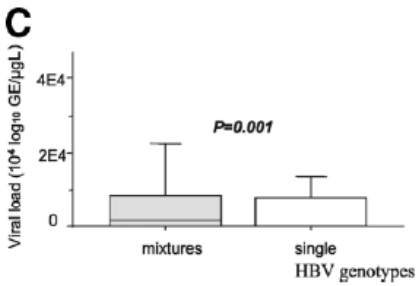




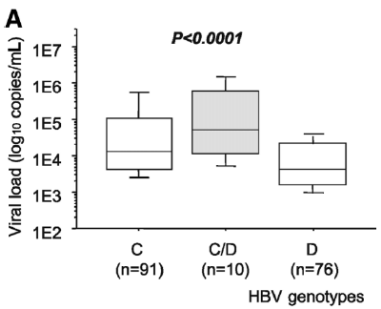
In patients

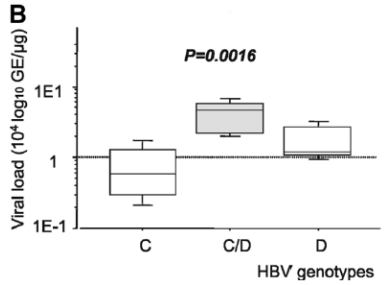


Genotypes	P value
A vs A/D	0.0001
A vs C	0.0002
A vs C/D	0.0009
A vs D	0.0009
A/C vs A/D	0.0015
A/C vs C	<0.0001
A/C vs C/D	0.0002
A/C vs D	0.0002
A/D vs C	0.0003
A/D vs D	0.0067
C vs C/D	0.0015
C vs D	0.0451
C/D vs D	0.0250



In vitro experiments

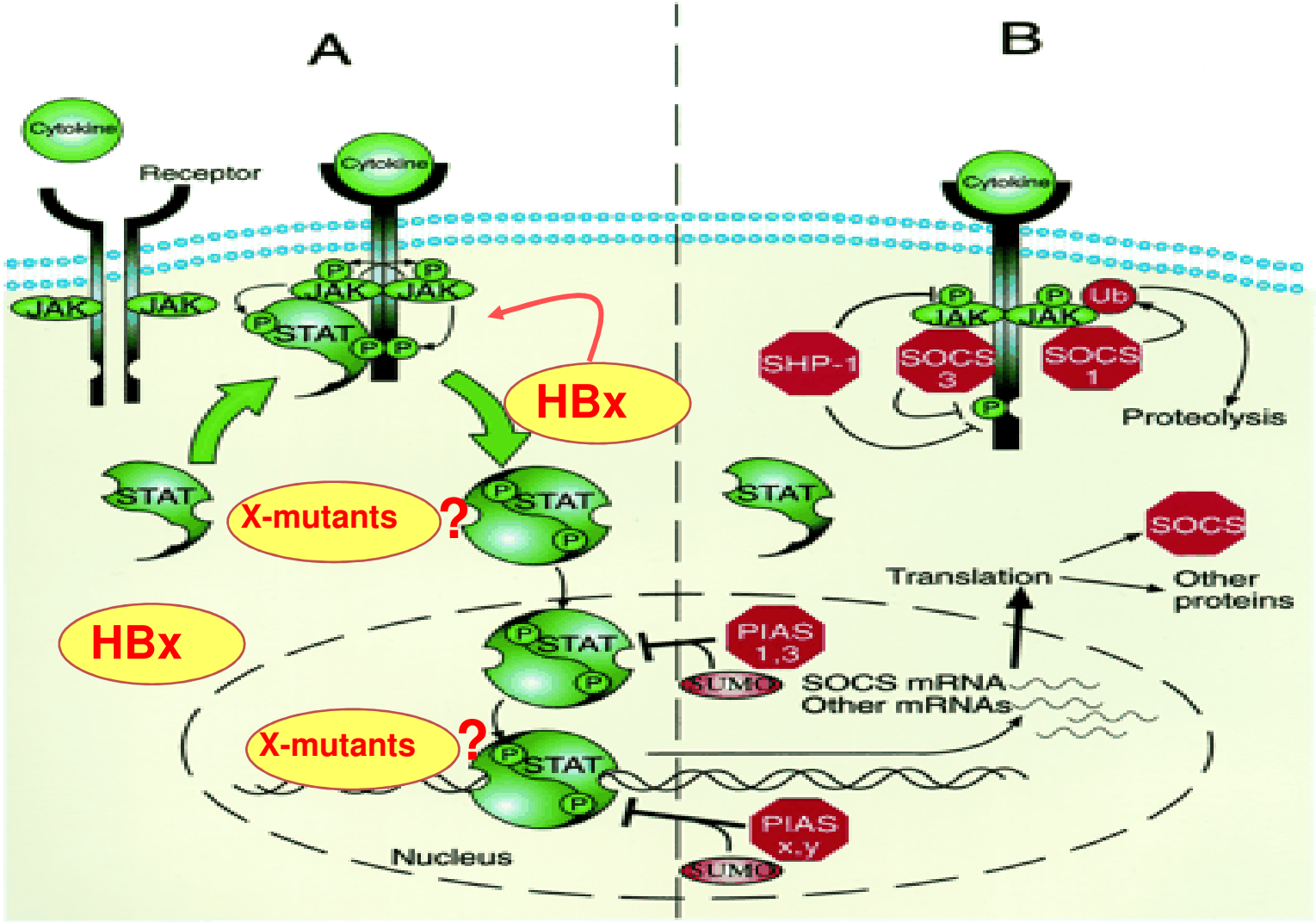




Conclusion: Mixed HBV-genotype infections are associated with altered pathogenesis and clinical outcome of liver disease

