

**平成25度第一回都道府県肝疾患診療連携拠点病院
医師向け研修会**

3)NASHの病態・診断・治療の現状と問題点

**2013年8月2日(国立国際医療研究センター病院)
大阪府済生会吹田病院 岡上 武**

4大肝疾患

- **B型肝炎:130万人**
- **C型肝炎:170万人**
- **アルコール性肝障害:250万人**
- **非アルコール性脂肪性肝疾患(NAFLD):1,500~2,000万人**

(NAFLD:nonalcoholic fatty liver disease)

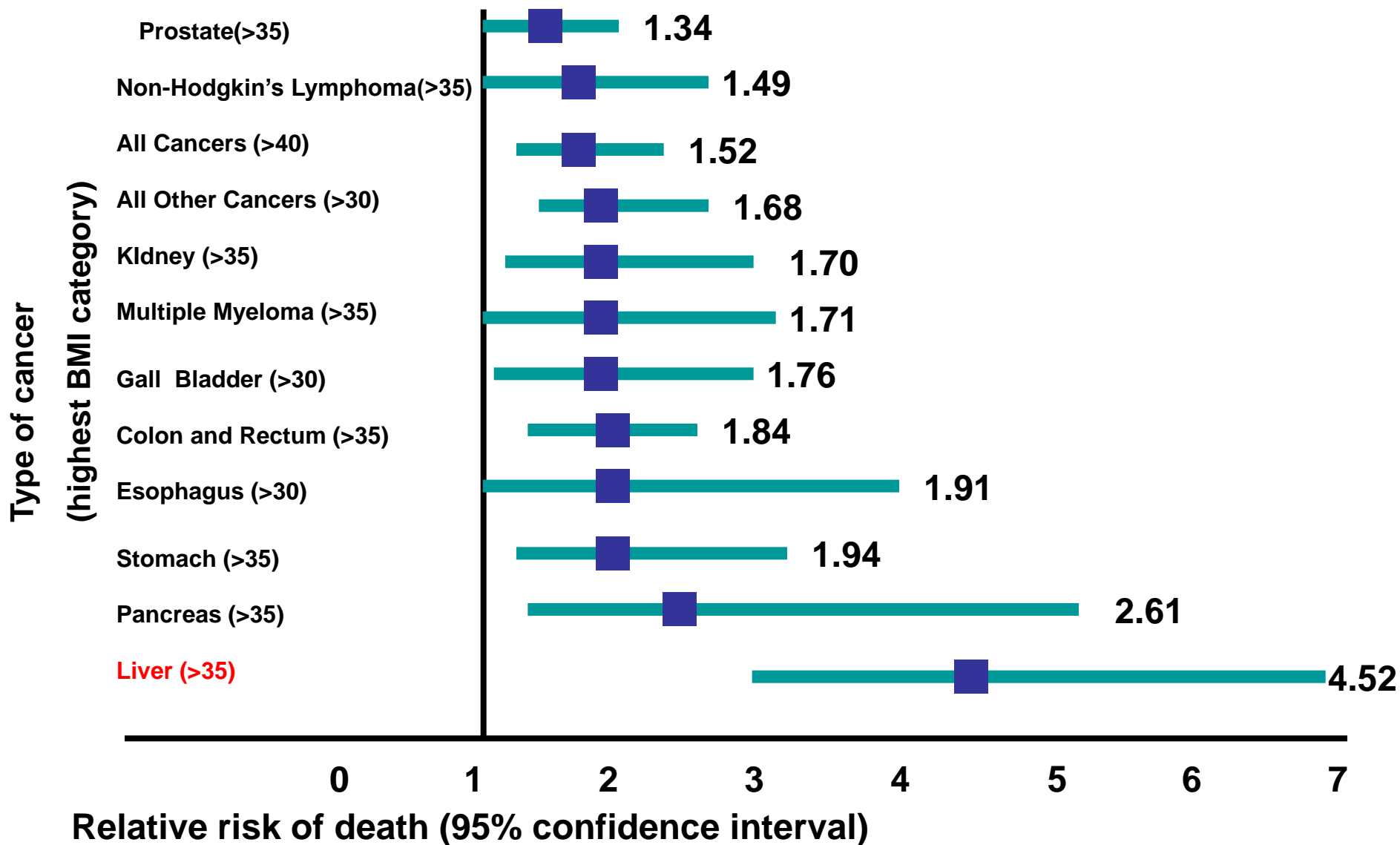
⇒ **肥満、糖尿病、脂質異常症、高血圧患者**に多い。

(life style-related disease: 生活習慣病の肝臓での表現型)

⇒ **単純性脂肪肝(nonalcoholic fatty liver: NAFL)**と、炎症を伴い進行性の脂肪肝である**非アルコール性脂肪肝炎 (nonalcoholic steatohepatitis: NASH)**の2種類ある。**NASHは国内に300~400万人存在と推定。**

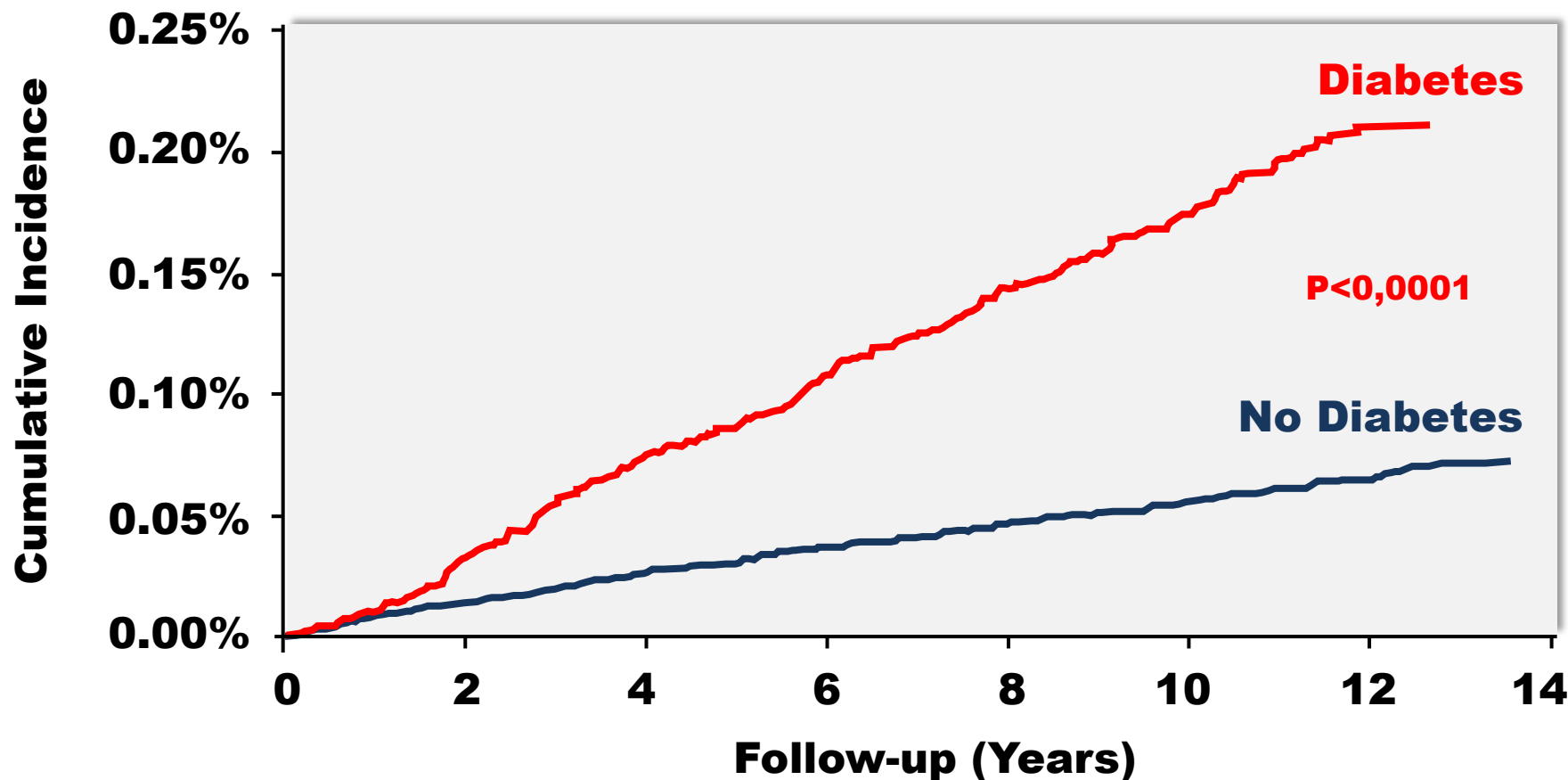
⇒ **NAFLDは日本、米国、オーストラリアなどで最も高頻度の肝疾患。**

BMI 35以上の男性では肝癌発生リスクが非肥満者の4.5倍高い



Calle EE, et al. N Engl J Med 2003

米国の糖尿病患者は非糖尿病患者に比して 累積肝癌発生率が3倍以上高い



糖尿病とがん罹患リスクに関する メタ解析とプール解析

日本癌学会・日本糖尿病学会による報告書(2013年)

がん種	メタ解析*	わが国の プール解析**	生涯がん罹患リスク (2007年)***		年間調整罹患率 (2007年) 人口10万対****	
			男性	女性	男性	女性
胃がん	1.19(1.08~1.31)	1.06(0.91~1.22)	10.9%	5.5%	78.9	28.6
大腸がん	1.30(1.20~1.40)	1.40(1.19~1.64)	8.5%	6.7%	63.4	35.9
肝がん	2.50(1.80~2.90)	1.97(1.65~2.36)	4.0%	2.2%	29.8	10.6
膵がん	1.82(1.66~1.89)	1.85(1.46~2.34)	2.2%	2.1%	15.1	9.3
乳がん	1.20(1.12~1.28)	1.03(0.69~1.56)	—	6.9%	—	67.1
子宮内膜がん	2.10(1.75~2.53)	1.84(0.90~3.76)	—	1.1%	—	10.5
前立腺がん	0.84(0.76~0.93)	0.96(0.64~1.43)	6.6%	—	43.5	—
膀胱がん	1.24(1.08~1.42)	1.28(0.89~1.86)	2.0%	0.7%	12.5	2.7

* : これまでに発表された代表的なメタ解析データ
 ** : 未発表データ(国立がん研究センター 津金昌一郎)
 *** : 日本人における生涯がん罹患リスク
 **** : 日本人におけるがんの年齢調整罹患率

解析対象患者約33万5000例のうち、がん罹患患者数は約3万3000例で、糖尿病罹患患者における全がんハザード比(HR)は1.2[男性1.19(95%CI 1.12~1.27)、女性1.19(同1.07~1.31)]であった。がん種別の相対リスク(RR)は、肝がん1.97、膵がん1.85、大腸がん1.40の順に有意に高いことなどが報告された。

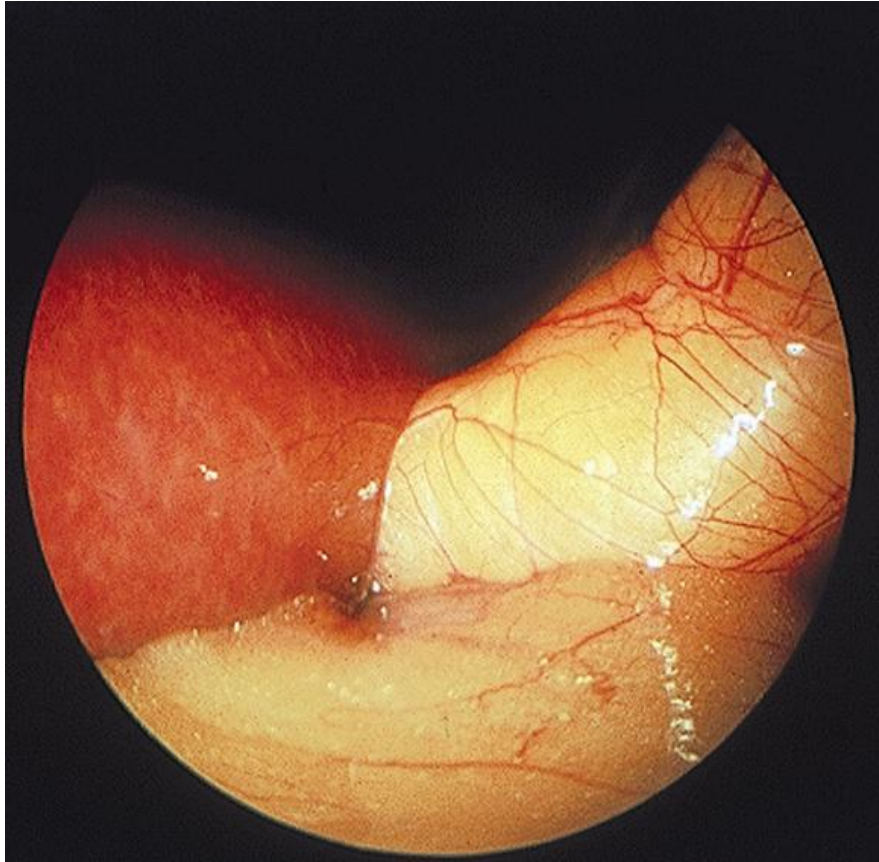
高血圧患者はNAFLDの頻度が高く、dipperに比してnondipperでは有意にNAFLDの罹患率が高い。

Fallo F, et al. Nonalcoholic fatty liver disease, adiponectin and insulin resistance in dipper and nondipper essential hypertensive patients.

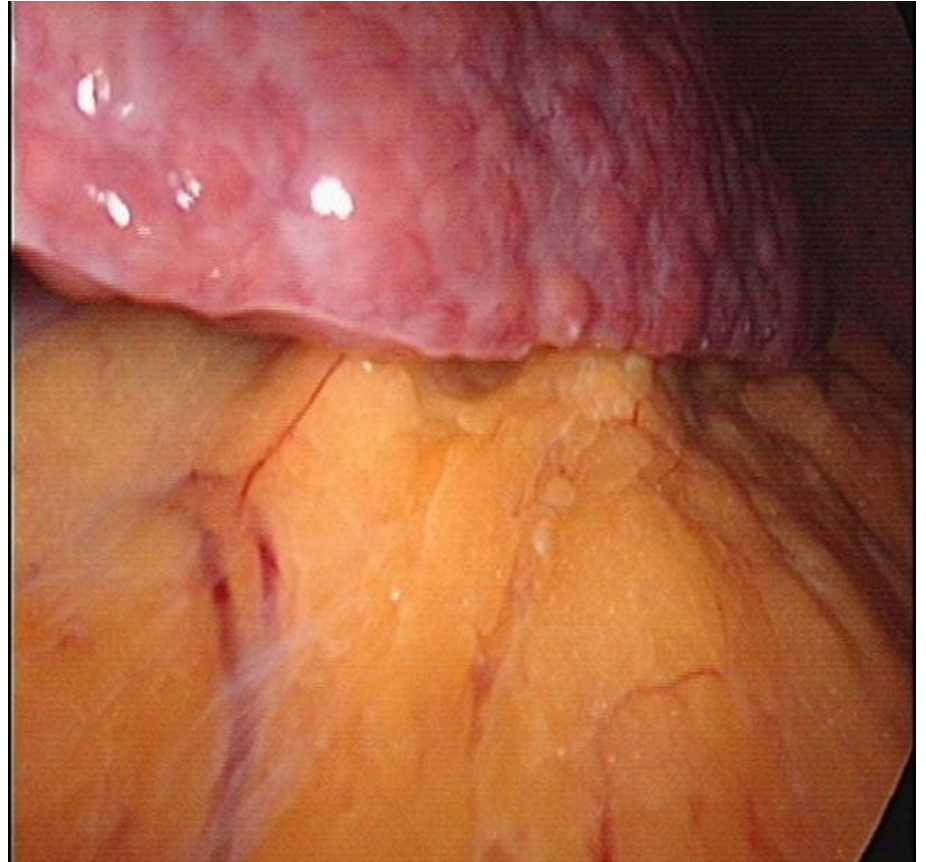
J Hypertens 26:2191-2197, 2008

	Number	Frequency of NAFLD	Insulin (pmol/l)	HOMA index	adiponectin (µg/ml)
Dipper	47	40.4%	55.4 ± 27.6	2.1 ± 1.2	17.7 ± 7.0
		p<0.005	<0.001	<0.001	<0.001
Nondipper	33	81.8%	86.5 ± 33.0	3.1 ± 1.2	10.1 ± 5.8

