Bilirubin as a predictor of oxidative stress-mediated diseases

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Outline

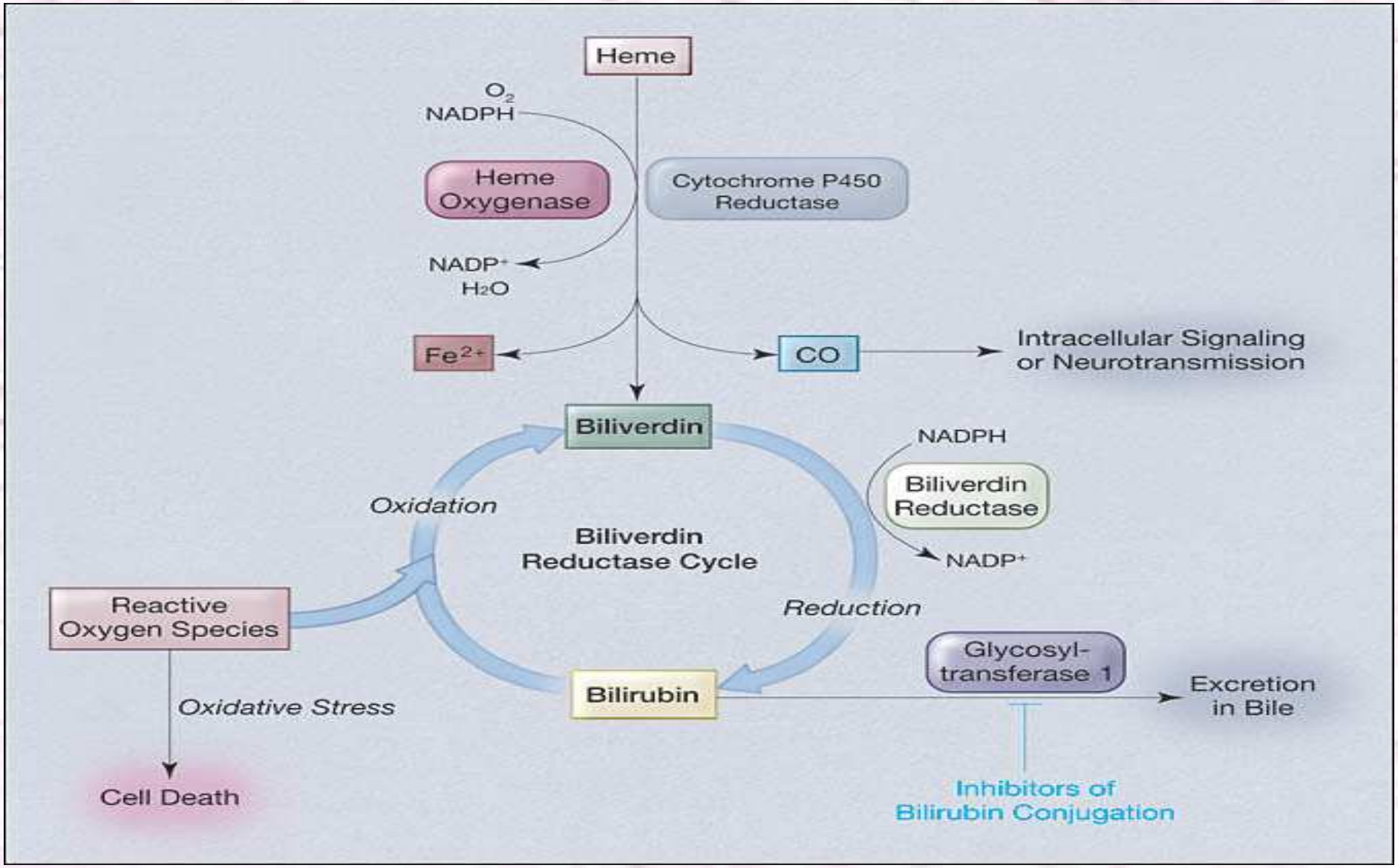
- Serum bilirubin and markers of
  - oxidative stress
  - inflammation
- Relationship of serum bilirubin to
  - rheumatological diseases
  - neuropsychiatric diseases