Toan NL et al. (2006) Hepatology

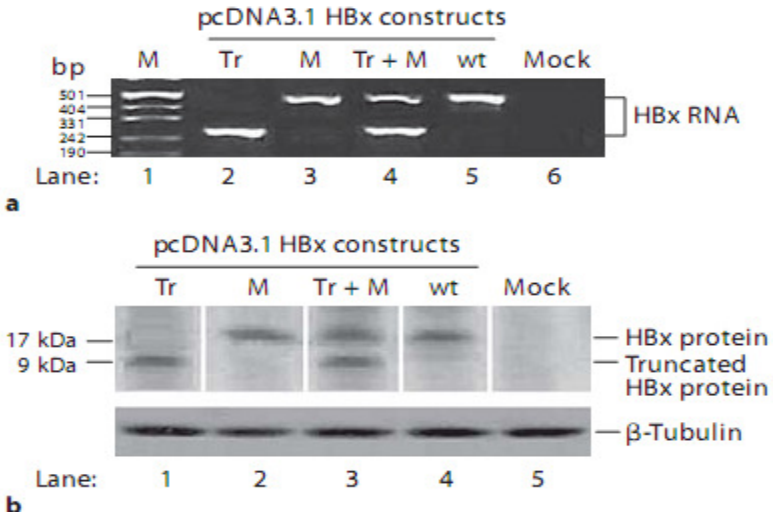
JAK/STAT/SOCS signaling



Wormald et al., 2004

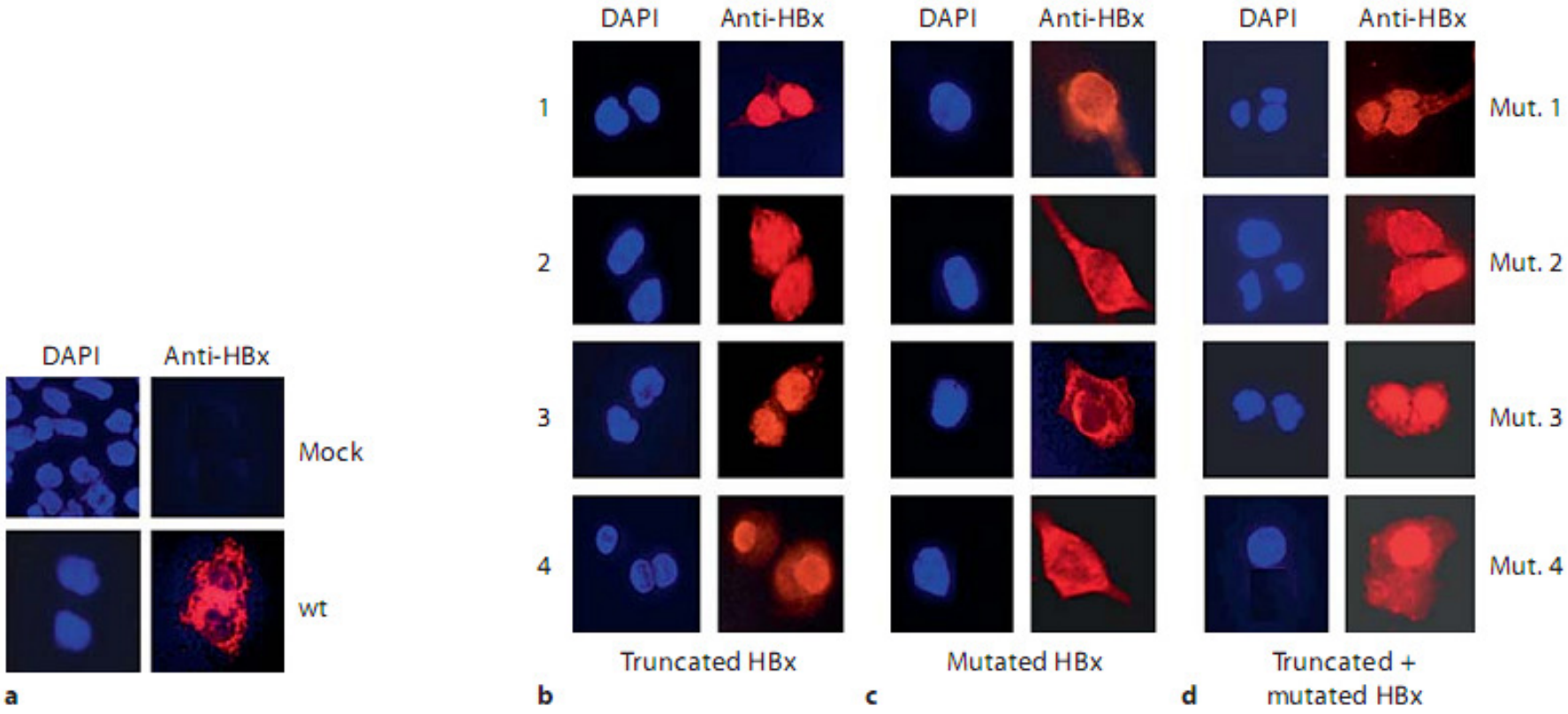
HBx Mutants Dysregulate STAT/SOCS Signaling

In HCC patients shown random mutations throughout HBx-gene, and 4/48 HCC patients were combination to stop codon insertions at codons 77, 78 and 131 together with HBx gene deletions.



Bock CT, Toan NL et al. (2008) Intervirology

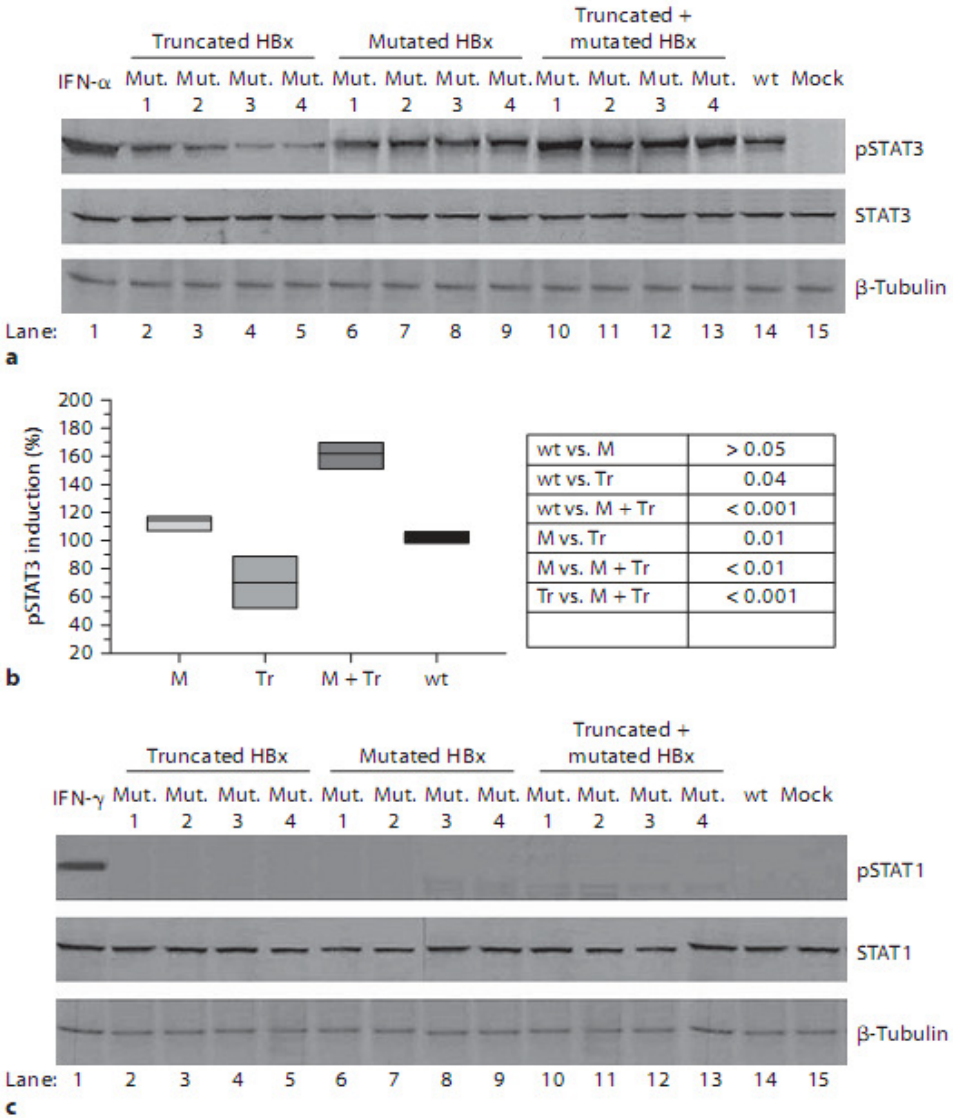
HBx Mutants Dysregulate STAT/SOCS Signaling



A different distribution of HBx mutant proteins in transfected cells, wt-HBx protein were predominant distributed in cytoplasm. The truncated HBx-protein is mainly distributed in the nuclei, randomly mutated HBx protein had a predominant perinuclear localization and the combination of truncated and randomly mutated HBx is distributed into both the nuclei and cytoplasm.