イタリア人の非高血圧患者のNAFLDの頻度は**20%**



NAFL
(脂肪肝)

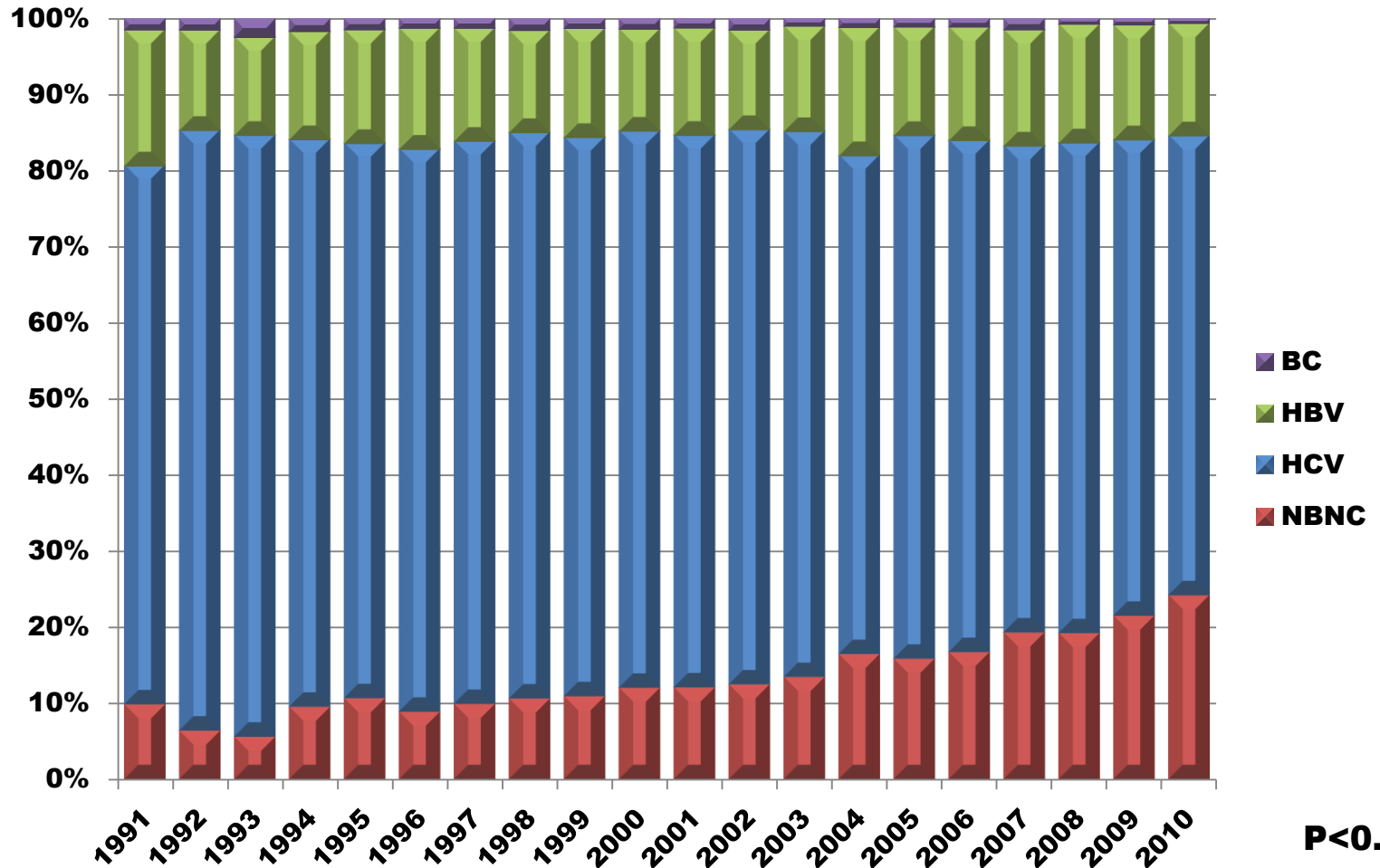


NASH肝硬変

肝癌背景肝疾患の推移

第29回犬山シンポジウム(2012年集計、責任者東大小池和彦教授)

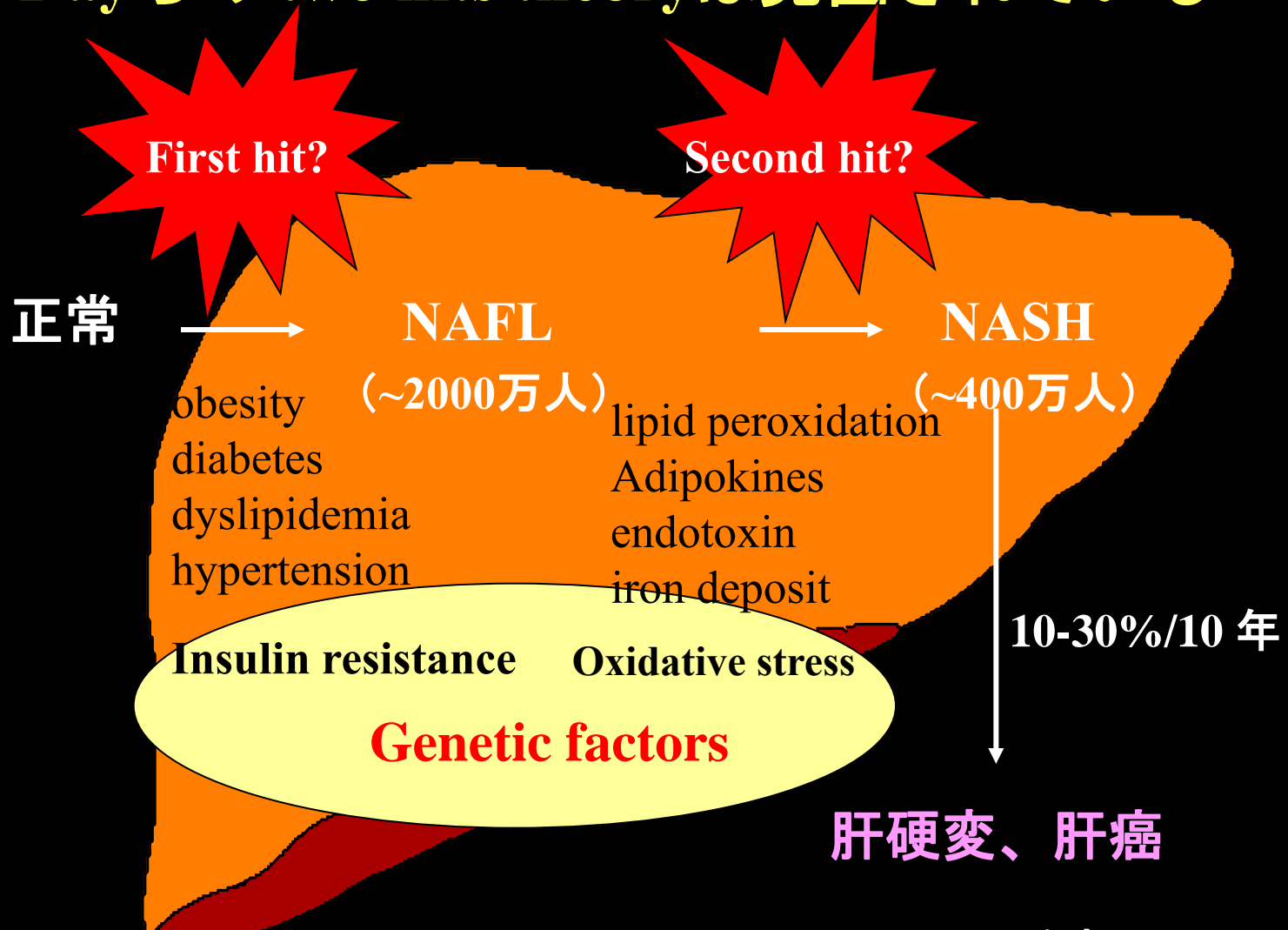
N=45,776



P<0.0001

NASH発症・進展の機序

-Dayらの two hits theoryは見直されている-



Day CP, James OFW. *Gastroenterology* 1998;114:842-5 (岡上、改変)

講演内容

1. 糖尿病患者の肝障害の実態
2. NAFL, NASHのscreening
3. 生活習慣病とNASH
4. NAFL, NASHにおける鉄蓄積
5. NASH発症・進展の感受性遺伝子
6. NASH肝癌の特徴
7. 治療の問題点

糖尿病5,642例の解析

厚生労働省NASH研究班と研究協力施設(9施設)

旭川医科大学、

東京大学、

福井県済生会病院、

済生会吹田病院、

鹿児島大学

山形大学

金沢大学

京都府立医科大学

済生会岡山総合病院

Shima T, Okanoue T, et al. J Gastroenterol 2013

糖尿病5,642例の患者背景

Characteristic	Total (5,642)	Male (3,238)	Female (2,404)	p
	M±SD	M±SD	M±SD	
Age (y)	63.3 ± 12.7	62.2 ± 12.5	64.8 ± 12.9	<0.001
BMI (kg/m²)	24.6 ± 4.7	24.5 ± 4.2	24.7 ± 5.2	0.629
AST (IU/L)	26.4 ± 17.2	27.1 ± 18.0	25.5 ± 15.9	<0.001
ALT (IU/L)	28.2 ± 24.5	30.6 ± 26.9	24.9 ± 20.5	<0.001
GGT (IU/L)	48.3 ± 72.5	59.6 ± 86.7	33.1 ± 42.9	<0.001
Alb (g/dL)	4.2 ± 0.4	4.2 ± 0.5	4.1 ± 0.4	<0.001
PLT (× 10⁴/μL)	21.3 ± 6.1	20.8 ± 6.0	21.9 ± 6.1	<0.001
FPG (mg/dL)	152.7 ± 61.7	156.0 ± 63.9	148.3 ± 58.2	<0.001
HbA1c (%)	7.2 ± 1.7	7.2 ± 1.7	7.2 ± 1.6	0.744
HOMA-IR (FPG<140)	2.55 ± 2.60	2.51 ± 2.59	2.61 ± 2.60	0.209
TCho (mg/dL)	195.1 ± 39.5	191.6 ± 40.0	199.6 ± 38.5	<0.001
TG(mg/dL)	136.3 ± 102.7	145.1 ± 111.9	124.5 ± 87.5	<0.001
HA (ng/mL)	74.5 ± 98.6	59.3 ± 73.0	94.6 ± 122.1	<0.001
TypeIV collagen 7S (ng/mL)	4.9 ± 2.0	4.8 ± 2.0	4.9 ± 1.9	0.544
Ferritin (ng/mL)	142.0 ± 157.0	171.9 ± 174.9	99.1 ± 114.1	<0.001
UA (mg/dL)	5.4 ± 1.5	5.7 ± 1.4	4.9 ± 1.4	<0.001

糖尿病5,642例の患者背景

Characteristic	Total (5,642) Positive(%)	Male (3,238) Positive(%)	Female (2,404) Positive(%)	P
HBsAg (+)	83 (1.7%)	50 (1.8%)	33 (1.6%)	0.604
Anti-HBc Ab(+)	958 (29.8%)	572 (31.4%)	386 (27.8%)	0.030
Anti-HCV Ab(+)	247 (5.1%)	144 (5.1%)	103 (5.0%)	0.834
Alcohol				<0.001
<20g/day	3,589 (80.8%)	1,769 (69.3%)	1,820 (96.3%)	
20-59g/day	661 (14.9%)	609 (23.8%)	52 (2.8%)	
≥ 60g/day	193 (4.3%)	176 (6.9%)	17 (0.9%)	
Hypertension	2,515 (51.0%)	1,419 (49.9%)	1,096 (52.3%)	0.100
Dyslipidemia	3,434 (63.3%)	1,882 (60.9%)	1,552 (66.6%)	<0.001
Diabetes mellitus (Intervention)				0.002
None	1,072 (20.5%)	629 (20.9%)	443 (20.0%)	
Oral drugs	2,495 (47.7%)	1,489 (49.4%)	1,006 (45.4%)	
Insulin	1,513 (28.9%)	810 (26.9%)	703 (31.8%)	
Oral drugs + insulin	147 (2.8%)	85 (2.8%)	62 (2.8%)	
HCC	67 (1.4%)	48 (1.8%)	19 (0.9%)	0.017

HBs原陽性者の血清HBV DNA量、 年齢、ALT値、血小板数

合計 (n:40)	% (n)	Age (y.o)	ALT (IU/L)	PLT ($\times 10^4/\mu\text{L}$)
陰性 (<2.6 log copy/ml)	72%(29)	63.6	25.3	20.5
陽性 (≥ 2.6 log copy/ml)	28%(11)	55.6	28.0	18.5
2.6 - 3.9	18% (7)	61.9	26.6	18.9
4.0 -	10% (4)	50.0	30.5	17.8
男性 (n:26)	% (n)	Age	ALT	PLT
陰性	69%(18)	63.4	26.5	21.3
陽性	31% (8)	52.8	32.1	19.5
女性 (n:14)	% (n)	Age	ALT	PLT
陰性	79% (11)	64.1	23.5	18.9
陽性	21% (3)	70.2	17.0	16.0

HCV体陽性者の血清HCV RNA値、 年齢、ALT値、血小板数

合計 (n:148)	% (n)	Age (y.o)	ALT (IU/L)	PLT ($\times 10^4/\mu\text{L}$)
陰性 (<2.7 log IU/ml)	38%(57)	67.2	28.2	17.7
陽性 (≥ 2.7 log IU/ml)	62%(91)	67.4	51.7	15.3
<hr/>				
2.7 - 4.9	3% (4)	65.1	28.0	16.4
5.0 -	59%(87)	67.5	52.7	15.2
<hr/>				
男性 (n:89)	% (n)	Age	ALT	PLT
陰性	36%(32)	65.3	28.1	16.3
陽性	64%(57)	66.9	53.0	16.0
<hr/>				
女性 (n=59)	% (n)	Age	ALT	PLT
陰性	42%(25)	69.5	28.3	19.4
陽性	58%(34)	68.3	49.4	14.0

糖尿病患者の肝機能異常($ALT \geq 31IU/L$)に 関連する因子の多変量解析

Male

	Regression coefficient	Standard error	Odds ratio	95%CI		P
Age	-0.394	0.048	0.674	0.613	- 0.741	<0.001
PLT	-0.216	0.049	0.806	0.732	- 0.886	<0.001
HCVAb	0.278	0.042	1.321	1.218	- 1.433	<0.001
BMI	0.411	0.048	1.509	1.374	- 1.657	<0.001