Bilirubin as an antioxidant

- in vitro studies -

- ***in vitro,*** **bilirubin is almost 30x more potent than vitamin E in preventing LDL oxidation** ***(Life Sci 1994)***
- **2.5 uM bilirubin inhibits lipid peroxidation by 32% in tissue homogenates from Wistar rats** ***(Can J Phys Pharm 1998)***
- **10 nM bilirubin eliminates 10 000x higher concentration of H_2O_2** ***(PNAS 2002)***

Katabolismus hemu



Sedlak, T. W. et al. JAMA 2006

Bilirubin as an antioxidant
- human studies -

- contribution of bilirubin on total antioxidant capacity
  - in adults as much as 20%
  - in newborns as much as 77%! *(Clin Sci 1993, FEBS Lett 1994, Biol. Neon. 1997)*
- higher neonatal jaundice protects from
  - circulatory failure, sepsis and asphyxia *(Lancet 1991)*
  - neonatal retinopathy *(NEJM 1989)*

Bilirubin and markers of oxidative stress: Total antioxidant capacity

(Vítek et al. Atherosclerosis 2002)

	bilirubin (μM/l)	TAS (mM/l)
GS	32.6±13.5*	1.43±0.12*
CHD	9.0±2.7	1.30±0.15
Control	9.1±2.7	1.32±0.13

*GS vs. CHD and controls: $p<0.05$

GS = Gilbert syndrome (benign hyperbilirubinemia)

CHD = coronary heart disease

TAS = Total antioxidant status (Randox, GB)

Influence of exogenous bilirubin on TAS

(Vítek et al. Atherosclerosis 2002)

UCB (μM/l)	total bilirubin (μM/l)	TAS (mM/l)
0*	24.5	1.37
6.25	31.9	1.42
12.5	40.6	1.46
25	55.8	1.51
50	79.6	1.57
100	133.0	1.76

* = serum with defined antioxidant capacity

These results are consistent with data mentioned above

(Clin Sci 1993, FEBS Lett 1994, Biol. Neon. 1997)

Bilirubin and markers of oxidative stress: Urinary biopyrrins

- **Tripyrrolic compounds formed by oxidative degradation of bilirubin**
- **Increased urinary excretion found in following conditions a/w increased oxidative stress:**
  - **acute myocardial infarction** ***(Int J Cardiol 2001, Am J Cardiol 2002)***
  - **congestive heart failure** ***(J Am Coll Cardiol 2004)***
  - **major and prolonged surgical procedures** ***(Clin Chem 1998; Surg Today 1998; HPB Surg 1999)***
  - **sepsis** ***(J Surg Res 2001)***
  - **alcohol consumption** ***(Ind Health 2001)***
  - **atopic dermatitis** ***(Life Sci 2003)***
  - **schizophrenia and depressions** ***(Eur Neuropsychopharm 2005)*** **or even psychological stress** ***(BBRC 2002)***

Urinary excretion of biopyrrins in GS

(Vítek et al. J Gastroent Hepatol 2006)

Parameter	**Controls** (bili ≤17.0 uM/L) (n=25)	**GS** (bili >17.0 uM/L) (n=33)
Serum bilirubin (μM/L)	**9.9 ± 3.0**	**27.8 ± 9.7; p<0.001**
Urinary biopyrrins (U/g creatinine)	**90.2 ± 139.1**	**19.9 ± 26.0; p<0.001**

Parameter	OR (95% CI)
Biopyrrins (adjusted for possible confounding factors)*	**0.18 (0.03-0.94) p=0.042**