Bock CT, Toan NL et al. (2008) Intervirology

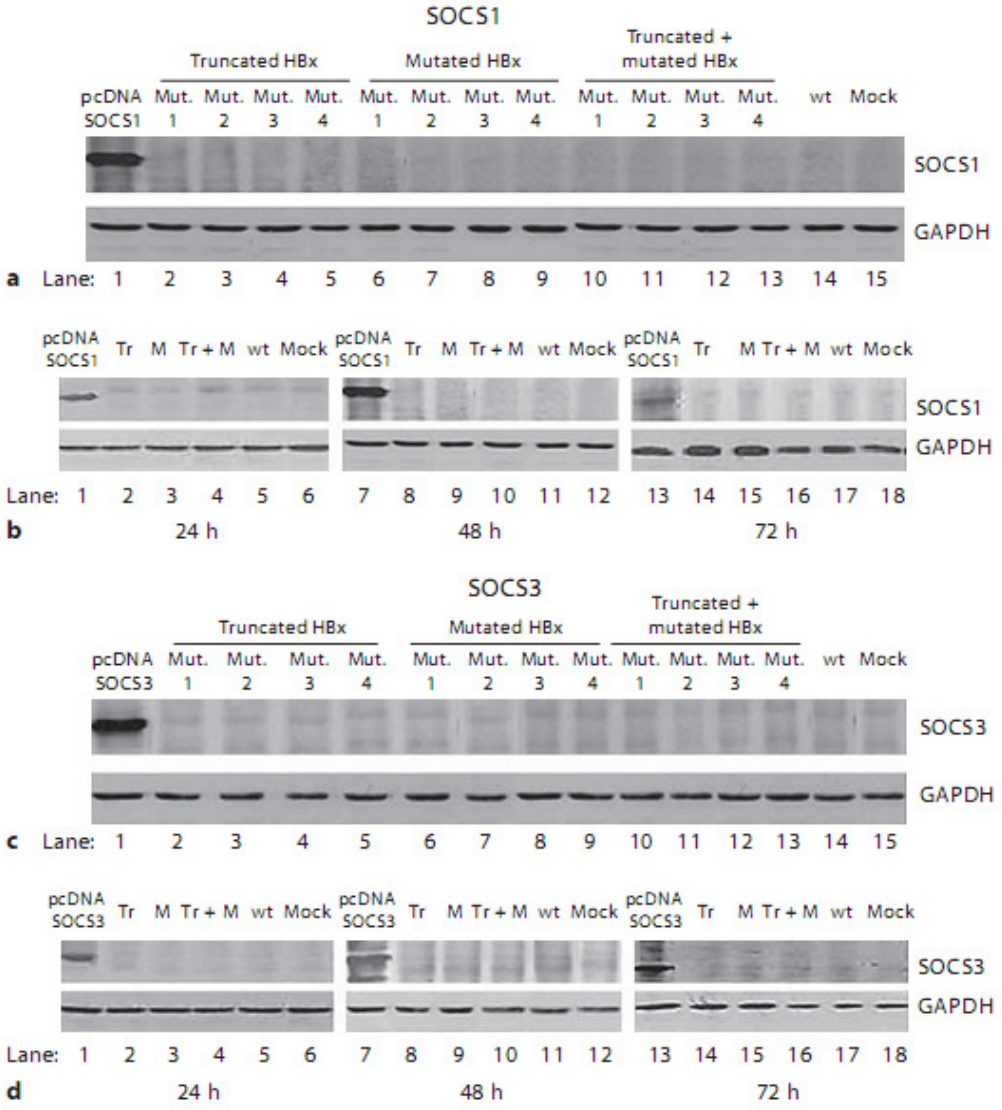
HBx Mutants Dysregulate STAT/SOCS Signaling



Conclusion: Mutated HBx-protein activates STAT-3 but not STAT-1.

Bock CT, Toan NL et al. (2008) Intervirology

HBx Mutants Dysregulate STAT/SOCS Signaling



There were no activation SOCS3 and SOCS1 proteins in Wt or mutated HBx-proteins.

Bock CT, Toan NL et al. (2008) Intervirology

Interferon alpha 2 and HBV infection

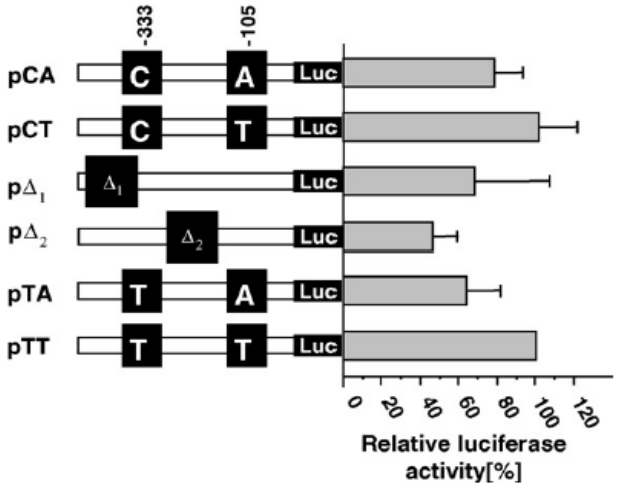
- IFNa is a critical mediator of immunity to hepatitis B virus (HBV) infection
- IFN has been used in the treatment of viral hepatitis. In this study we investigated the mutated IFNa gene in 344 HBV-infected patients and 293 HC control

Allelic distribution of variants in *INF-α* promoter region in patients and controls

	Vietnamese			
	HBV−		HBV+	
	Wild type	Variant	Wild type	Variant
C-105T	1	0	1	0
T-333A	1	0	0.99	0.01
Δ_1	1	0	1	0
Δ_2	0.89	0.11	0.81	0.19

Allelic frequency and OR of the deletion in *INF-α* promoter region in patients and controls

Diagnosis (n)	Δ_2 n (frequency)	OR (CI)	P
AHB ($n=82$)	17 (0.21)	2.2 (1.1–4.1)	0.020
CHB ($n=124$)	29 (0.23)	2.6 (1.5–4.3)	<0.001
LC ($n=180$)	28 (0.16)	1.6 (0.9–2.6)	n.s.
HCC ($n=230$)	44 (0.19)	2.0 (1.3–3.1)	0.002
Asym ($n=72$)	8 (0.11)	1.1 (0.4–2.3)	n.s.
Total HBV-infected ($n=688$)	126 (0.18)	1.9 (1.4–2.7)	<0.001
HC ($n=586$)	62 (0.11)	n.a.	n.a.