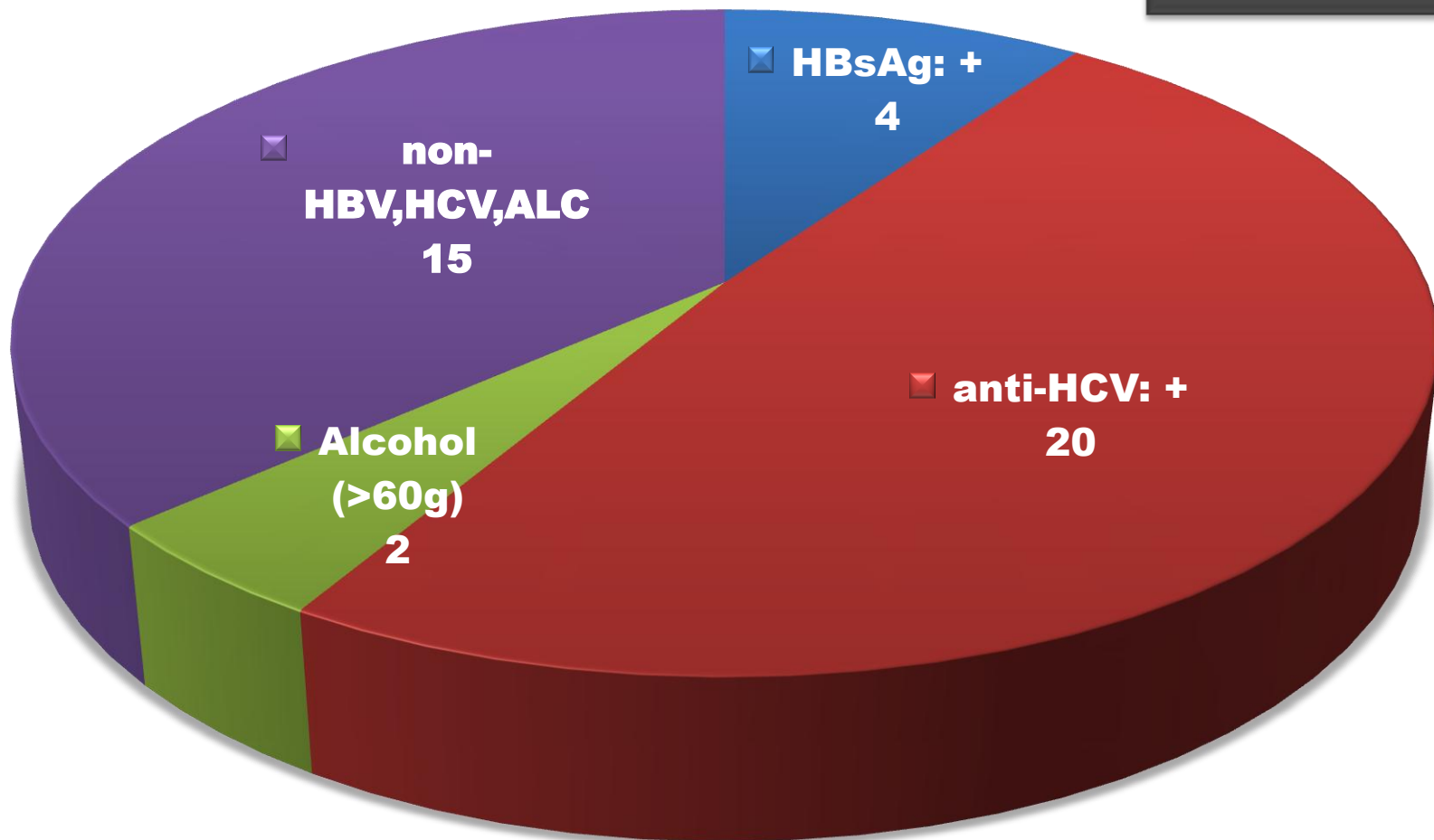
Female

	Regression coefficient	Standard error	Odds ratio	95%CI		P
Age	-0.265	0.059	0.767	0.683	- 0.861	<0.001
PLT	-0.336	0.063	0.714	0.632	- 0.808	<0.001
HCVAb	0.208	0.050	1.232	1.117	- 1.359	<0.001
BMI	0.397	0.057	1.487	1.330	- 1.663	<0.001

(ロジスティック回帰分析) (独立変数: BMI, Age, Drinking, HBs Ag, anti-HCV, PLT count, HL, HT)