*** positive LFT, uric acid, albumin, total cholesterol and glucose intolerance**

Bilirubin and markers of oxidative stress: Advanced glycation-end products

- AGEs, such as N-carboxymethyl lysine (CML) or pentosidine, are formed through the interaction of plasma proteins with sacharide oxidation products *(Nephrol Dial Transpl 1997)*
- AGEs activate NF-κB with subsequent overexpression of proinflammatory genes including CRP *(Kidney Int 2001)*
- related also to chronic inflammation and correlate with inflammatory markers, such as CRP, ESR, leukocyte or platelet count *(BBRC 1998)*

AGEs levels in subjects with GS

(Kalousova et al. Cell Mol Biol 2005)

Parameter *(median; 25-75%)*	GS (bili >17.0 uM/L) (n=33)	Controls (bili ≤17.0 uM/L) (n=21)	significance
pentosidine *(nmol/g total protein)*	*1.12 (0.90-1.28)*	*1.31 (1.18-1.58)*	*p<0.005*
CML *(mg/g total protein)*	*6.7 (6.1-7.34)*	*7.33 (6.76-8.20)*	*p=0.01*

Parameter	*OR (95% CI)*	*Parameter*	*OR (95% CI)*
Pentosidine *(adjusted for possible confounding factors)**	*0.04 (0.003-0.67), p=0.02*	CML *(adjusted for possible confounding factors)**	*0.20 (0.03-1.30), p=0.09*

* positive LFT, uric acid, albumin, total cholesterol and glucose intolerance

Bilirubin vs. inflammation

- has anticomplement effects *(BBA 1999)*
- attenuates liver injury in a rat model of endotoxemia *(Hepatology 2004)*
- blocks oxidant-mediated activation of leukocytes *(Circ Res 1999)* and attenuates vascular endothelial dysfunction *(ATVB 2005)* via inhibition of NF-kB *(J Immunol 2004)*
- inhibits transendothelial leukocyte migration via suppresion of VCAM signaling *(J Immunol 2005)*, a process implicated in the pathogenesis of numerous diseases, including
  - IBD *(Gut 1995)*
  - conjunctivitis *(Allergy 2002)*
  - nephropathy *(Nephron 1999)*
  - arthritis *(Arthr Rheum 2001)*
  - systemic colagenoses *(Clin Nephrol 1995)*
  - and possibly cancer *(Br J Cancer 1998)*