Conclusion: Deletion in the promoter of the *IFN-α 2* gene reduces the transcription in vitro and was susceptibility to hepatitis B

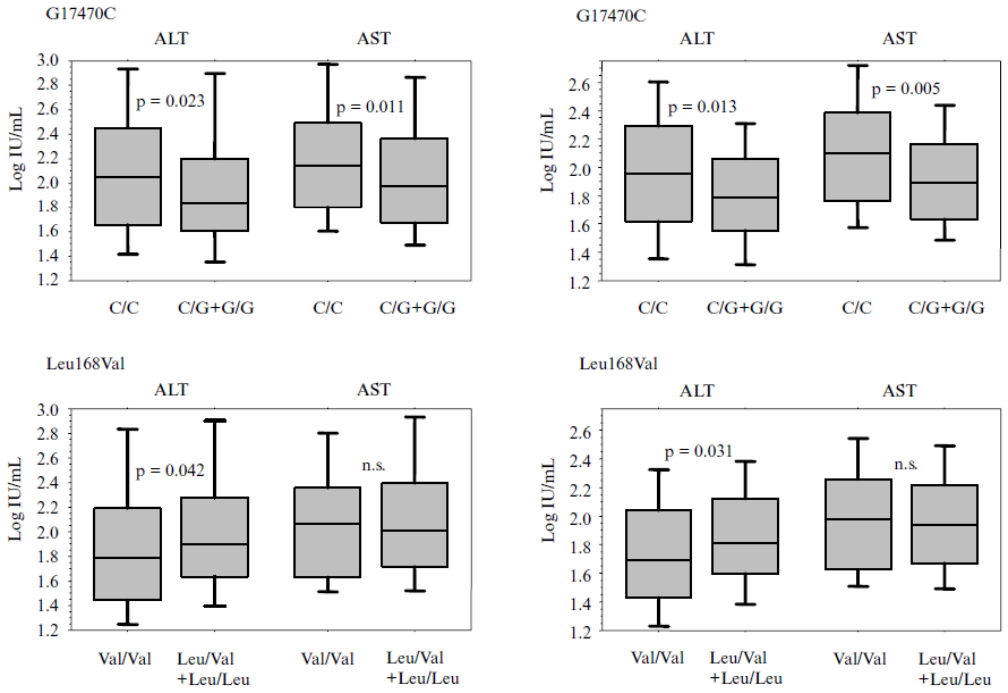
Song LH et al. (2006) Mutation research

Interferon alpha receptor 1 and HBV infection

• *17470C* allele (rs1012335) in *IFNAR1* was more frequent in HBV-infected patients (OR: 2.6; p < 0.001)

• *G* allele (rs2257167, cause Val to Leu) was more frequent in the healthy control (OR: 0.54, p = 0.004)

Conclusion: two variants of *IFNAR1* gene are associated with clinical outcomes of HBV infection

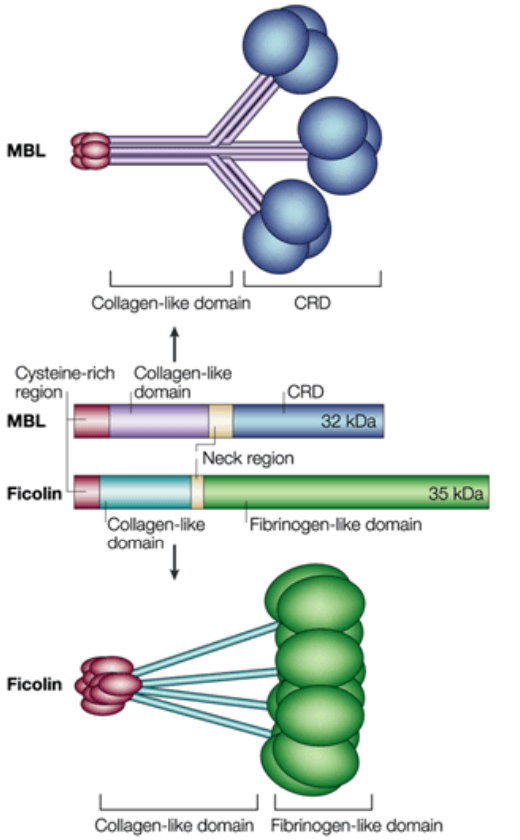


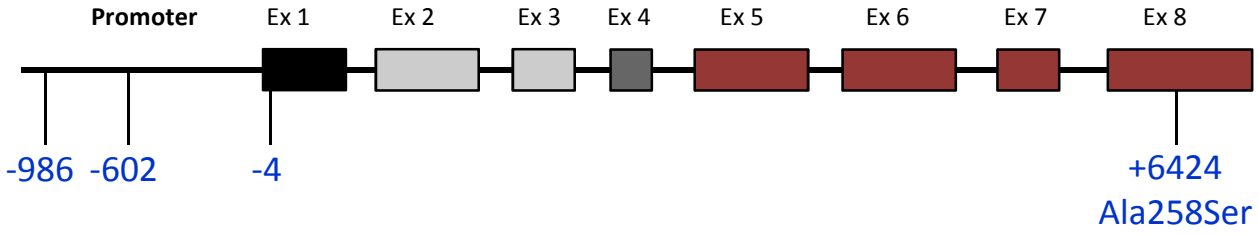
Song LH et al. (2008) Eur. Cytokine Netw.

Ficolin-2 and *FCN2* polymorphism

- Soluble pattern recognition molecules
- Produced mainly in liver
- Recognize pathogen recognition receptors (PRRs)
- Activate complement system via lectin pathway
- Clearance of apoptotic cells
- SNPs (-986G/A, -602G/A -4A/G and +6424G/T)