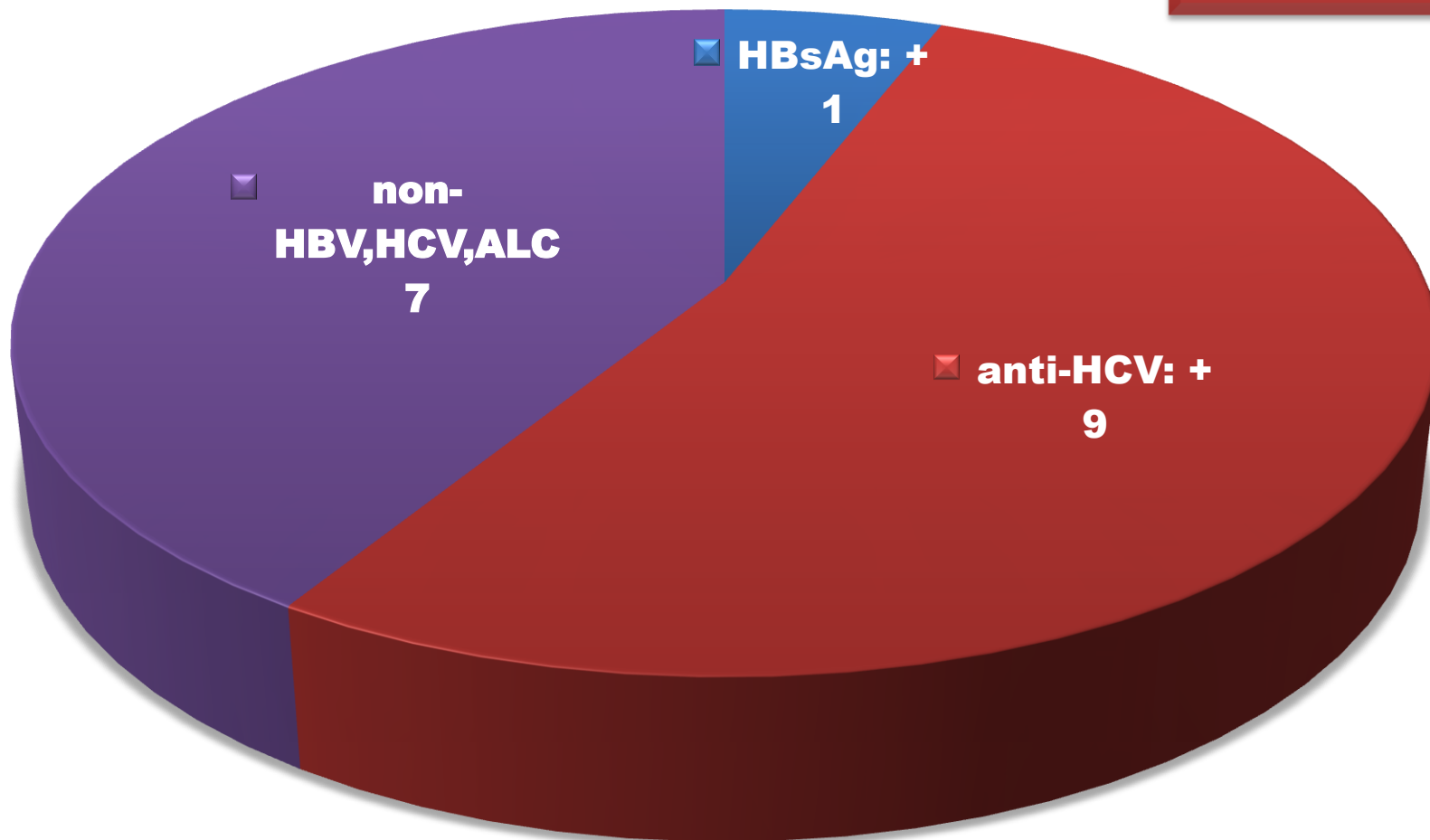
肝癌合併糖尿病患者の背景肝疾患の割合

男性 41例



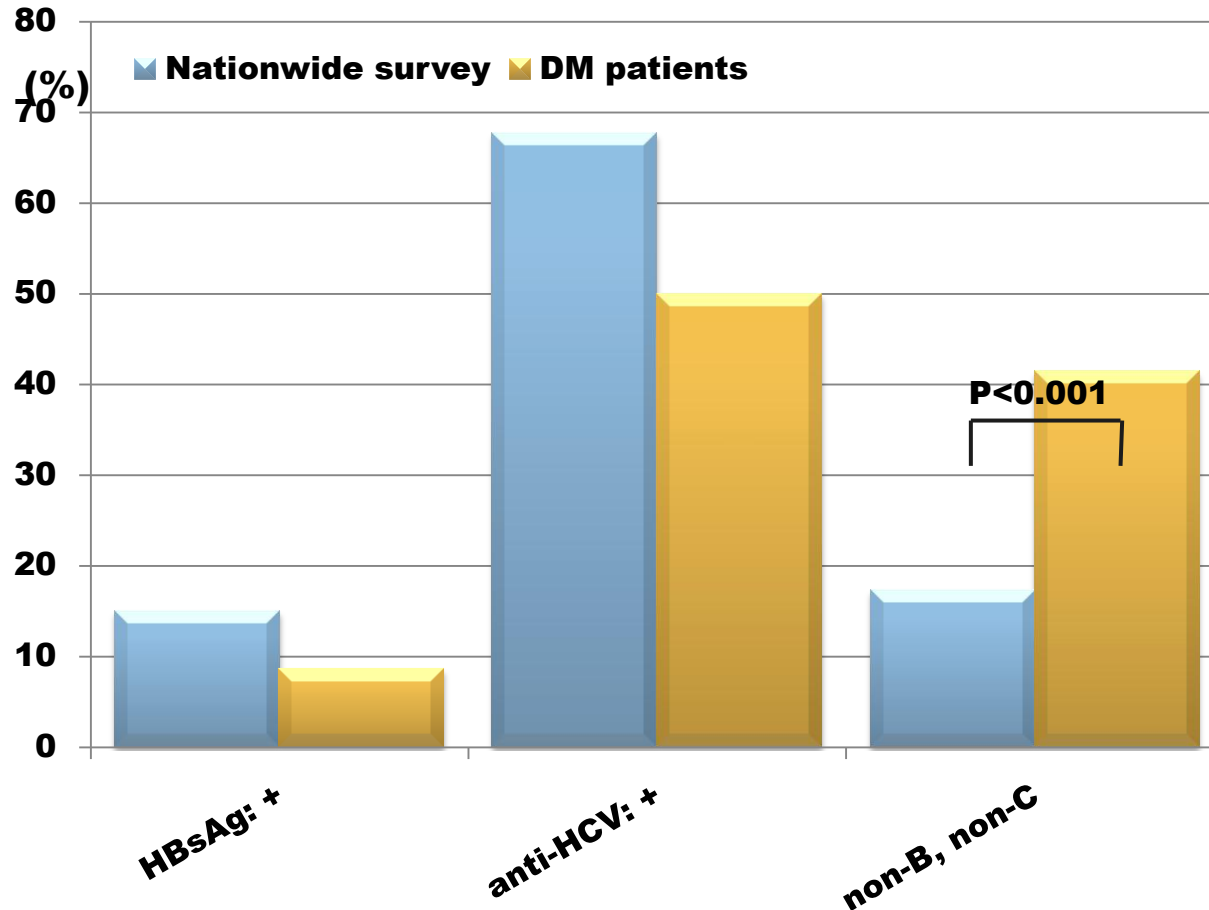
肝癌合併糖尿病患者の背景肝疾患の割合

女性 17例



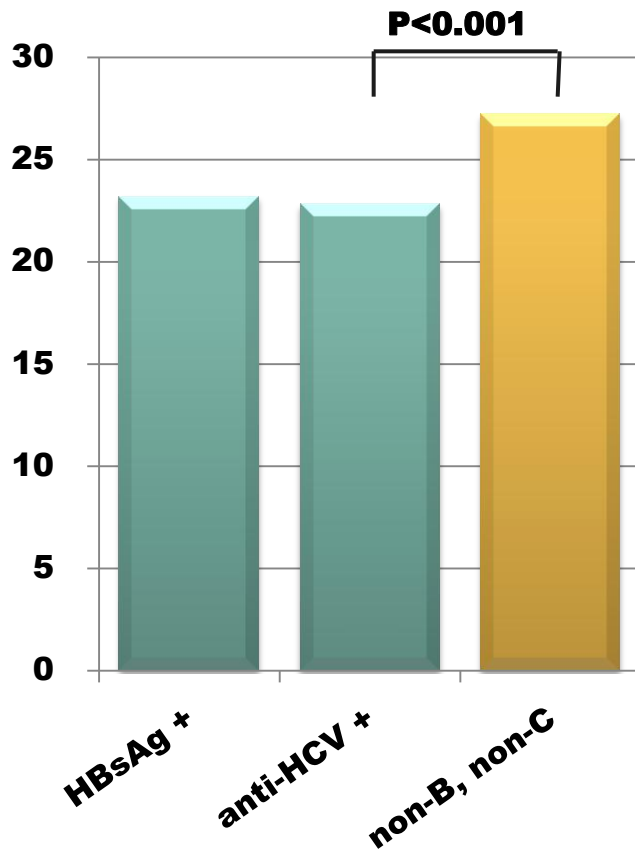
肝癌患者の背景肝疾患の割合

全国集計(日本肝癌研究会)と糖尿病患者の比較

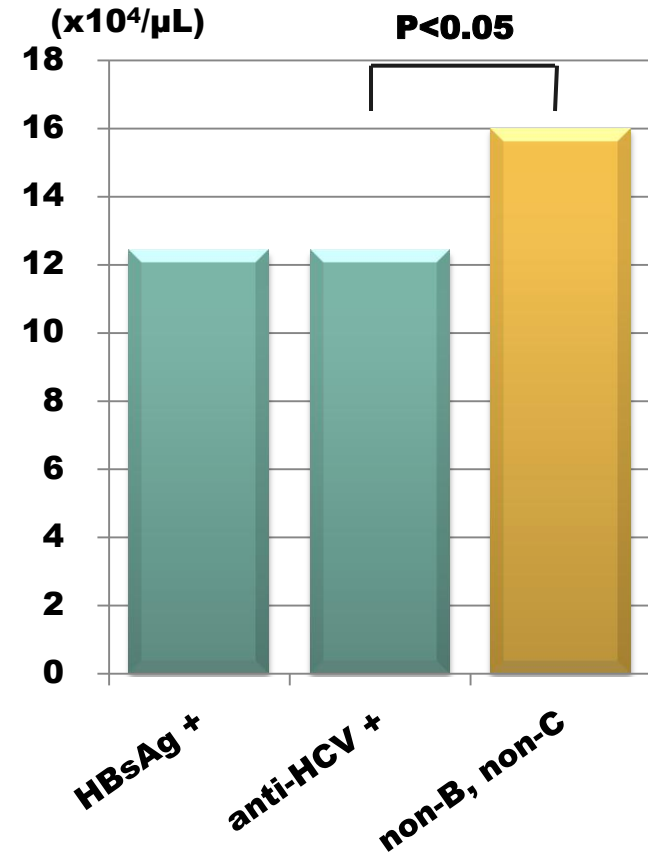


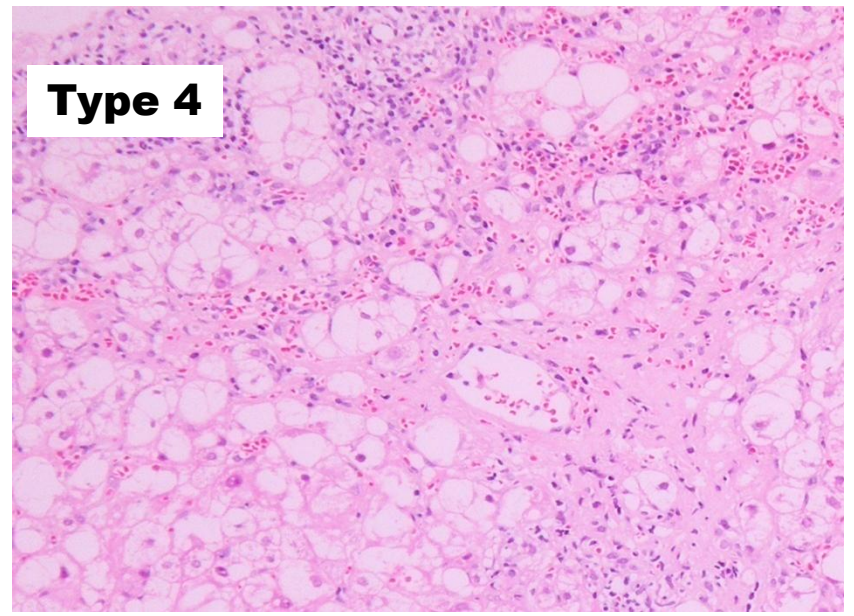
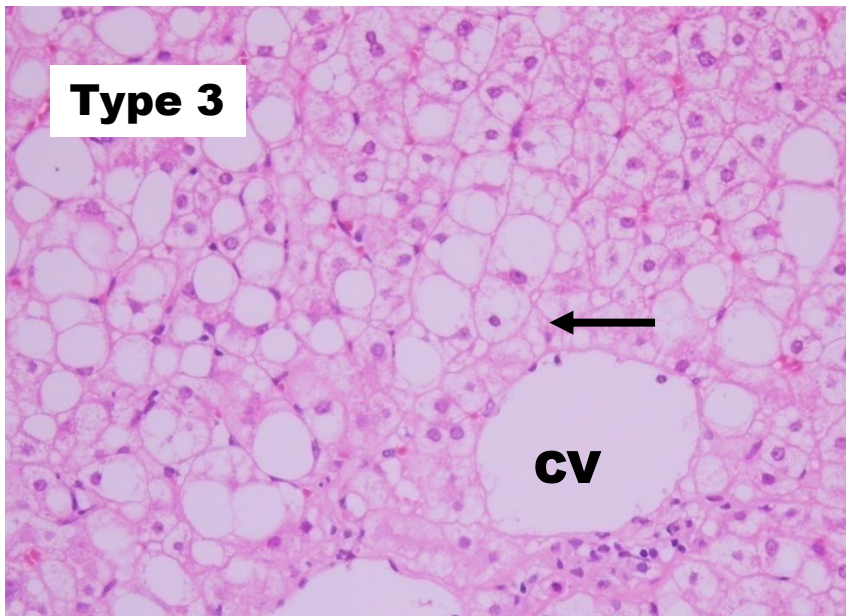
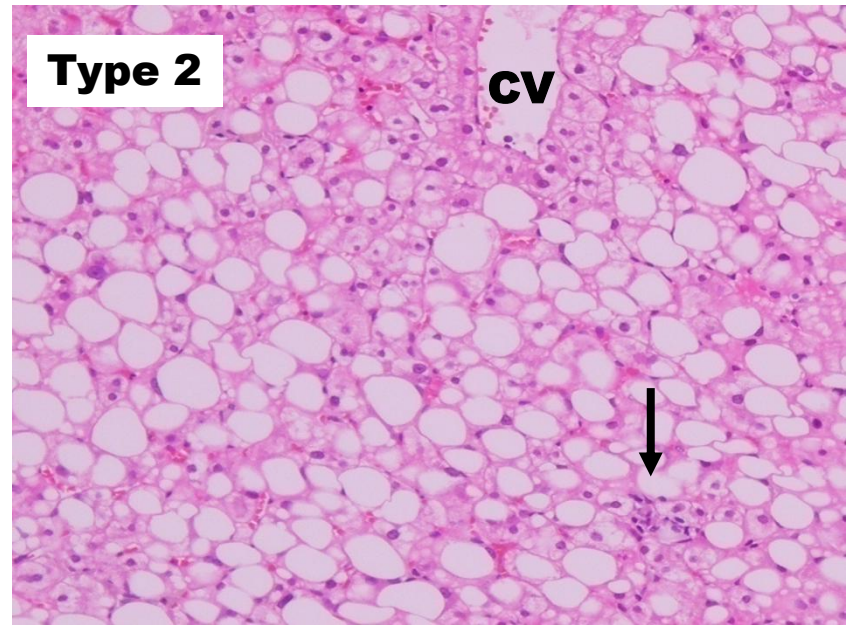
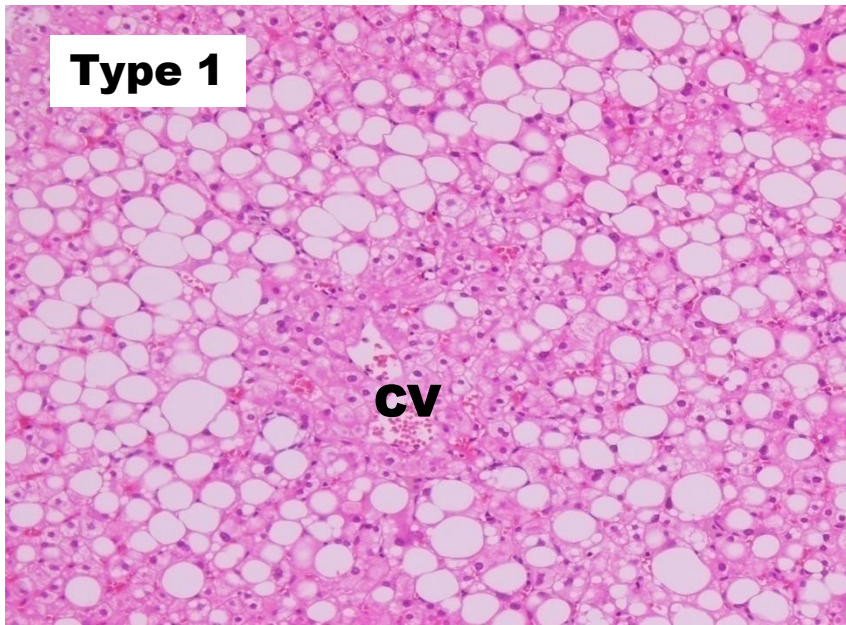
肝癌合併糖尿病患者の背景肝疾患 ウイルス別のBMIと血小板数

BMI

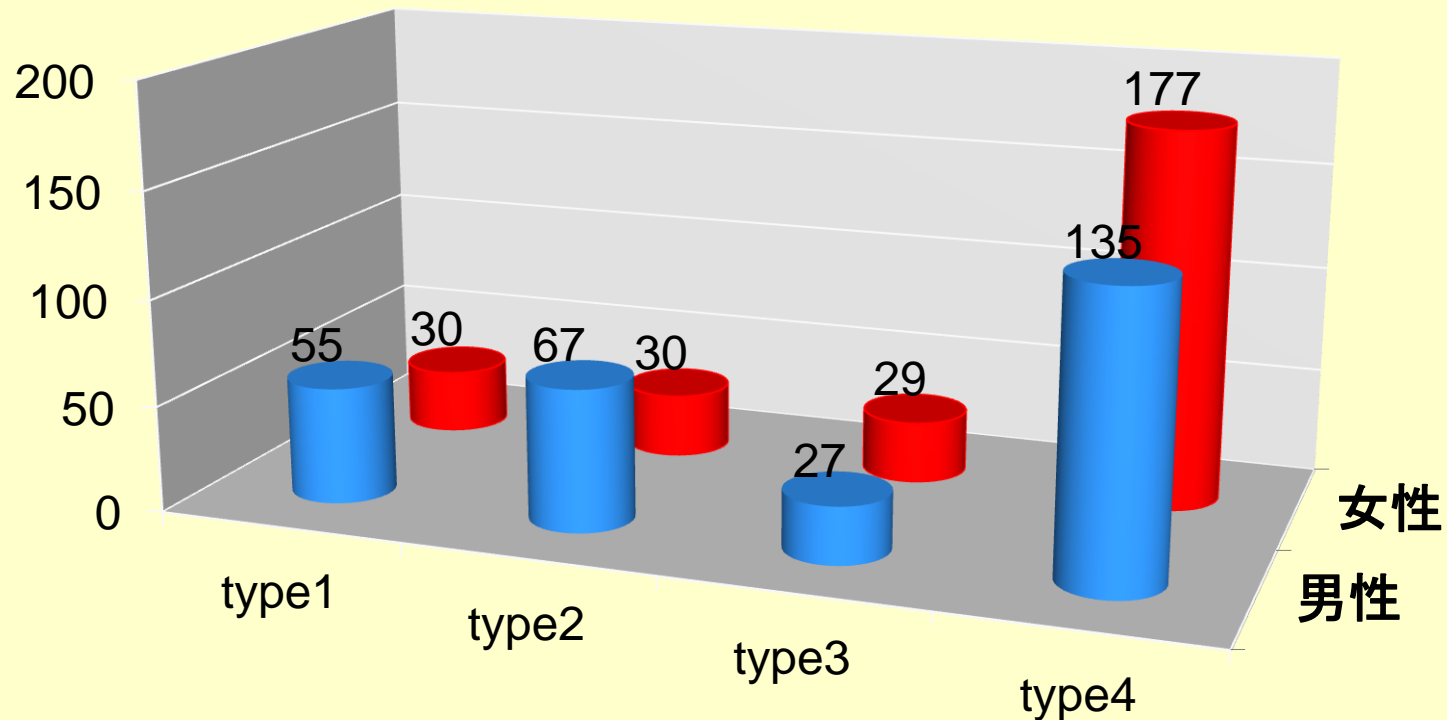


Platelet count



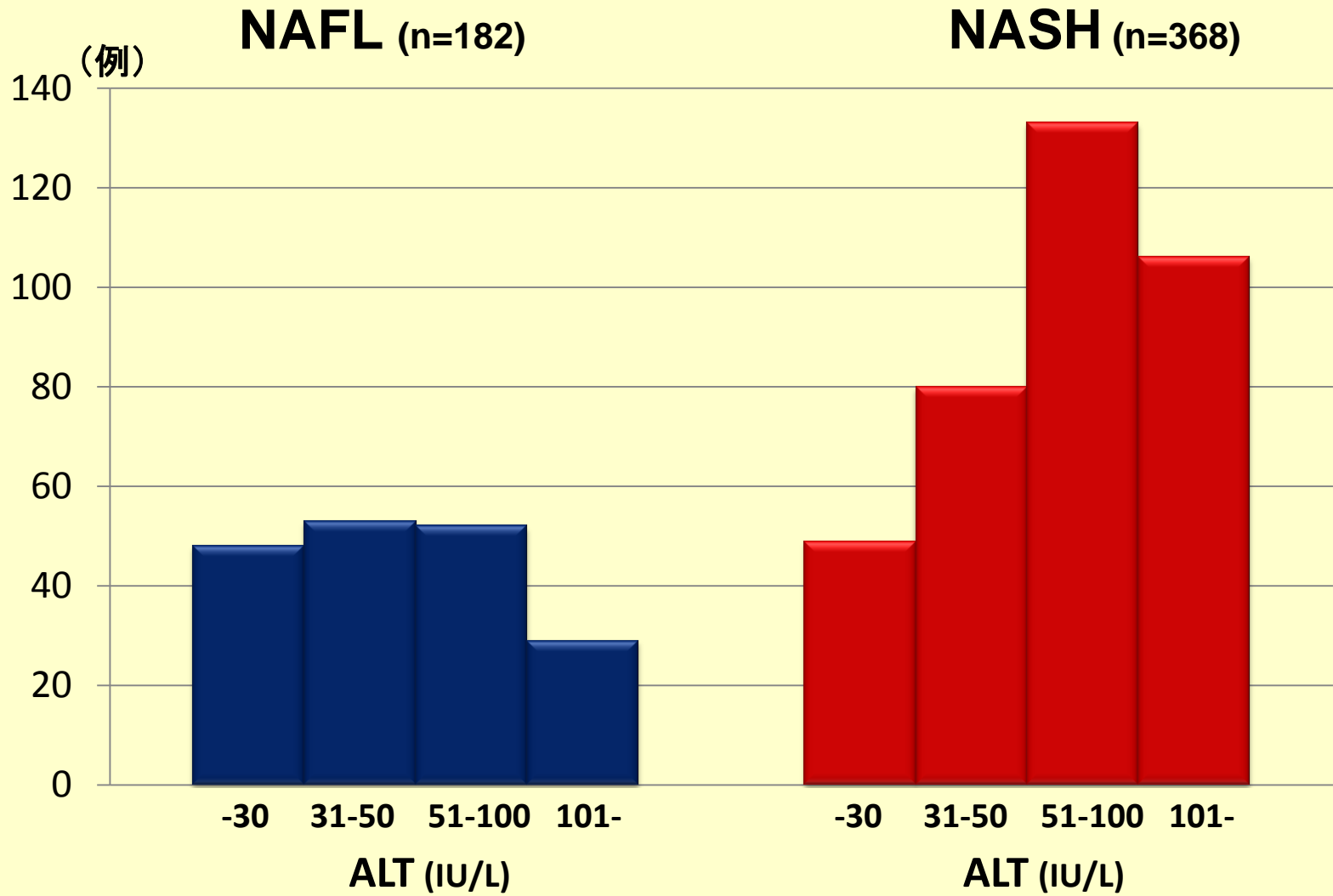


Matteoni分類に基づく肝組織所見の男女分布の比較 (2007/4-2012/12 大阪府済生会吹田病院:550例)

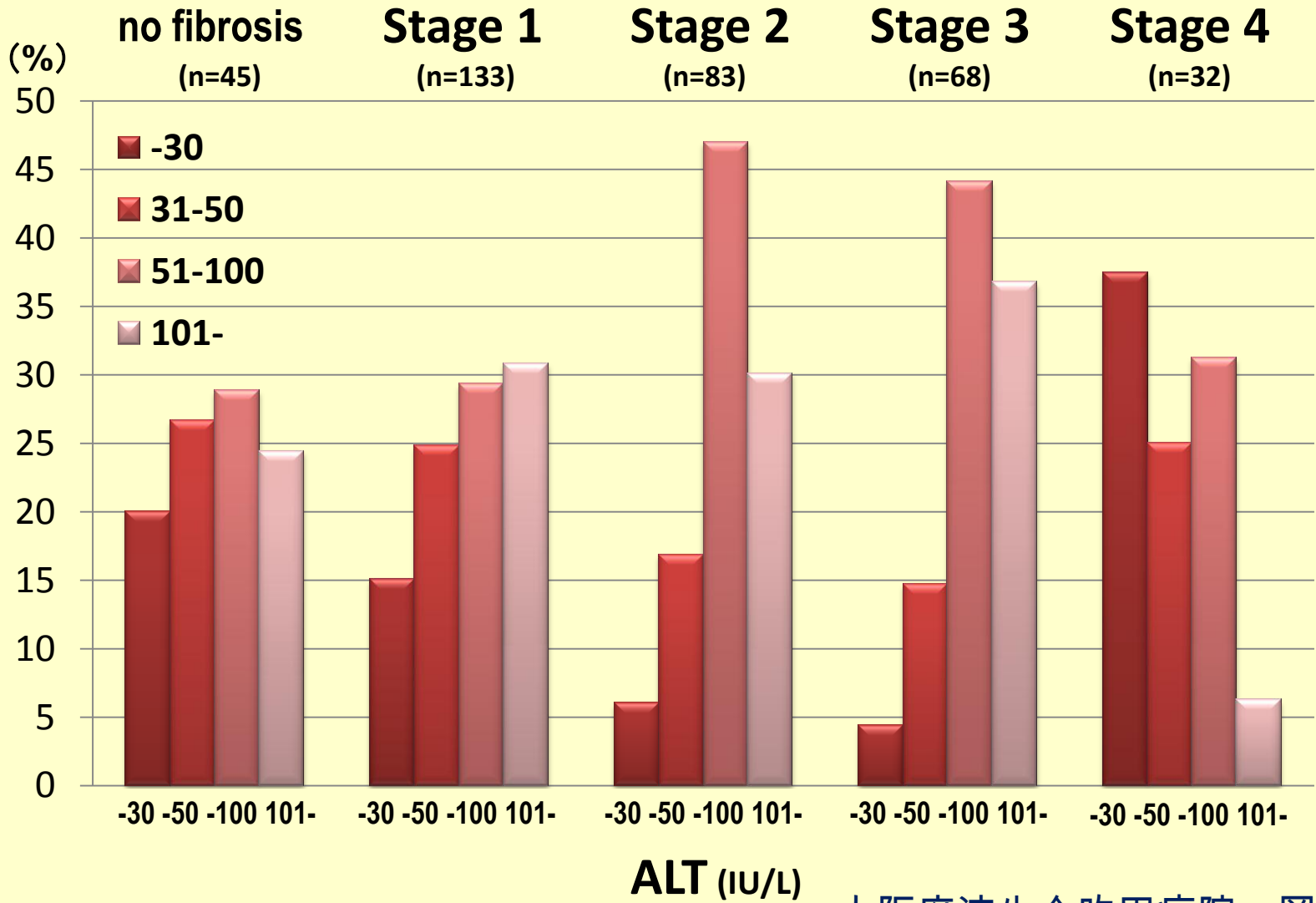


	NAFL	NASH
female	23% (60)	77% (206)
male	43% (122)	57% (162)

単純性脂肪肝とNASHにおけるALT値の分布



NASHにおけるstage別のALT値の分布



肝生検なしにNASHの診断は可能か？

Clinical scoring systems in the West

NASH vs. simple steatosis

Index	Author (Nation)	N	Population	AUROC	Predictors
HAIR score	Dixon JB (Australia)	105	Obese	0.9	HTN, ALT, IR (1/QUICKI)
	Palekar (USA)	80	NAFLD	0.763	Age, female, BMI, AST, AAR, HA
NashTest (NT)	Poynard (France)	257	NAFLD	0.79 ^a 0.79 ^b	Combination of 13 parameters ^c
	Gholam (USA)	97	Obese	0.82	AST, DM
NASH clinical scoring system	Campos (USA)	200	Obese	NR	HTN, DM, AST, ALT, sleep apnea, non-black race
Nice model	Anty (France)	464	Obese	0.88 ^a 0.83 ^b	ALT, CK-18, MetS

^a Estimation group, ^b Validation group

^c Age, sex, height, weight, TG, AST, ALT, TC, α 2-macroglobulin, apolipoproteinA1, haptoglobin, GGT, and T-Bil