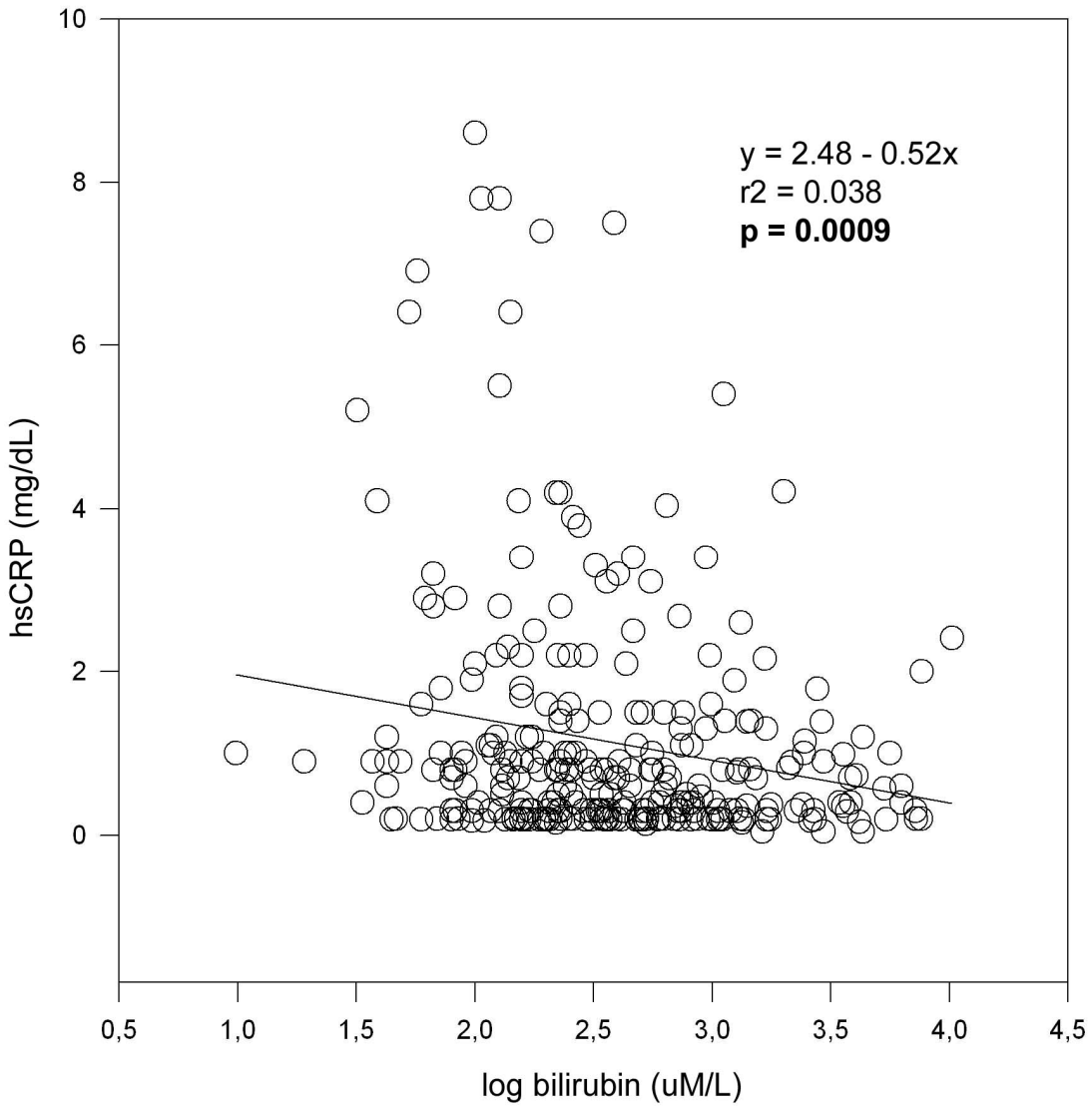
hsCRP levels and serum bilirubin

(Vítek et al. Prev Control 2005, abstract)

	Bilirubin (range; μM/L)	hsCRP (median; 25-75%; mg/L)	significance*
Quartile 1 (n=78)	*2.7-9.0*	*0.90* *(0.3-2.1)*	
Quartile 2 (n=67)	*9.1-12.5*	*0.70* *(0.2-1.4)*	*p=0.089**
Quartile 3 (n=71)	*12.6-18.8*	*0.40* *(0.2-1.0)*	*p=0.010**
Quartile 4 (n=72)	*18.9-55.2*	*0.44* *(0.2-1.2)*	*p=0.013**

* vs. Quartile 1

Relationship between serum bilirubin and hsCRP levels



Summary of part I

- Inverse relationship between serum bilirubin and hsCRP levels was found also in a study by Erdogan et al. *(Atherosclerosis 2006)*
- Bilirubin inhibits protein kinase C activity *(Clin Chim Acta 1993)* implicated in pathogenesis of inflammatory *(J Biochem 2002)*, but also atherosclerotic *(Cardiol Rev 2005)*, and cancer diseases *(Arch Pharm Res 2005)*
- Elevated serum bilirubin levels are associated not only with increased serum antioxidant capacity, but also with low degree of inflammation

Bilirubin and rheumatological diseases

Recently, we found inverse relationship between bilirubin levels and:

- **systemic lupus erythematosus (SLE)** ***(Vítek et al. J Am Soc Nephr 2003 + Liver Int 2006, abstracts)***
- **rheumatoid arthritis** ***(Schwertner and Vítek. Clin Chem 2004, abstract)***
- **Wegener granulomatosis** ***(Vítek et al. unpublished results)***