are associated with ficolin-2 levels and diseases





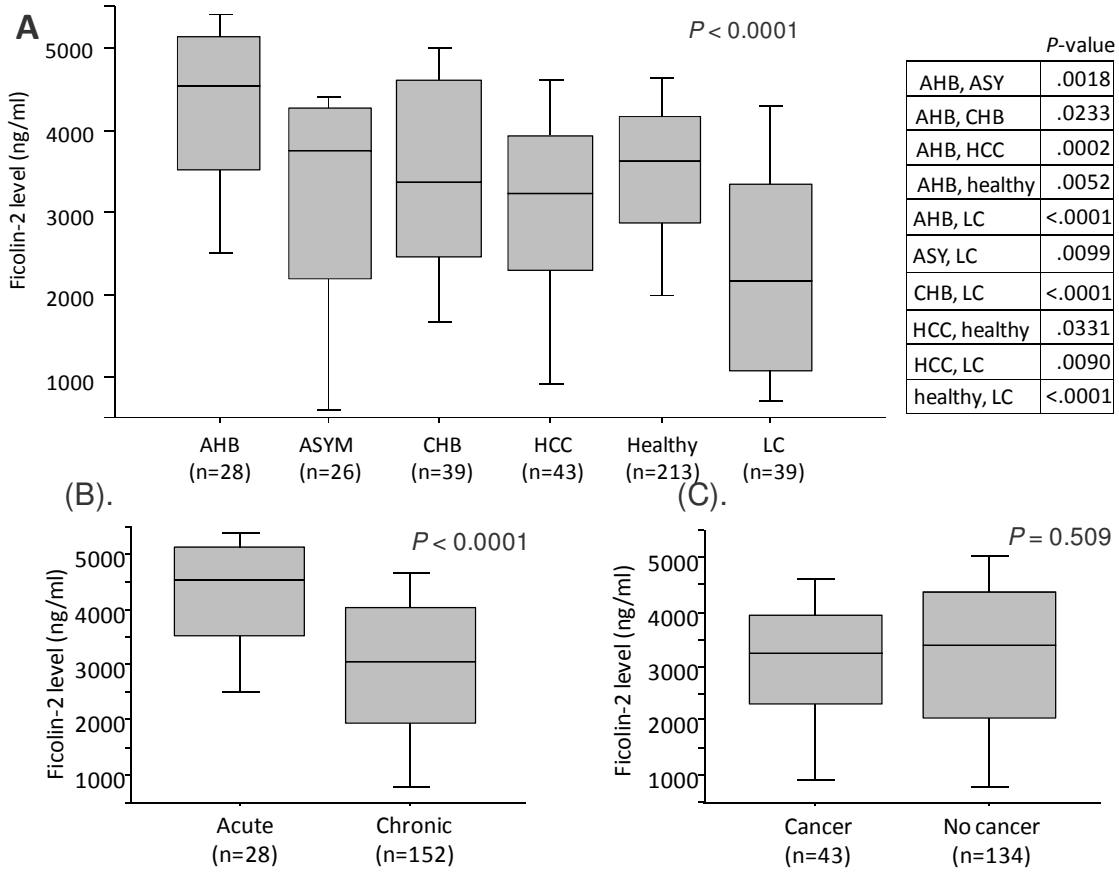
Garred P. et al. J Innate Immun. 2010;2(1):3-16

FCN2 polymorphism and HBV infection

Haplotype -986/-602/-4/+6424	Cases (%)	Controls (%)	OR (95%CI)	*P*
	AHB (n=92)	**Controls (n=606)**		
GGAG	64 (69.6)	411 (67.8)	NA	NS
GGAT	24 (26.1)	122 (20.1)	NA	NS
AAAG	2 (2.2)	15 (2.5)	NA	NS
AGGG	2 (2.2)	58 (9.6)	**0.2 (0.02-0.8)**	**0.02**
	HCC (n=224)	**Controls (n=606)**		
GGAG	159 (71)	411 (67.8)	NA	NS
GGAT	56 (25)	122 (20.1)	NA	NS
AAAG	8 (3.6	15 (2.5)	NA	NS
AGGG	1 (0.4)	58 (9.6)	**0.04 (0.001-0.25)**	**<0.0001**
	HBV Patients (n=796)	**Controls (n=606)**		
GGAG	561 (70.5)	411 (67.8)	NA	NS
GGAT	172 (21.6)	122 (20.1)	NA	NS
AAAG	27 (3.4)	15 (2.5)	NA	NS
AGGG	36 (4.5)	58 (9.6)	**0.4 (0.28-0.7)**	**0.0002**

Tong HV et al. PLoS One. 2011;6(11):e28113

Ficolin-2 levels in HBV infection



	P-value
AHB, ASY	.0018
AHB, CHB	.0233
AHB, HCC	.0002
AHB, healthy	.0052
AHB, LC	<.0001
ASY, LC	.0099
CHB, LC	<.0001
HCC, healthy	.0331
HCC, LC	.0090
healthy, LC	<.0001

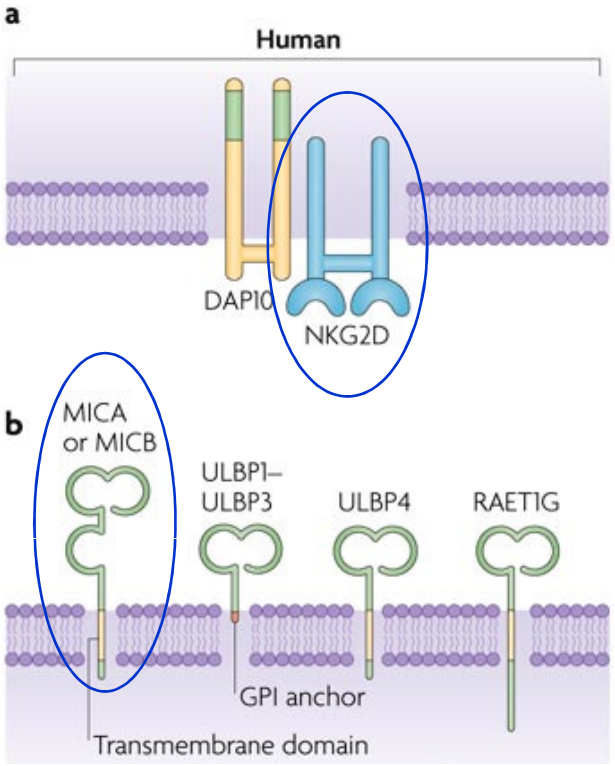
Soluble ficolin-2 levels were significantly increased in acute groups and significantly decreased in CHB, LC and HCC.

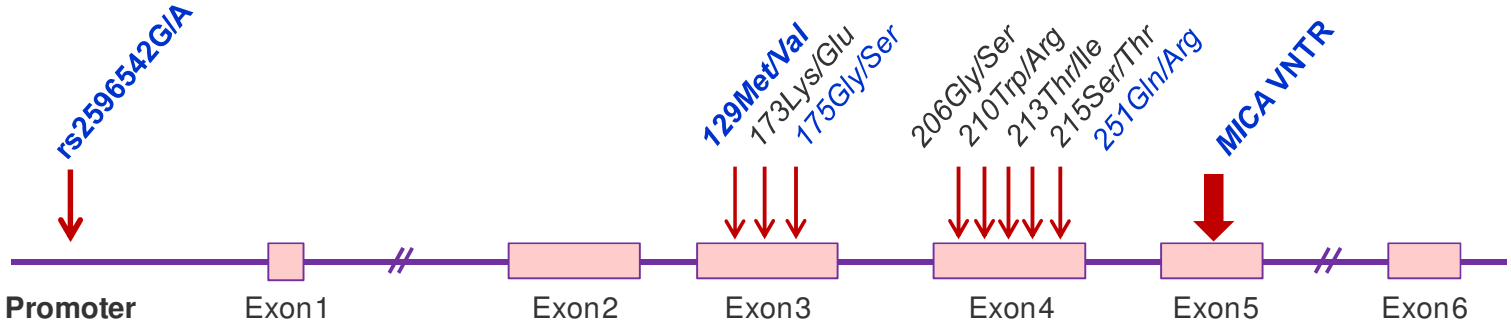
sFicolin-2 protein was significantly modulated during infection, and the high level of serum ficolin-2 significantly contributed to immune response against HBV infection.