Severe fibrosis vs. mild fibrosis

Index	Author (Nation)	N	Fibrosis stage	AUROC	Predictors
BAAT	Ratziu (France)	93	F0-1 vs. F2-4	0.84	BMI, age, ALT, TG
FibroTest (FT)	Ratziu (France)	267	F0-2 vs. F3-4	0.84	Combination of 13 parameters ^c
NAFLD fibrosis score	Angulo (USA, UK, Australia, Italy)	480 ^a 253 ^b	F0-2 vs. F3-4	0.88 ^a 0.82 ^b	Age, BMI, AST/ALT ratio, IFG/DM, PLT, Alb
ELF panel	Guha (UK)	192	F0 vs. F1-4 F0-1 vs. F2-4 F0-2 vs. F3-4	0.82 0.90 0.93	TIMP1, HA, P3NP
BARD score	Harrison (USA)	827	F0-2 vs. F3-4	0.81 ^a 0.78 ^b	BMI, AST/ALT, DM
FibroMeter	Calès (France)	235	F0-1 vs. F2-4	0.936 ^a 0.952 ^b	Glucose , AST, ferritin, PLT, ALT, BW, age
FIB-4 index	Shah (USA)	541	F0-2 vs. F3-4	0.802	Age, AST, ALT, PLT
PAF	Hossain (USA)	432	F0-1 vs. F2-4	0.742	Male , Caucasian, DM, ALT, AST

日本人NAFLD症例における NASHの拾い上げ

**Simple reliable markers
applicable to Japanese patients**

Sumida Y, Okanoue T, et al. J Gastroenterol, 2011

Independent predictors of NASH in the estimation group (n=177)

肝生検で診断したNAFLD (NASH vs non-NASH 98:79)
(市立奈良病院 角田圭雄)

Variables	Odds ratio	95% confidence interval	P-value
Ferritin ≥ 200 ng/ml (female) or 300 ng/ml (male)	4.01	1.07—15.02	0.0396
Insulin ≥ 10 μU/ml	5.59	1.71-18.31	0.0045
TypeIV collagen 7S ≥ 5.0 ng/ml	15.54	1.49—162.39	0.0219

NAFIC score

(NASH, Ferritin, Insulin, typeIV collagen 7S)

Variables	Cutoff values	Score values
1. Ferritin (ng/mL) Female/ Male	$\geq 200/300$	1 point
2. Insulin (μ U/mL)	≥ 10	1 point
3. Type IV collagen 7S (ng/mL)	≥ 5.0	2 points

This score ranges from 0 to 4

Sumida Y, Okanoue T, et al. J Gastroenterol, 2011

Clinical parameter	NASH (n=244 (55%))	Non-NASH (n=198 (45%))	P-value
Hemoglobin (g/dl)	14.7±2.5	14.7±1.5	0.9758
Platelet count (10 ⁴ /μl)	21.2±6.7	24.1±5.7	<0.0001
AST (IU/l)	88±387	38±22	0.0694
ALT (IU/l)	111±217	65±43	0.0001
AST/ALT ratio	0.75±0.32	0.65±0.21	0.0001
GGT (IU/l)	85±73	85±102	0.9852
Cholinesterase (IU/l)	364±89	387±85	0.0091
Total cholesterol (mg/dl)	207±43	210±39	0.5121
Triglyceride (mg/dl)	172±106	173±86	0.9038
Ferritin (ng/ml)	346±989	183±159	0.0221
Glucose (mg/dl)	113±63	105±39	0.1081
IRI (μU/ml)	16.8±12.9	11.9±8.3	<0.0001
Hyaluronic acid (ng/ml)	67±74	34±37	<0.0001
Type IV collagen 7S (ng/ml)	5.2±2.1	3.9±0.8	<0.0001
Histological fibrosis 0-1/2/3/4	143/56/30/15 (59%/23%/12%/6%)		

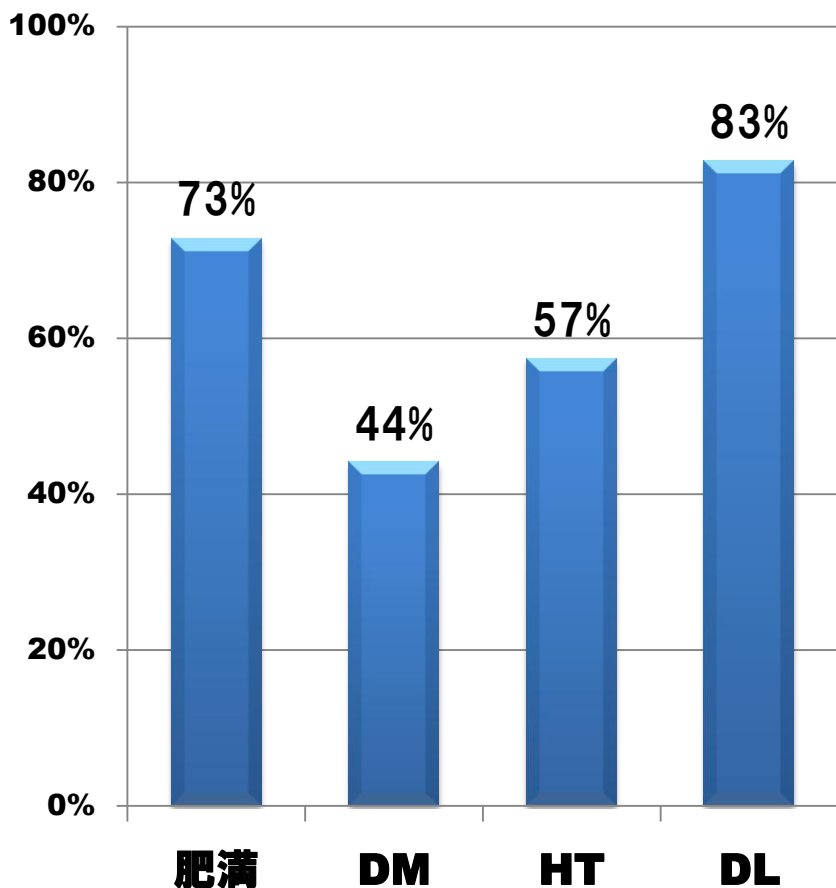
AUROC for NAFIC score and various scoring systems

	N	AUROC for NASH	AUROC for Significant fibrosis (stage 2 to 4)	AUROC for Advanced fibrosis (stage 3 to 4)
NAFIC	619	0.791	0.802	0.832
HAIR	609	0.631	0.593	0.566
Palekar's score	563	0.733	0.799	0.835
Gholam's score	619	0.777	0.786	0.729
BAAT	604	0.647	0.585	0.526
BARD	604	0.627	0.688	0.734
NAFLD fibrosis score	588	0.685	0.817	0.853
N (Nippon) score	585	0.668	0.720	0.704
FIB4-index	608	0.742	0.848	0.871

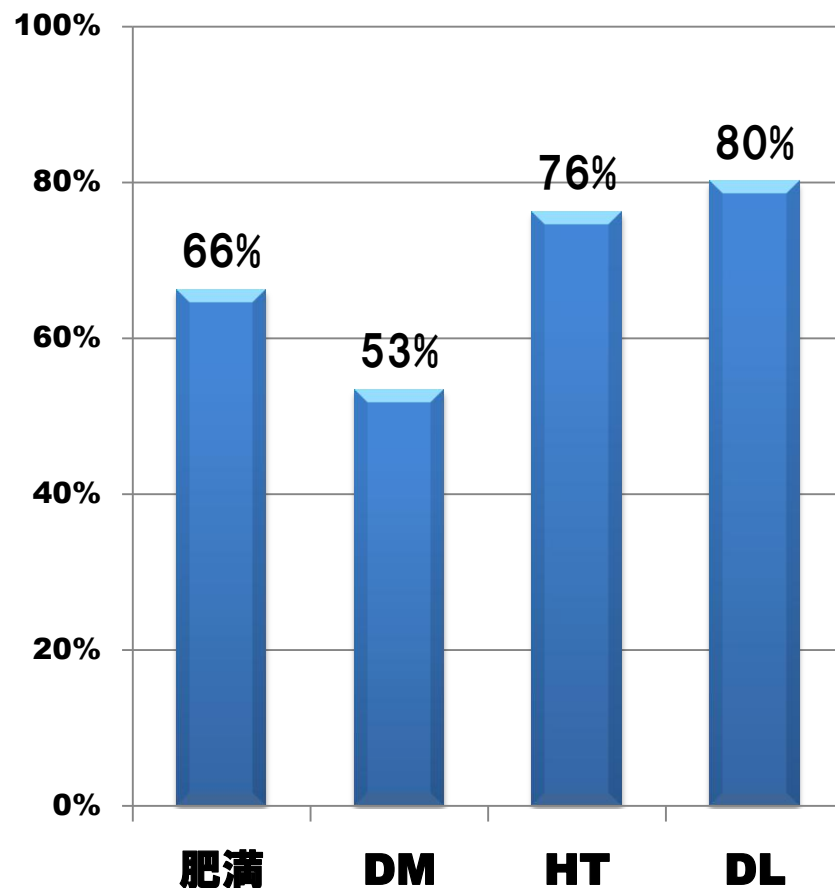
NAFLD症例における生活習慣病合併の 有無別NASHの比率(若年、高齢別)

大阪府済生会吹田病院550例NAFLD の生活習慣病合併頻度

男性 (n=284)

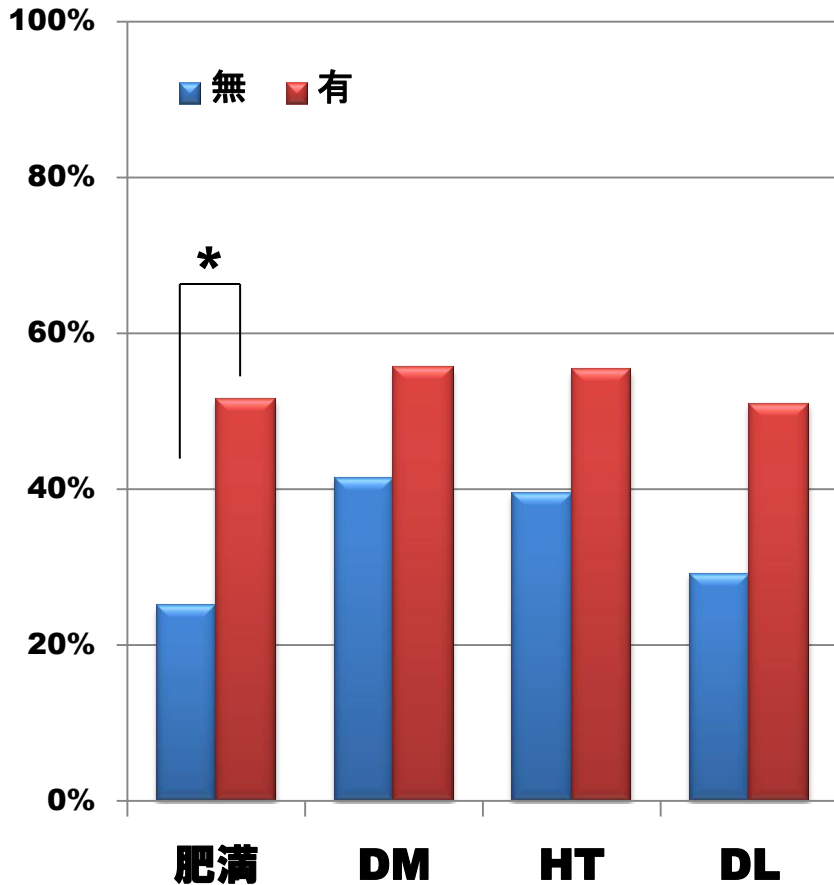


女性 (n=266)



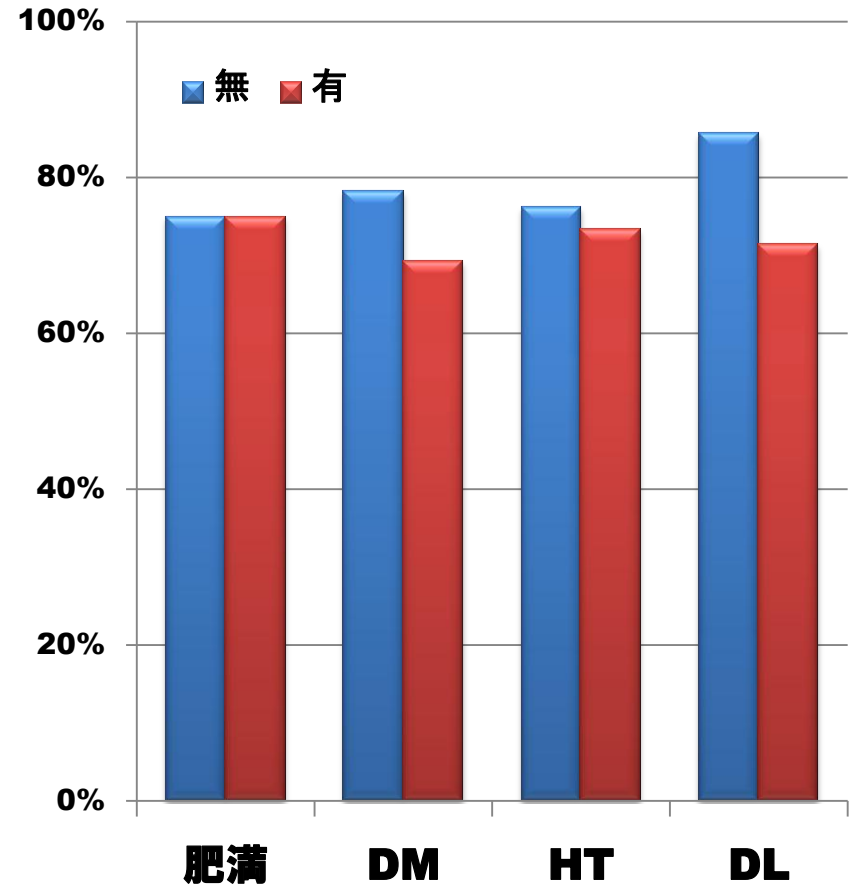
生活習慣病合併の有無別のNASHの比率 (49歳以下)

男性 (n=130)



* p<0.05

女性 (n=36)

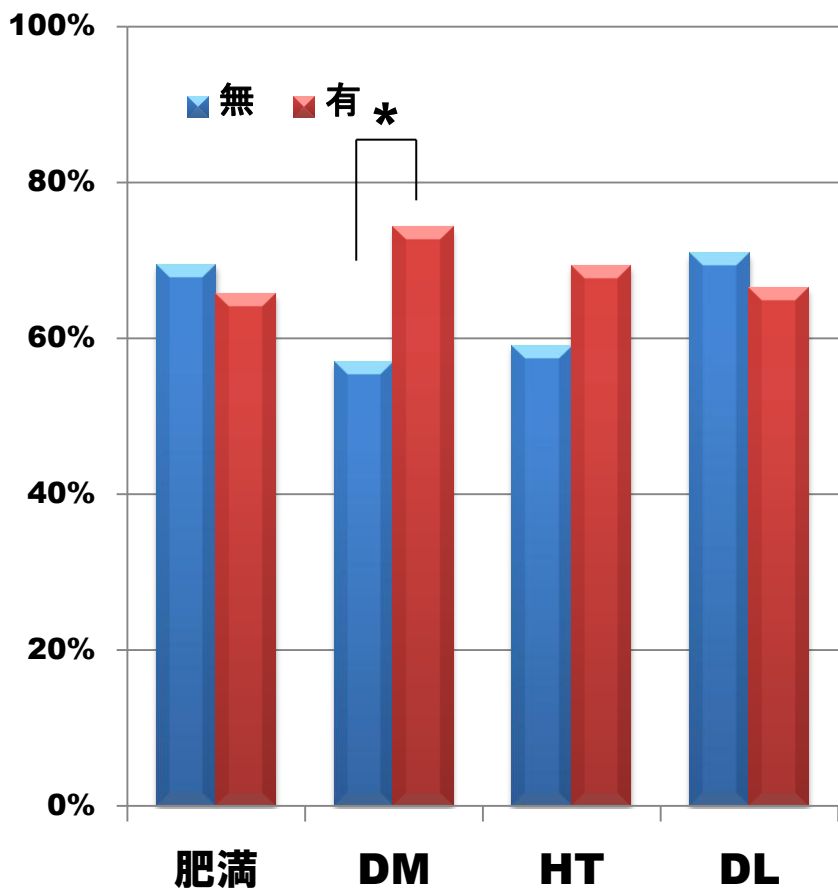


NAFLD症例における生活習慣病合併の有無別のNASH頻度

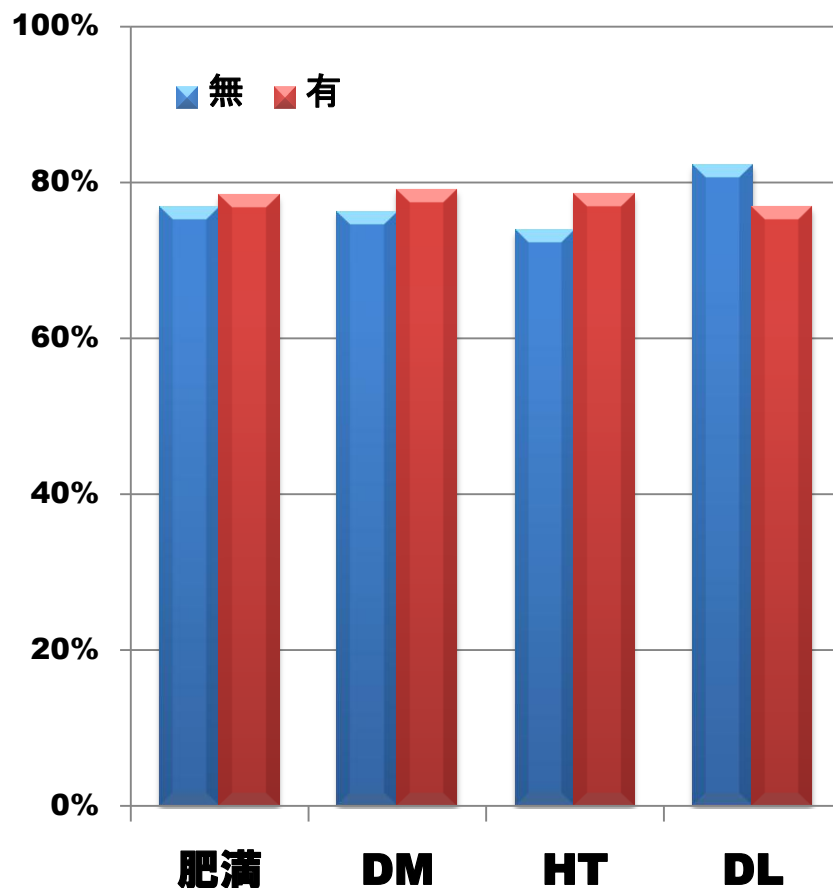
(50歳以上)