Serum bilirubin in SLE

Parameter	Bilirubin (μM/L, *median; 25-75%)*	Significance (vs. controls)
Controls (n=115)	10.6 (8.3-15.0)	Reference
SLE ¨plain¨ (n=25)	8.2 (6.1-13.1)	p=0.049
Neurolupus (n=75)	7.3 (5.7-9.6)	p<0.00001
SLE nephropathy (n=95)	7.1 (5.4-8.0)	p<0.001
SLE total	8.0 (6.3-10.9)	p<0.00001
	ORs for bilirubin above median of controls OR (95% CI)	
SLE ¨plain¨	0.57 (0.23-1.41), p=0.22	
Neurolupus	0.22 (0.11-0.43), p=0.00001	
SLE nephropathy	0.02 (0.005-0.09), p<0.00001	
SLE total	0.14 (0.08-0.25), p<0.00001	

Bilirubin and SLE

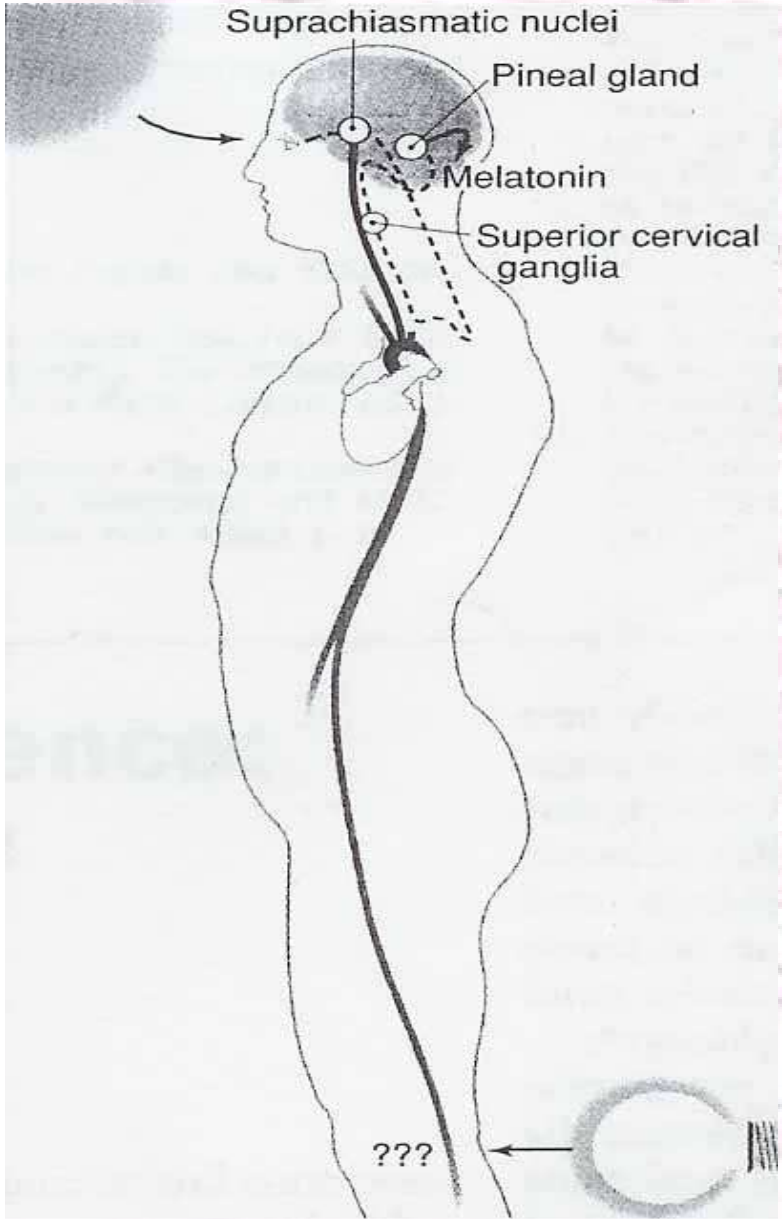
- Our results demonstrate striking inverse association between serum bilirubin levels and SLE
- Antioxidant defense systems efficiency is related to the prognosis of patients with SLE *(Clin Chem Lab Med 2002)*
- Further studies must reveal whether low serum bilirubin levels are caused by genetic predisposition of affected subjects (as evidenced by early onset of the disease) or due to the consumption of bilirubin during severe SLE-mediated oxidative stress *(Rheumatology 1999)*
- The same principle might also apply for RA and Wegener granulomatosis, where similar results have been detected