Tong HV et al. PLoS One. 2011;6(11):e28113

MICA : gene and protein

- The human major histocompatibility complex class I (MHC) chain-related gene A/B (***MICA/MICB***)
- Ligands for NKG2D receptors of NK, NKT cells
- Members of *MIC* gene family including ***MICA***, MICB and *MICC* ….to *MICG*
- *MICA* polymorphism is associated with many diseases (autoimmune, infectious diseases and cancer)





Frigoul A et al. 2005*, Recent Res. Devel. Human Genet.* Robert A. Eagle & John Trowsdale *Nature Reviews Immunology* **7**, 737-744

MICA polymorphisms and HBV-related HCC

HCC versus LC

	OR	95% CI	P-value	Best fit model
rs2596542G/A	1.6	1.13-2.3	0.006	Allelic
MICA-129 (rs1051792)	1.5	1.04-2.1	0.03	Allelic
MICA-175 (rs1131896)	1.7	0.97-2.9	0.05	Gly/Gly (GG)
MICA-251 (rs1063635)	1.6	1.1-2.3	0.008	allelic
A5/A5.1	0.4	0.2-0.9	0.015	Genotype
A6/A9	5.4	1.2-49.9	0.02	Genotype
A4	1.8	1.02-3.2	0.04	Allele
A5	0.7	0.5-0.99	0.03	Allele
GGAG-A5	0.64	0.45-0.9	0.009	Haplotype
AAGA-A4	2.3	1.04-5.4	0.04	Haplotype
AGAA-A6	3	0.93-12.7	0.05	Haplotype

HCC versus CHB

	OR	95% CI	P-value	Best fit model
rs2596542G/A	1.5	1.1-2.1	0.01	Allelic
MICA-129 (rs1051792)	2.3	1.1-5	0.02	Recessive
MICA-175 (rs1131896)	0.47	0.3-0.8	0.0015	Heterozygote advantage
A5/A5.1	0.35	0.15-0.8	0.005	Genotype
A5/A6	4.25	1.3-17.7	0.006	Genotype
AAGA-A4	2.1	1-4.6	0.04	Haplotype
GGAA-A5	0.06	0.001-0.4	0.0001	Haplotype

Variants in MICA gene were significantly associated with HCC susceptibility from CHB and LC patients. Some variants contributed to an increased risk of HCC (Odds ratio > 1), Exp. promoter variant rs2596542 contributed to increased risk of HCC with the OR is 1.6.

Tong HV et al. J Viral Hepat. 2013 Oct;20(10):687-98

MICA polymorphisms and HBV persistence

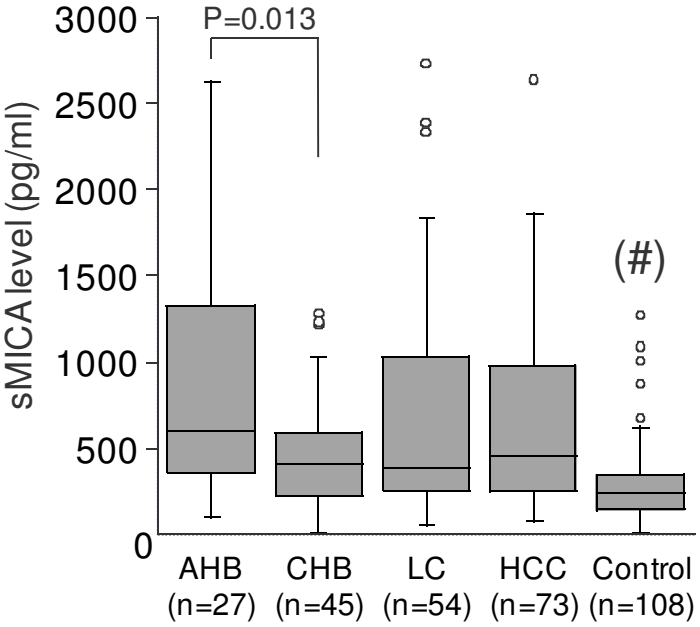
HBV cases vs. healthy control

	OR	95% CI	P-value	Best fit model
MICA-175 (rs1131896)	1.3	1.1-1.5	0.0095	Allelic
A5/A5	1.6	1.14-2.34	0.005	Genotype
A9/A9	0.47	0.24-0.9	0.02	Genotype
A5	1.24	1.02-1.5	0.02	Allele
A6	1.5	1.02-2.2	0.04	Allele
A9	0.8	0.6-0.97	0.02	Allele
GGGG-A5	2.4	1-6.7	0.051	Haplotype

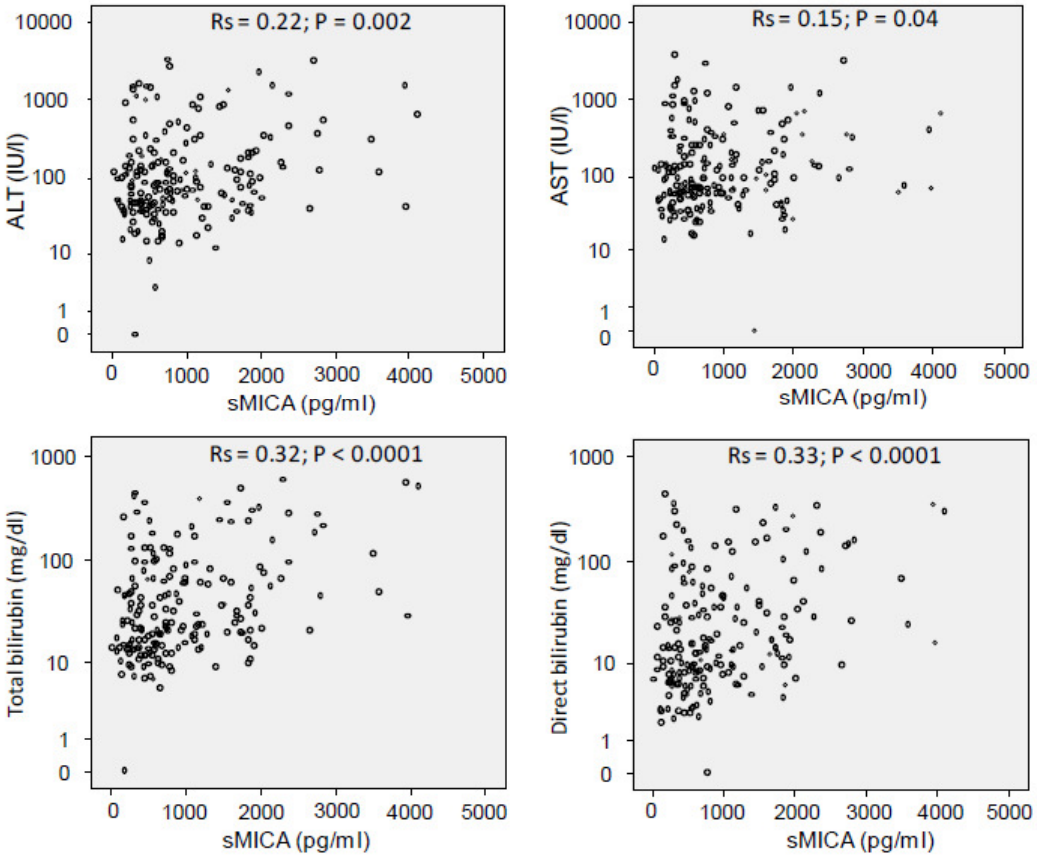
To comparison between groups of patients infected with HBV and healthy controls, the polymorphisms in MICA were significantly associated with HBV infection and HBV persistence.