男性 (n=154)

女性 (n=230)

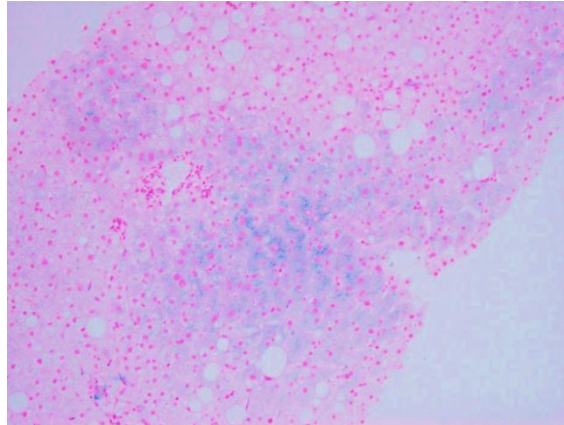


* p<0.05



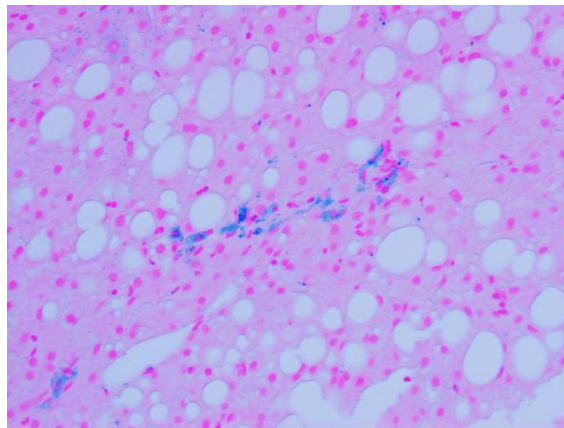
NAFLDにおける鉄蓄積パターンと病態との相関

肝細胞型(HC)



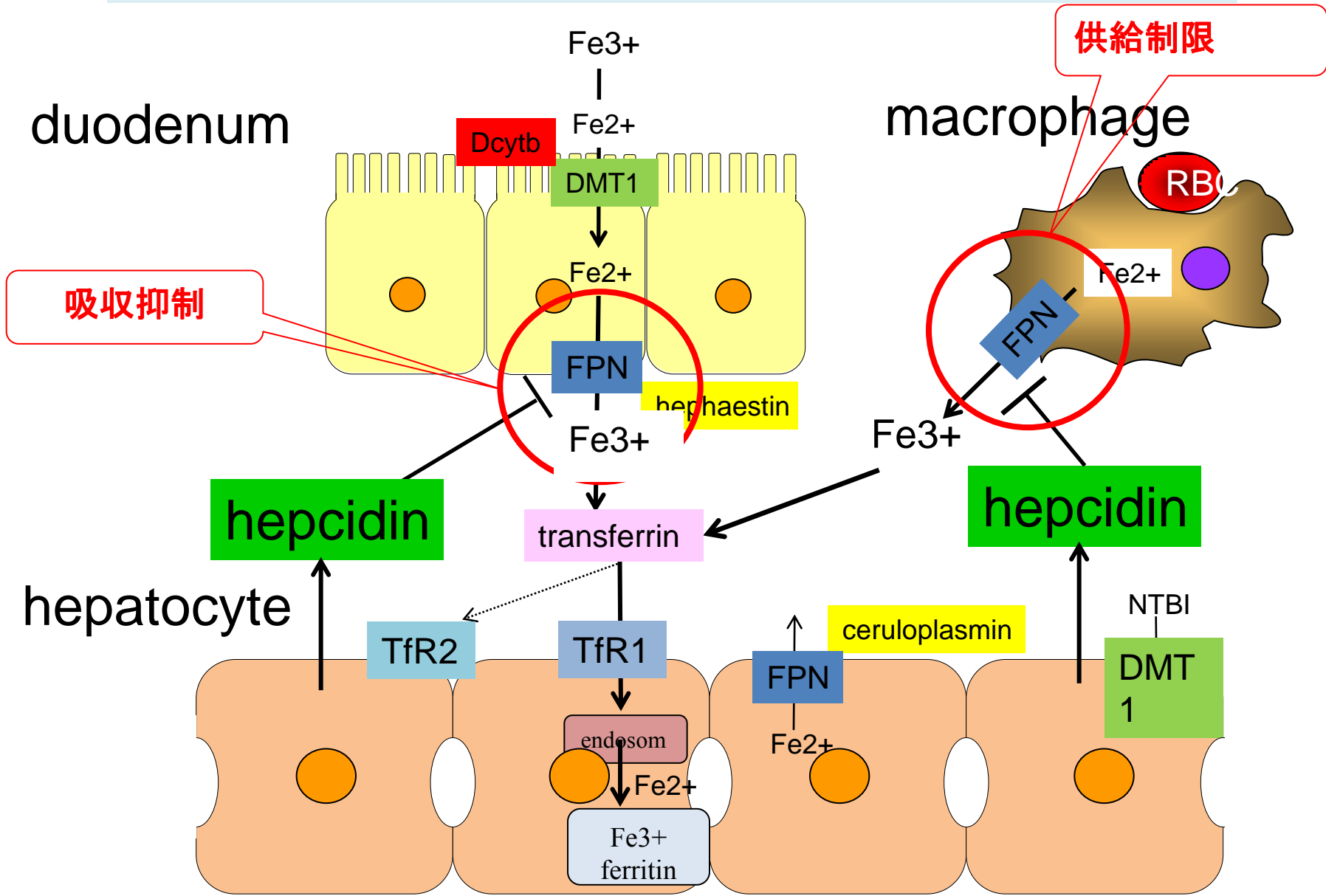
Valenti L et al. *HFE* Genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology* 2010;138:905–912 (N = 587)

網内皮型(REC)



Nelson JE et al. Relationship between the pattern of hepatic iron deposition and histological severity in nonalcoholic fatty liver disease. *Hepatology* 2011;53:448-457 (N = 849)

Hepcidinによる鉄代謝制御メカニズム



目的

1. NASH/NAFLDの鉄蓄積パターンと組織学的進展度の関係を調べる
2. 鉄蓄積パターンにおけるhepcidinの役割を明らかにする

方法

対象: 143名のNAFLD症例 (simple steatosis/NASH = 58/85, M/F = 89/54)

肝生検組織: Bruntの基準 (simple steatosisはstage=0、grade=0)

肝細胞(HC)鉄スコア=0-4

細網内皮系細胞(REC)鉄スコア=0-2

Hepcidin発現: 肝生検組織からRT-PCRにより定量

鉄蓄積スコアとHFE遺伝子変異

		REC score			Total
		0	1	2	
HC score	0	44	3	0	47
	1	21	17	6	44
	2	9	6	11	26
	3	2	8	15	25
	4	0	0	1	1
Total		76	34	33	143

HC単独=32名

REC単独=3名

HC+REC=64名

蓄積なし=44名

$p = 7.2 \times 10^{-15}$ (Chi-square test)

C282Y/C282Y	C282Y/wild	wild/wild	risk allele frequency
0	0	123	0%
H63D/H63D	H63D/wild	wild/wild	risk allele frequency
0	2	121	0.008%

非鉄蓄積群(NONE) 肝細胞蓄積群(HC) 混合型蓄積群(MIX)

	NONE (n = 44)	HC (n = 32)	MIX (n = 67)	ANOVA
Age	55.6 ± 15.8	47.9 ± 16.7	54.6 ± 14.4	0.071
Sex (M/F)	20/24	18/14	51/16	*0.003
BMI (kg/m ²)	26.4 ± 4.5	25.5 ± 3.4	27 ± 3.6	0.214
DM (yes/no)	12/32	11/21	20/47	*0.801
Fe (µg/ml)	105.9 ± 37	112.7 ± 35.2	116 ± 40	0.663
%Tf	29.4 ± 11.6	31 ± 9.9	35.5 ± 12.4	0.208
Ferritin (ng/ml)	142.5 ± 124.3	144.2 ± 67.1	^{#1,2} 393.7 ± 303.4	3.1 × 10⁻⁸
AST (IU/L)	^{#3} 65.2 ± 43.4	44.8 ± 20.8	^{#4} 64.4 ± 37.6	0.028
ALT (IU/L)	93 ± 85.7	69 ± 36.9	103.1 ± 68.2	0.073
Total cholesterol (mg/dl)	204.6 ± 46.2	229.4 ± 51.1	208.9 ± 32	0.03
Triglyceride (mg/dl)	130.9 ± 67.4	180.1 ± 109.9	^{#5} 170.1 ± 90.6	0.039
HDL-cholesterol (mg/dl)	51.4 ± 14.2	52.5 ± 10.4	46.7 ± 10.4	0.087
IRI (µU/ml)	11.6 ± 6.9	12.9 ± 6.7	16.1 ± 9.3	0.054
Glucose (mg/dl)	109.9 ± 32.6	111.8 ± 38.1	111.6 ± 27.1	0.957
HOMA-IR	3.3 ± 2.3	3.2 ± 1.7	4.2 ± 2.5	0.184
HbA1c	5.9 ± 1	6.3 ± 1.4	6.1 ± 1.2	0.605

#1 p = 2.8 × 10⁻⁷ vs NONE, #2 p = 8.8 × 10⁻⁸ vs HC, #3 p = 0.027 vs HC, #4 p = 0.004 vs HC, #5 p = 0.041 vs NONE: Dunnett T3 test, *: Chai-square test

鉄蓄積症例における線維化促進因子(n = 99)

	Early stage n = 80			Advanced stage n = 19			univariate	multivariate
Age	53.5	[18 - 77]		61	[35 - 76]		0.012	0.192
Sex (M/F)	59/21			10/9			0.067	-
BMI (kg/m ²)	25.9	[19.8 - 34.5]		27.3	[22.5 - 39.6]		0.284	-
DM (yes/no)	19/61			12/7			0.002	0.031
Fe (µg/ml)	102	[60 - 202]		125	[56 - 226]		0.392	-
%Tf	28.9	[18.8 - 62.3]		39.5	[19.4 - 59.9]		0.268	-
Ferritin (ng/ml)	216	[34 - 1954]		412	[87 - 1112]		0.028	0.864
AST (IU/L)	42	[19 - 166]		73	[35 - 201]		0.0003	0.04
ALT (IU/L)	75	[16 - 386]		104	[27 - 193]		0.199	-
Total cholesterol (mg/dl)	218.5	[136 - 344]		194	[140 - 239]		0.015	0.032
Triglyceride (mg/dl)	158	[41 - 502]		120.5	[51 - 287]		0.1	-
HDL-cholesterol (mg/dl)	49	[25 - 79]		44.5	[33 - 67]		0.156	-
IRI (µU/ml)	12.6	[1.0 - 40.1]		16.8	[8.7 - 25.4]		0.048	0.981
Glucose (mg/dl)	102	[79 - 280]		110	[74 - 195]		0.085	-
HOMA-IR	3.1	[0.2 - 9.1]		4.3	[1.6 - 9.2]		0.059	-
HbA1c	5.8	[4.6 - 10.3]		5.9	[4.5 - 8.9]		0.838	-
HC iron (yes/no)	78/2			18/1			0.476	-
REC iron (yes/no)	50/30			17/2			0.019	0.048

univariate: Mann-Whitney U test, multivariate: Logistic regression analysis

Hepcidin発現レベルと相関する因子

	coefficient	univariate	multivariate
Age	-0.012	0.905	-
BMI (kg/m ²)	0.167	0.121	-
Fe (μg/ml)	0.001	0.993	-
%Tf	0.109	0.526	-
Ferritin (ng/ml)	0.398	0.0002	0.248
AST (IU/L)	-0.190	0.068	-
ALT (IU/L)	-0.168	0.107	-
Total cholesterol (mg/dl)	0.259	0.013	0.067
Triglyceride (mg/dl)	0.118	0.279	-
HDL-cholesterol (mg/dl)	0.136	0.282	-
IRI (μU/ml)	-0.008	0.954	-
Glucose (mg/dl)	-0.095	0.396	-
HOMA-IR	-0.002	0.989	-
HbA1c	0.078	0.597	-
Stage	-0.151	0.146	-
Grade	-0.108	0.301	-
Steatosis	-0.212	0.048	0.153
HC score	0.485	7.3 × 10⁻⁷	0.0003
REC score	0.314	0.002	0.354

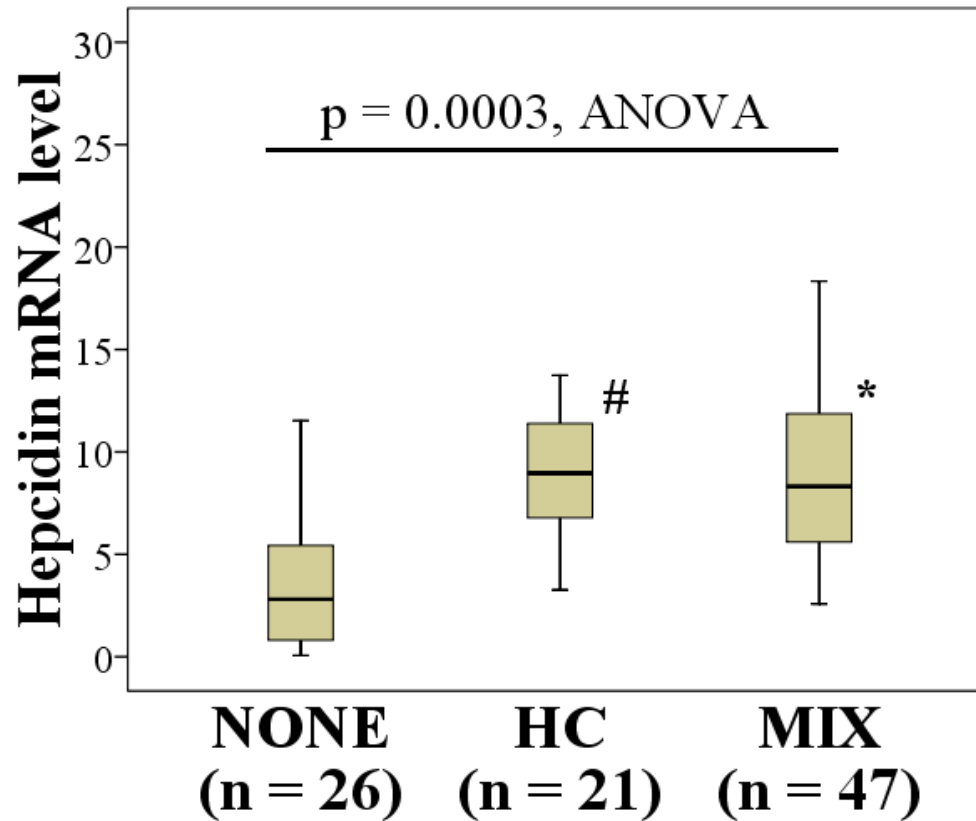
univariate: Spearman test, multivariate: Regression analysis