Bilirubin vs. neurologic and psychiatric diseases

Inverse association between serum bilirubin levels and

- **amyotrophic lateral sclerosis** *(Clin Neurol Neurosurg 2003)*
- **depressions** *(Biol Psychiatr 2002)* **- patients with winter depressions have lower nocturnal bilirubin concentrations compared to controls.**

Bilirubin and circadian rhytms?



Popliteal illumination with a visible-spectrum light source, originally developed to treat pathologic neonatal jaundice, can shift human circadian rhythms without any transduction of light through the eye *(Science 1998)*

Bilirubin was suggested as a chronobiological photoreceptor involved in human circadian rhythms

adapted from Oren et al. Science 1998

Schizophrenia and serum bilirubin

- **Controversial data:**
  - **positive association between hyperbilirubinemia and schizophrenia described by Müller *et al.* *(Pharmacopsych 1991)* and also Miyaoka *et al*. *(J Clin Psychiatry 2000 a,b)***
  - **on contrary, reduced levels of serum bilirubin *(Psychiatry Res 2000, Schizophr Res 2003, Neuropsychobiol 2004)* in patients with schizophrenia were demonstrated in several other studies**
  - **In none of these studies GS genotype has been analyzed in patients with schizophrenia**

Results

(Vítek et al. Liver Int 2005, abstract)

	Schizo n = 97	Controls n = 128	significance
Bilirubin, males + females (μM/L, median; 25%-75% range)	6.5 (4.8-8.5)	12.2 (9.0-16.1)	<0.001
Prevalence of bili >17 μM/L (%)	3.1	21.1	<0.001
UGT1A1*28 prevalence (%)	11.3	16.4	0.376
-3263G/G (PBREM) prevalence (%)	17.5	26.6	0.149
Serum bilirubin in subgroups according to individual genotypes (uM/L,median; 95 CI)			
TATA 6/6	5.0 (4.2-7.2)	10.9 (7.8-12.6)	<0.00001
TATA 6/7	7.0 (5.2-8.8)	12.9 (8.8-15.0)	<0.00001
TATA 7/7 (GS genotype)	12.7 (6.2-18.2)	21.8 (16.9-30.4)	0.015
-3263T/T	5.0 (4.2-6.0)	10.9 (7.6-12.2)	<0.00001
-3263G/T	6.8 (4.8-8.2)	14.1 (9.3-15.8)	<0.00001
-3263G/G (GS genotype)	9.8 (6.4-13.5)	16.9 (11.3-25.3)	0.004

Summary

- Patients with schizophrenia have neither higher prevalence of common GS polymorphisms nor hyperbilirubinemia >17 μM/L (1 mg/dL)
- Furthermore, all genotype subgroups of patients with schizophrenia exhibit substantially lower serum bilirubin levels
- Low serum bilirubin levels seems to be a
  1. predisposing factor for schizophrenia
  2. but also a result of its consumption during increased oxidative stress

Conclusions

Serum bilirubin is an important predictor of oxidative stress-mediated diseases including autoimmune, neurologic and psychiatric conditions

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- **1st, 3rd and 4th Department of Internal Medicine**
- **Institute of Hygienic Medicine and Medical Epidemiology**

and

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- **Research Institute of Rheumatology**