Tong HV et al. J Viral Hepat. 2013;20(10):687-98

sMICA levels and HBV outcomes



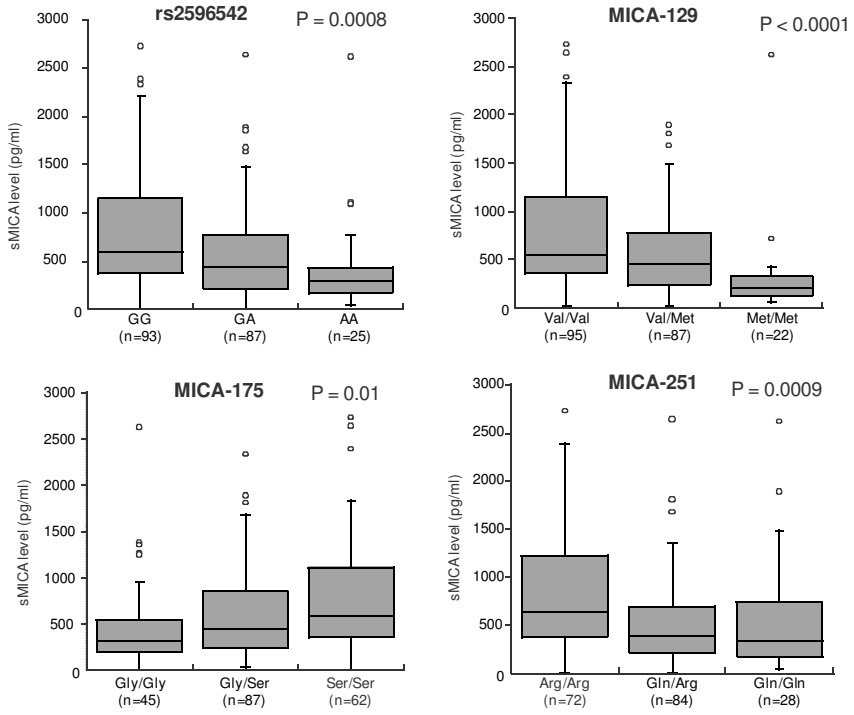
(#): P<0.0001 when compared with other groups



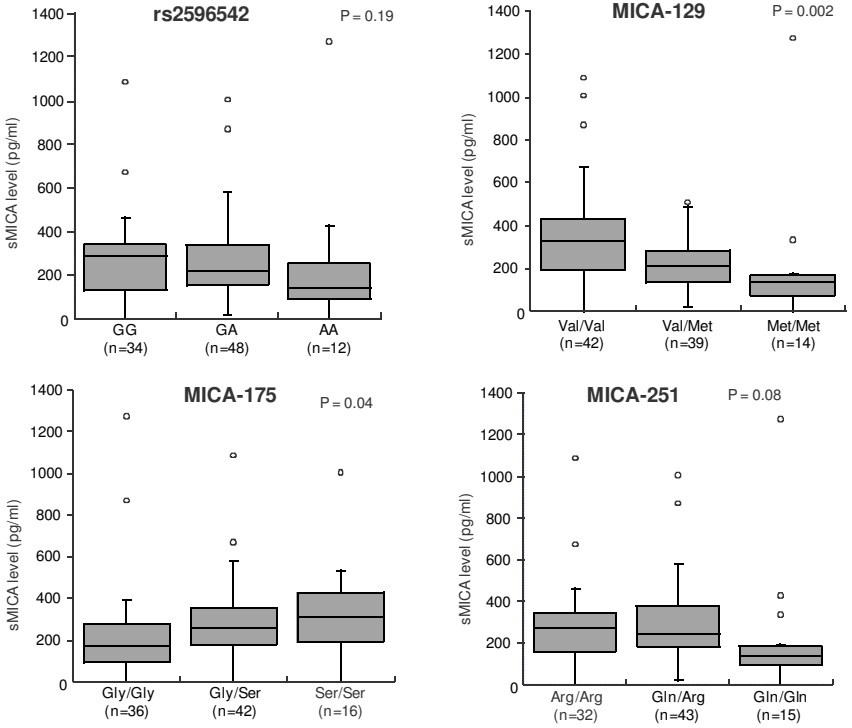
Tong HV et al. J Viral Hepat. 2013;20(10):687-98

sMICA levels according to *MICA* SNPs

in patients



in healthy controls



Tong HV et al. J Viral Hepat. 2013;20(10):687-98

Main message: MICA

❖ **Polymorphisms in *MICA* gene are associated with HBV persistence, HBV-induced HCC**

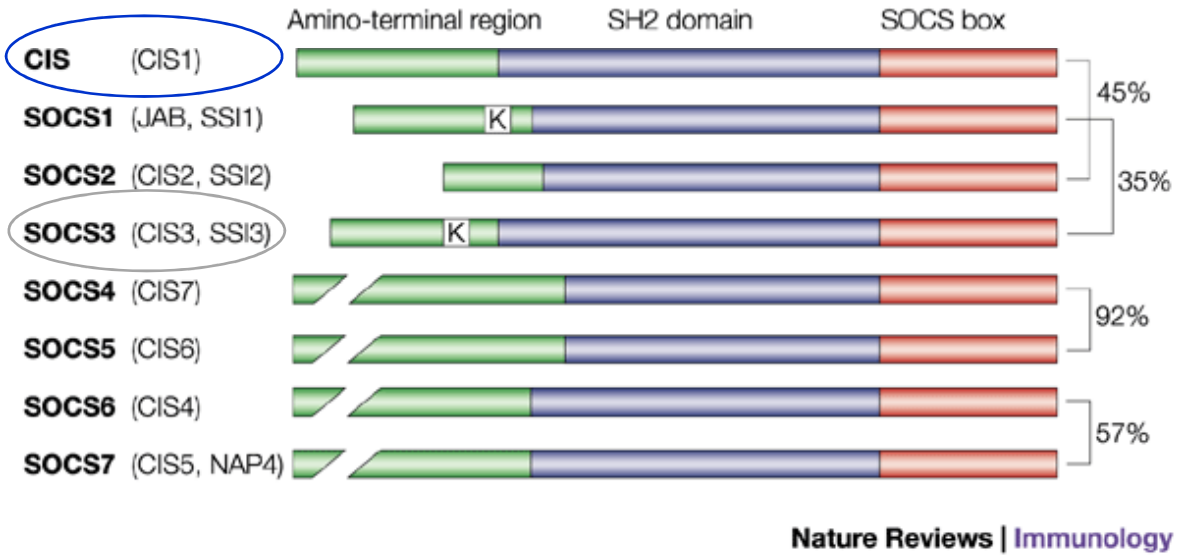
❖ ***MICA* polymorphisms modulate MICA protein serum levels**