REC鉄蓄積に寄与する因子

	REC iron (-)			REC iron (+)			univariate	multivariate
	n = 76			n = 67				
Age	54	[18 - 78]		58	[22 - 77]		0.491	-
Sex (M/F)	38	38		51	16		0.001	0.068
BMI (kg/m ²)	25.6	[18.6 - 39.7]		26.7	[20.0 - 39.6]		0.060	-
DM (yes/no)	23	53		20	47		0.552	-
Fe (μg/ml)	107	[40 - 167]		113	[56 - 226]		0.573	-
%Tf	30.3	[11.6 - 60.3]		31.5	[19.4 - 62.3]		0.175	-
Ferritin (ng/ml)	124	[14 - 452]		321	[35 - 1954]		2.6 × 10⁻¹²	0.056
AST (IU/L)	43	[16 - 170]		55.5	[19 - 201]		0.092	-
ALT (IU/L)	65	[12 - 398]		90	[16 - 386]		0.012	0.430
Total cholesterol (mg/dl)	208	[54 - 344]		208.5	[140 - 302]		0.499	-
Triglyceride (mg/dl)	124	[41 - 459]		155	[42 - 502]		0.069	-
HDL-cholesterol (mg/dl)	52	[14 - 85]		45	[25 - 75]		0.014	0.820
IRI (μU/ml)	11.2	[1.3 - 31.2]		14.6	[1 - 40.1]		0.045	0.094
Glucose (mg/dl)	101	[79 - 280]		106	[74 - 220]		0.502	-
HOMA-IR	3.0	[0.3 - 10.9]		3.9	[0.2 - 9.2]		0.085	-
HbA1c	5.8	[4.3 - 10.3]		5.9	[4.5 - 9.2]		1.000	-
Stage: 0/1/2/3/4	37	15/10/12/2		21	18/11/14/3		0.083	-
Grade: 0/1/2/3	38	22/13/3		21	29/12/5		0.077	-
Steatosis: 1/2/3	23	26/22		26	20/20		0.582	-
HC score: 0/1/2/3/4	44	21/9/2/0		3	23/17/23/1		4.2 × 10⁻¹³	0.005

univariate: Mann-Whitney U test, multivariate: Logistic regression analysis

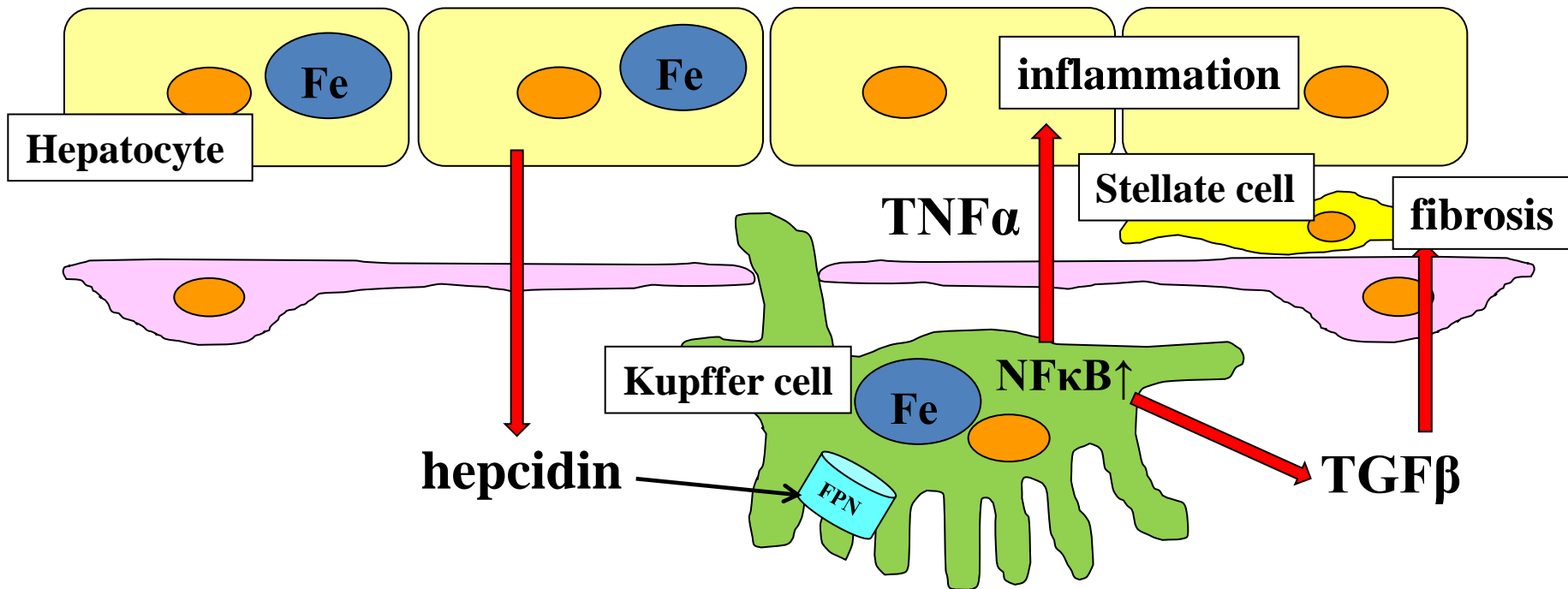
肝組織中のhepcidin発現レベル(n = 94)



$p = 0.02$ vs NONE, * 5.4×10^{-7} vs NONE: Dunnett T3 test

結語

1. NASH/NAFLDでは網内皮系細胞の鉄蓄積が肝病態進展と密接に関連すると考えられた。
2. 肝細胞の鉄蓄積はhepcidinの発現を増加させ、網内皮系細胞の鉄蓄積を促進させている可能性が示唆された。



genome-wide association study(GWAS)によるNASH発症・進展感受性遺伝子の同定

Kawaguchi T, Okanoue T , et al. Genetic polymorphisms of the human PNPLA3 gene are strongly associated with severity of nonalcoholic fatty liver disease in Japanese. PLoS One 7(6): e38322, 2012

PNPLA3 の機能

- The normal physiologic role of PNPLA3 and how the I148M isoform causes liver disease remain obscure.
- PNPLA3 is expressed primarily in liver and adipose tissue, in which it partitions to membranes and lipid droplets.
(He *et al. J Biol Chem* 2011)
- During fasting, low levels of SREBP-1c result in low transcription of *PNPLA3* mRNA and efficient degradation of PNPLA3 protein, ensuring very low levels of PNPLA3 in the liver. With carbohydrate refeeding, insulin upregulates SREBP-1c, which in turn stimulates *PNPLA3* transcription and concomitantly upregulates fatty acid biosynthesis.
(Huang *et al. Proc Natl Acad Sci USA* 2010)
- It is unclear whether PNPLA3 functions as a lipase or a lipogenic enzyme.
- In vitro data suggest that PNPLA3 normally functions as an acylglycerol hydrolase and that the I148M substitution causes a loss of hydrolytic function, resulting in accumulation of excess triacylglycerol (TAG).
(Huang *et al. J Biol Chem* 2011)
- Data from genetically manipulated mice are difficult to reconcile with a loss-of-function model. Inactivation of *Pnpla3* in mice does not result in any change in hepatic TAG content.
(Chen *et al. Hepatology* 2010)
- Overexpression of *PNPLA3*^{I148M}, but not *PNPLA3*^{WT}, in mouse liver causes hepatic steatosis. In *PNPLA3*^{I148M} transgenic mice, formation of fatty acids and TAG was increased and hydrolysis of TAG was impaired.
(Li *et al. J Clin Invest* 2012)

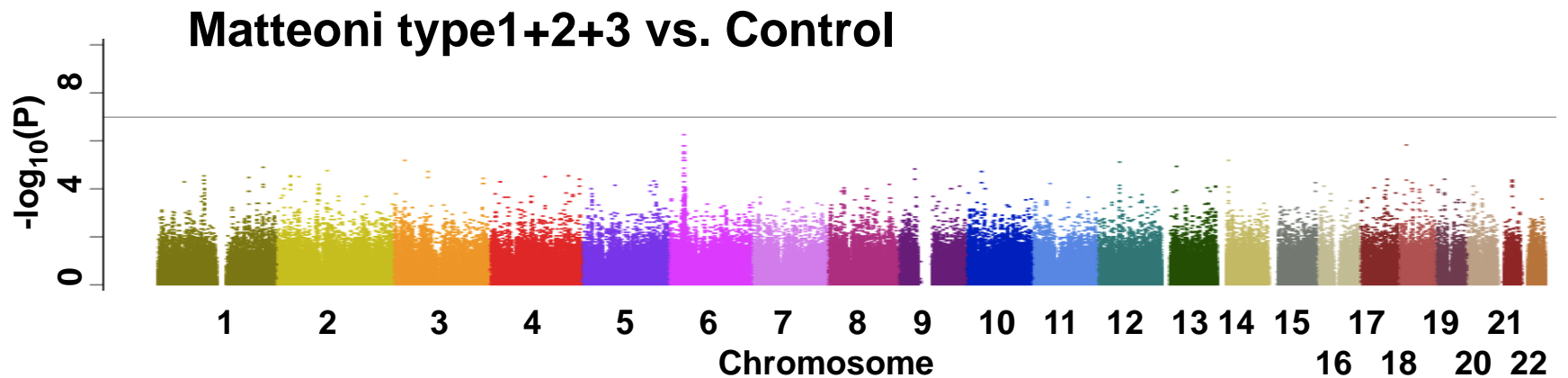
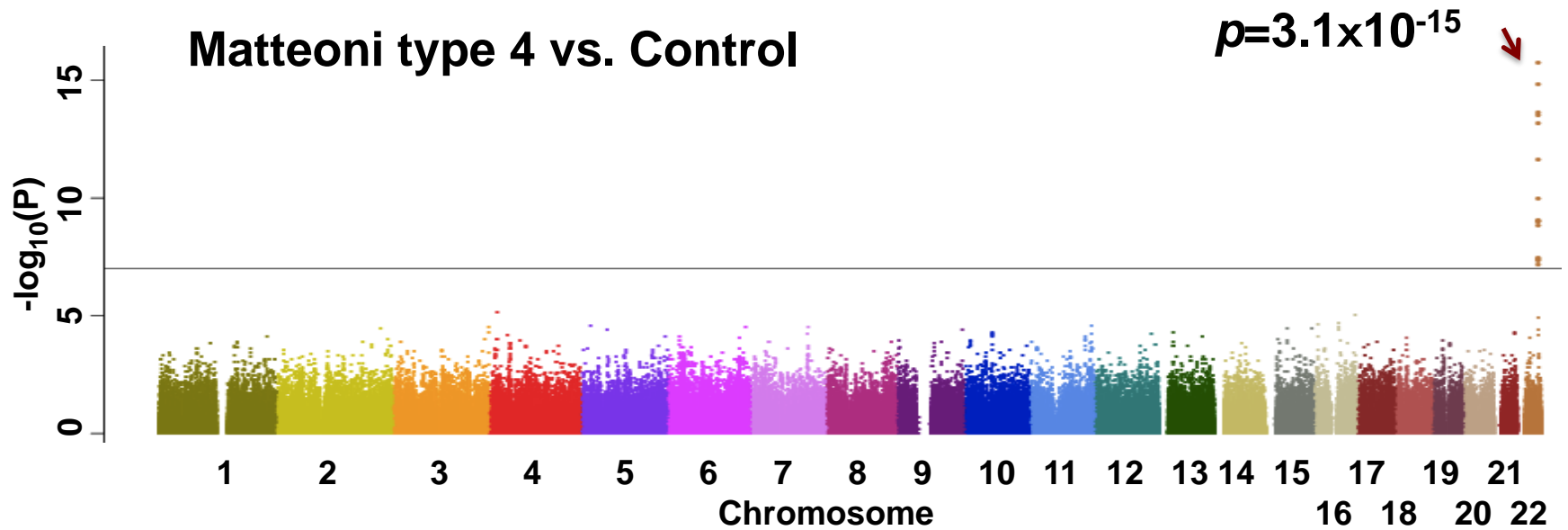
Clinical characteristics according to the histological classification (1)

Phenotype	Matteoni classification of NAFLD					
	Type 1	Type 2	Type 3	Type 4	Control	p-value
Number of samples	100	73	29	327	932	0.0023
Sex (Male/Female)	59/41	47/26	13/16	130/197	471/461	<0.001
Age (year)	49.7±15.3	51.5±15.3	49.4±14.0	57.6±14.8	48.8±16.3	
Physical measurement						
BMI	26.2±4.3	27.7±4.8	27.6±3.5	27.7±5.2		0.054
Amount of visceral fat (cm ²)	146.8±65.3	154.3±47.7	136.8±53.8	151.7±57.4		0.46
Abdominal circumscript (cm)	90.9±9.9	94.1±10.0	88.5±10.2	94.1±11.8		0.10
Biochemical trait						
AST(IU/L)	31.1±14.6	36.4±18.5	52.4±35.1	57.7±48.4		<0.001
ALT(IU/L)	48.6±30.8	62.8±47.6	81.5±46.9	74.9±48.4		<0.001
GGT(IU/L)	71.0±62.5	67.1±66.9	96.1±91.3	76.6±73.9		0.25
Albumin (g/dL)	4.5±0.4	4.4±0.3	4.5±0.3	4.3±0.4		<0.001
Total bilirubin(mg/dL)	0.9±0.5	0.9±0.5	0.9±0.6	0.8±0.4		0.063
Cholinesterase (unit)	389.1±97	354.3±97.2	371.1±109.9	348.9±93.2		<0.001
Type IV collagen 7s (ng/dL)	3.8±0.7	3.9±0.9	3.9±0.8	5.1±1.7		<0.001
Hyaluronic acid (ng/dL)	25.6±22.5	33.6±29.5	31.5±24.0	80.9±84.3		<0.001
Triglycerides (mg/dL)	151.9±73.8	154.0±92.1	166.1±86.5	161.2±85.7		0.23
Total cholesterol (mg/dL)	209.1±32.8	194.0±38.0	203.0±39.9	200.3±39.0		0.093
HbA1c(%)	6.1±1.1	5.9±1.2	6.5±1.8	6.2±1.3		0.13
IRI (μg/dL)	9.1±5.4	11.4±9.0	10.4±6.3	14.9±9.9		<0.001