❖ **MICA protein modulate HBV infection, liver disease progression and tumor surveillance**

Tong HV et al. J Viral Hepat. 2013 Oct;20(10):687-98 *Tong HV et al. (unpublished)*

CISH and HBV infection

- Cytokine-inducible SRC homology 2 (SH2) domain containing protein (CISH)
- SOCS family of proteins (suppressor of cytokine signaling)
- CISH controls the signaling of a variety of cytokines, in particular **IL-2**
- *CISH* polymorphism (e.g. **rs414171**) associated with the susceptibility to different infectious diseases



Yoshimura A. et al. Nat Rev Immunol. 2007 Jun;7(6):454-65

CISH and HBV infection

-292A>T (#rs414171)	**HBV Patients** (n=473)	**Healthy controls** (n=416)	**OR (95% CI)**	***P* value**
Genotype				**0.029**
AA	177 (37.4)	169 (40.6)	Reference	
AT	214 (45.3)	201 (48.3)	1.02 (0.76-1.37)	0.91
TT	82 (17.3)	46 (11.1)	**1.7 (1.1-2.65)**	**0.012**
Allele			**1.22 (1-1.49)**	**0.04**
Allele A	568 (60.04)	539 (64.8)		
Allele T	378 (39.96)	293 (35.2)		
Dominant			1.14 (0.87-1.51)	0.33
Wild type	177 (37.4)	169 (40.6)		
AT+TT	296 (62.6)	247 (59.4)		
Recessive			**1.69 (1.23-2.54)**	**0.0078**
AA+AT	391 (82.7)	370 (88.9)		
Mutant	82 (17.3)	46 (11.1)		

No significant association of *CISH* variant rs414171 with clinical parameters

Tong HV et al. Immunogenetics. 2012 Apr;64(4):261-5

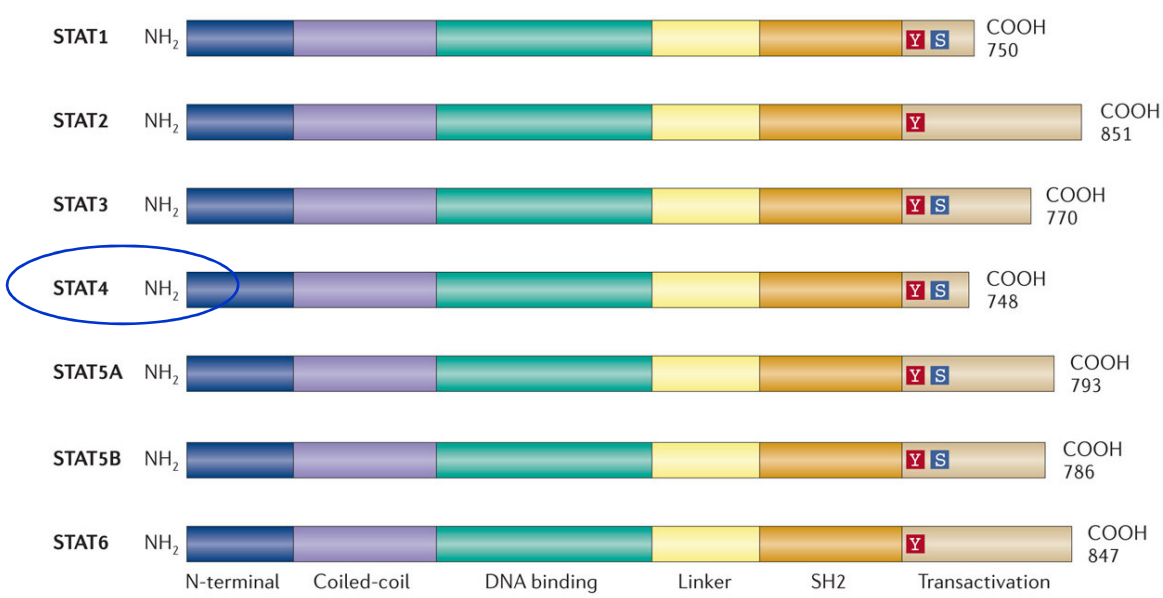
STAT4 and HBV-induced HCC

STAT protein family (signal transducer and activator of transcription)

Transduce signals from cytokine-receptor complexes and regulate transcription of specific genes

Response mainly to IL-12, IL-23, interferon type I and regulates the transcription of various genes including IFN-y via the JAK/STAT pathway

Variants in *STAT4* (e.g. **rs7574865**) are associated with several autoimmune diseases



Gabriella Miklossy et al. (2013) Nature Reviews Drug Discovery 12, 611–629

STAT4 and HBV-induced HCC

STAT4 **rs7574865G/T**	**CHB n(%)**	**LC n(%)**	**HCC n(%)**	**HCC vs. CHB** OR(95% CI)	P value
GG	86 (41.7)	112 (50.5)	117 (49)	reference	
GT	92 (44.7)	87 (39.2)	102 (42.7)	0.9 (0.7-1.15)	0.41
TT	28 (13.6)	23 (10.3)	20 (8.3)	**0.7 (0.45-0.98)**	**0.039**
Allele					
G	264 (64.1)	311 (70)	336 (70.3)	**reference**	
T	148 (35.9)	133 (30)	142 (29.7)	**0.84 (0.7-0.99)**	**0.048**
Dominant					
GG	86 (41.7)	112 (50.5)	117 (49)	reference	
GT+TT	120 (58.3)	110 (49.5)	122 (51)	0.85 (0.7-1.1)	0.16
Recessive					
GG+GT	178 (86.4)	199 (89.6)	219 (91.6)	**reference**	
TT	28 (13.6)	23 (10.4)	20 (8.4)	**0.7 (0.5-0.99)**	**0.047**

Not significant

- ❖HCC vs. LC
- ❖HCC vs. LC+CHB
- ❖LC vs. CHB

❖No significant association of *STAT4* variant rs7574865 with clinical parameters

❖Variant rs9275319 in *HLA-DQ* is not in Hardy-Weinberg disequilibrium in Vietnamese population

Clark A, Gerlach F, Tong HV, et al. Immunogenetics. 2012 Apr;64(4):261-5

Summary and Conclusion

❖ **HBV genotype and genotype mixtures are associated with altered pathogenesis and clinical outcome of HBV infection.**

❖ **Atypical nuclear/perinuclear localization of HBx mutants might be responsible for an enhanced activation of STAT3, inhibition of STAT1 and silencing of SOCS1/SOCS3 expression.**

❖ **Polymorphisms in host immune genes (INF-α, *INF-α* receptor, *FCN2, MICA, CISH, STAT4*) are associated with clinical outcomes of HBV infection in Vietnamese population.**

❖ **Ficolin-2, MICA play an important role in immune modulation of HBV infection, liver disease progression and tumor surveillance.**

Acknowledgments

Thanks for your attention!

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