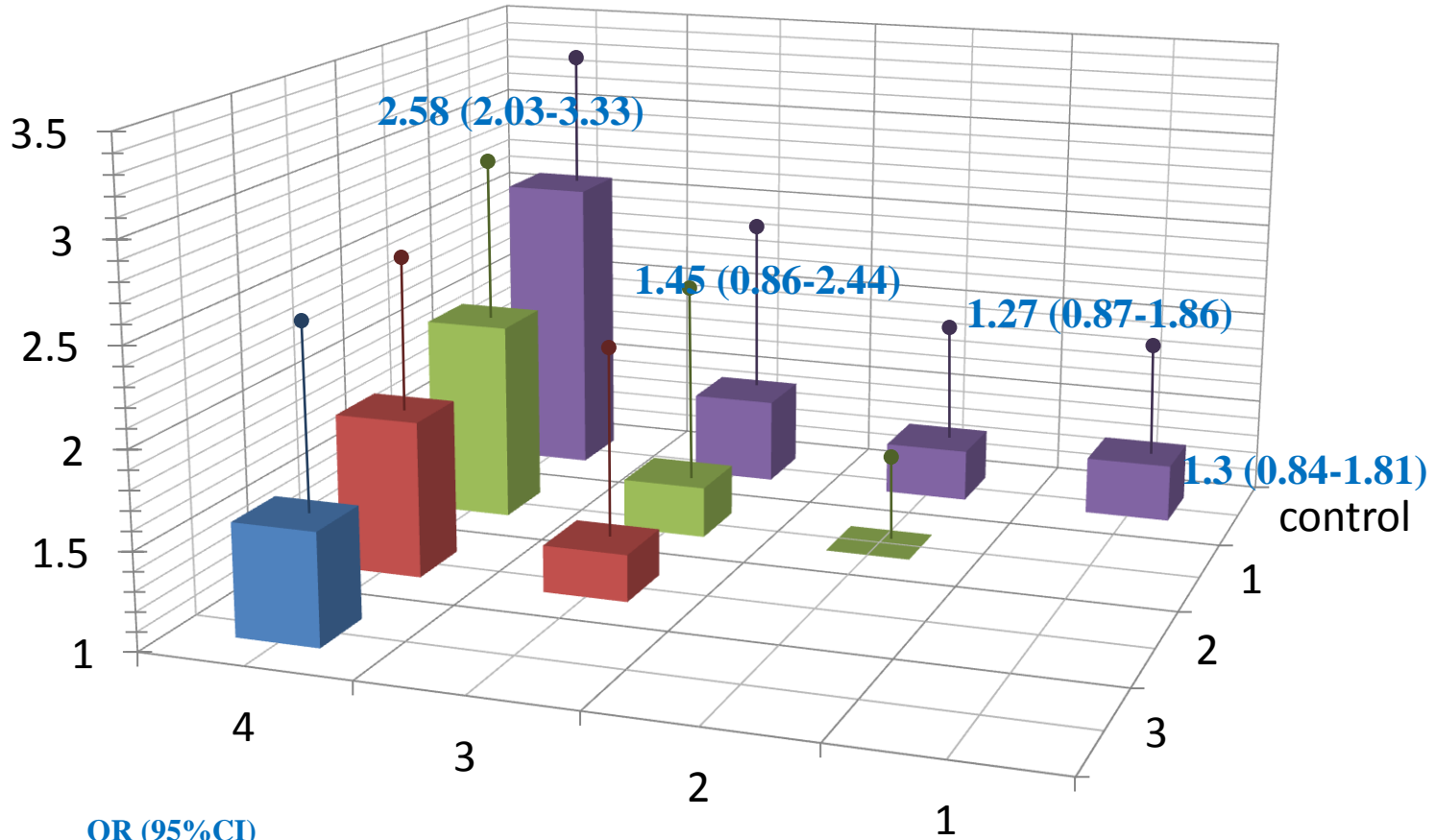
Clinical characteristics according to the histological classification (2)

Phenotype	Matteoni classification of NAFLD				Control	p-value
	Type 1	Type 2	Type 3	Type 4		
Number of samples	100	73	29	327	932	0.0023
FPG (mg/dL)	112.9±33.7	107.3±27.4	109.9±27.7	114.8±33.8		0.14
HOMA-IR	2.4±1.5	2.9±2.4	3.0±2.1	4.2±3.0		<0.001
hs-CRP (mg/dL)	1078.9±1407	1048.3±1185.0	865.8.3±658.4	1579.2±2377.9		0.027
Adiponectin (µg/mL)	7.4±4.4	8.5±6.6	6.6±2.6	6.9±4.3		0.24
Leptin (ng/mL)	9.9±7.4	9.1±6.2	11.3±9.4	12.4±7.9		<0.001
Ferritin (ng/mL)	145.8±101.1	176.5±134.0	271.2±307.0	208.3±180.3		0.027
Uric acid (mg/dL)	5.9±1.5	5.7±1.2	5.4±1.9	5.7±1.6		0.77
PLT(×10 ⁴ /µL)	23.0±5.9	22.9±4.9	21.9±6.7	15.7±1.6		<0.001
ANA (0/1/2/3/4)	42/17/4/0/0	31/8/4/1/2	15/6/2/0/0	147/76/31/8/12		0.015
Clinical history						
Diabetes (NGT/IGT/DM)	36/11/34	24/7/27	12/8/7	103/35/119		0.45
dyslipidemia (+ /-)	31/68	31/42	9/20	120/206		0.6
Hypertension (+ /-)	64/35	33/40	19/10	155/172		0.013
Liver biopsy feature						
Brunt grade (1/2/3)			19/3/2	149/133/44		<0.001
Brunt stage (1/2/3/4)				123/74/105/24		
Fat droplet (1/2/3/4)	38/32/19/11	14/29//18/7	7/3/10/4	51/99/104/52		<0.001
Iron deposition (0/1/2/3/4)	30/14/21/10/1	24/9/12/2/1	10/5/2/2/0	132/56/29/29/11		0.16

Rs738409はMatteoni type 4にのみ特異的



Odds ratio between each type of NAFLD classified by Matteoni's classification for rs2896019



OR (95%CI)	Genotype counts (TT/TG/GG)				
control	NAFLD (Matteoni index)				
	1	2	3	4	
279/482/179(19%)	20/59/23(23%)	22/30/22(30%)	10/17/13(33%)	40/134/147(46%)	

NASH肝癌の特徴

Yasui K, Okanoue T, et al. *Characteristics of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma: a multicenter study in Japan.*

Clin Gastroenterol Hepatol 2011; 9:428-433

Characteristic	Total (n = 87)	Male (n = 54)	Female (n = 33)	P^a
Age (y)	72 (68–75)	72 (69–75)	72 (68–75)	0.52
BMI (kg/m²)	26.0 (23.8–28.3)	26.0 (23.8–28.8)	26.2 (23.9–27.7)	0.54
Obesity	54 (62%)	35 (65%)	19 (58%)	0.50
Diabetes	51 (59%)	31 (57%)	20 (61%)	0.77
Dyslipidemia	24 (28%)	13 (24%)	11 (33%)	0.35
Hypertension	47 (54%)	22 (41%)	25 (76%)	0.001
Platelet count (×10⁴/μL)	13.9 (10.1–18.0)	14.5 (11.7–18.0)	10.9 (7.8–18.0)	0.05
AST (IU/L)	47 (30–59)	46 (27–60)	47 (35–58)	0.45
ALT (IU/L)	36 (26–55)	43 (26–69)	34 (26–42)	0.11
γ-GTP (IU/L)	75 (40–115)	68 (36–177)	75 (40–115)	0.90
Fasting glucose (mg/dL)	114 (99–145)	112 (99–144)	120 (97–152)	0.59
HbA1C (%)	6.1 (5.4–7.1)	5.9 (5.4–7.0)	6.3 (5.2–7.1)	0.78
Total cholesterol (mg/dL)	169 (147–202)	169 (147–202)	169 (147–202)	0.62
HDL cholesterol (mg/dL)	50 (41–60)	45 (41–58)	55 (50–73)	0.03
Triglyceride (mg/dL)	100 (76–138)	118 (80–147)	96 (74–116)	0.06
Ferritin (ng/dL)	197 (74–401)	273 (154–703)	98 (23–172)	0.005
Hyaluronic acid (ng/mL)	166 (67–241)	151 (69–244)	174 (61–332)	0.85
AFP (ng/mL)	7.1 (5.0–18.0)	6.0 (4.0–14.7)	10.8 (5.9–18.0)	0.02
DCP (mAU/mL)	66 (22–298)	48 (22–243)	81 (21–942)	0.42

Characteristic	Total (n = 87)	Male (n = 54)	Female (n = 33)	<i>P</i> ^a
HCC tumor size (cm)	3.0 (2.0–4.0)	3.1 (2.2–4.5)	2.6 (1.9–4.0)	0.18
Numbers of HCC tumor				0.78
1	65 (75%)	39 (72%)	26 (79%)	
2 or 3	16 (18%)	11 (20%)	5 (15%)	
≥ 4	6 (7%)	4 (8%)	2 (6%)	
Background liver tissue				
Steatosis grade ^b				0.64
0: <5%	1 (1%)	1 (2%)	0 (0%)	
1: 5-33%	60 (69%)	36 (67%)	24 (73%)	
2: 34-66%	19 (22%)	11 (20%)	8 (24%)	
3: >66%	7 (8%)	6 (11%)	1 (3%)	
Necroinflammatory grade ^c				0.22
1: mild	31 (35%)	22 (41%)	9 (27%)	
2: moderate	45 (52%)	26 (48%)	19 (58%)	
3: severe	11 (13%)	6 (11%)	5 (15%)	
Fibrosis stage ^c				0.003
1	10 (11%)	10 (19%)	0 (0%)	
2	15 (17%)	10 (19%)	5 (15%)	
3	18 (21%)	13 (25%)	5 (15%)	
4	44 (51%)	21 (39%)	23 (70%)	

NASH薬物治療のEvidence

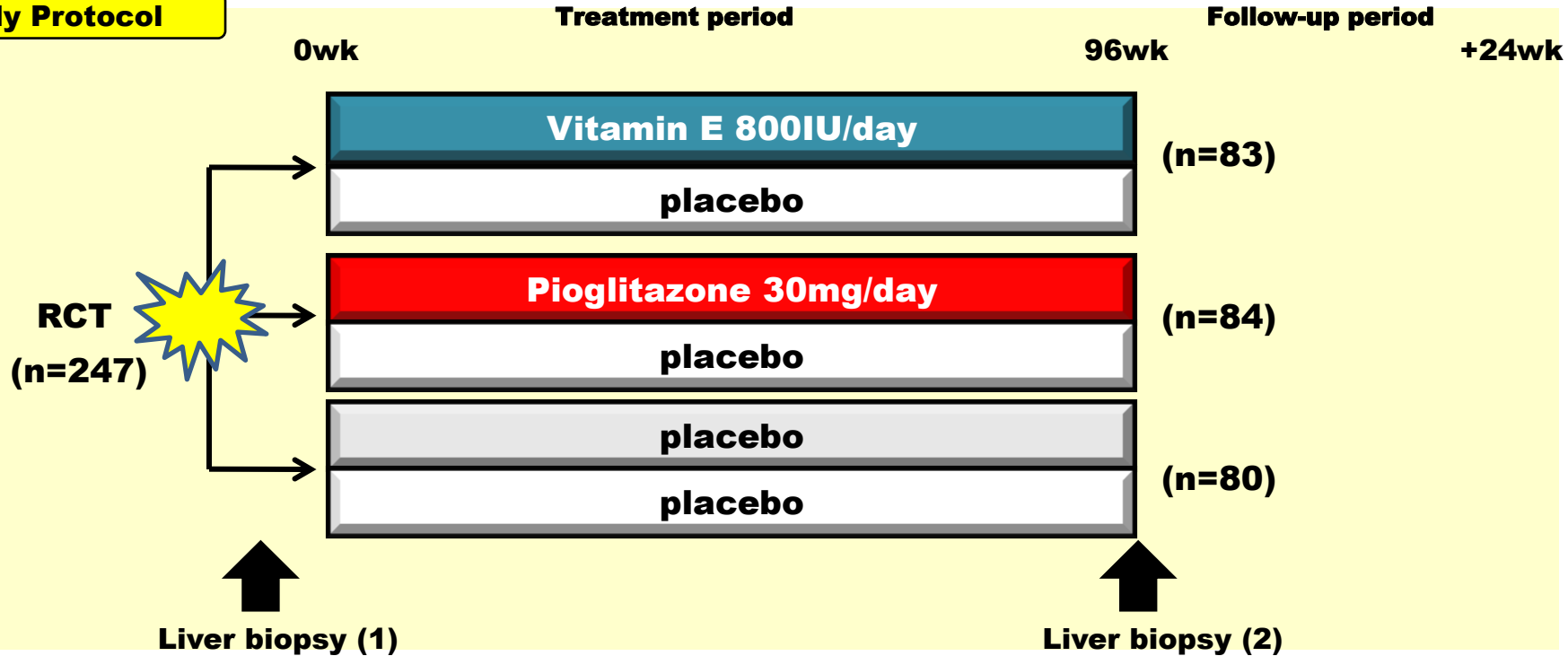
References:

- 1) Musso G, Gambino R, Cassader M, Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology*. 2010 ;52:79-104.
- 2) Hojo M, Watanabe S. Pharmacological therapy of nonalcoholic steatohepatitis. *Hepatol Res*. 2011 ;41:209-16.
- 3) Nobili V, Sanyal AJ. *J Gastroenterol*. 2011 Oct 8. [Epub ahead of print]
- 4) Marchesini G, Moscatiello S, Agostini F, Villanova N, Festi D. Treatment of non-alcoholic fatty liver disease with focus on emerging drugs. *Expert Opin Emerg Drugs*. 2011 ;16:121-36.

PIVENS trial

～ NASH治療における代表的大規模臨床試験 ～

Study Protocol



Outcomes

1 次評価項目(組織学的改善):

- **Hepatocyte ballooning**の1ポイント以上の改善
- 線維化の悪化なし
- 炎症, 脂肪化の1ポイント以上の改善を伴い, **NAS 3点以下**への改善あるいは**NAS 2ポイント以上**の改善

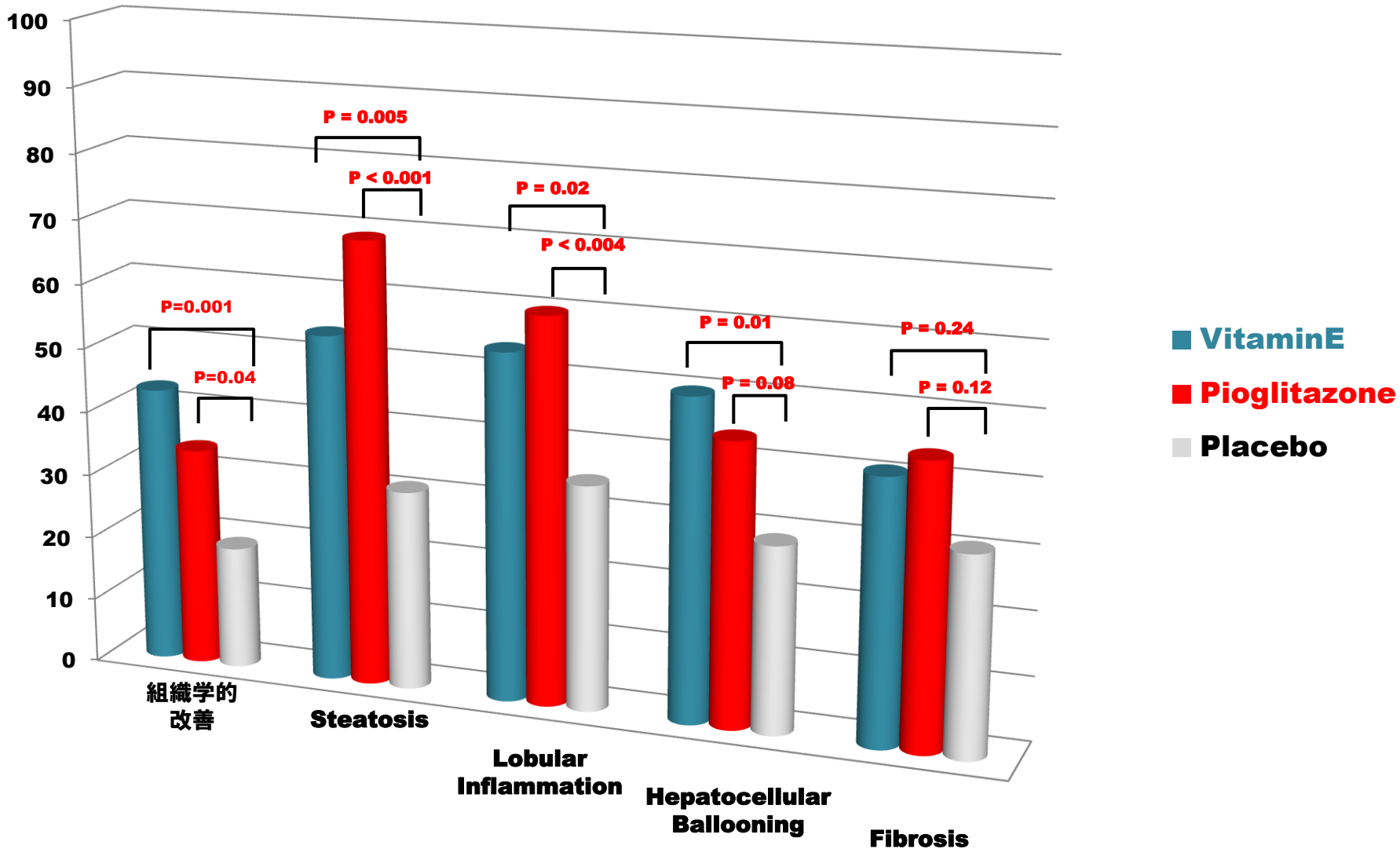
2 次評価項目:

- (1) **NAS**, 組織所見の各項目の評価、線維化
- (2) **AST, ALT**, 身体計測, インスリン抵抗性, 脂質プロファイル
- (3) **QOL (SF-36)**

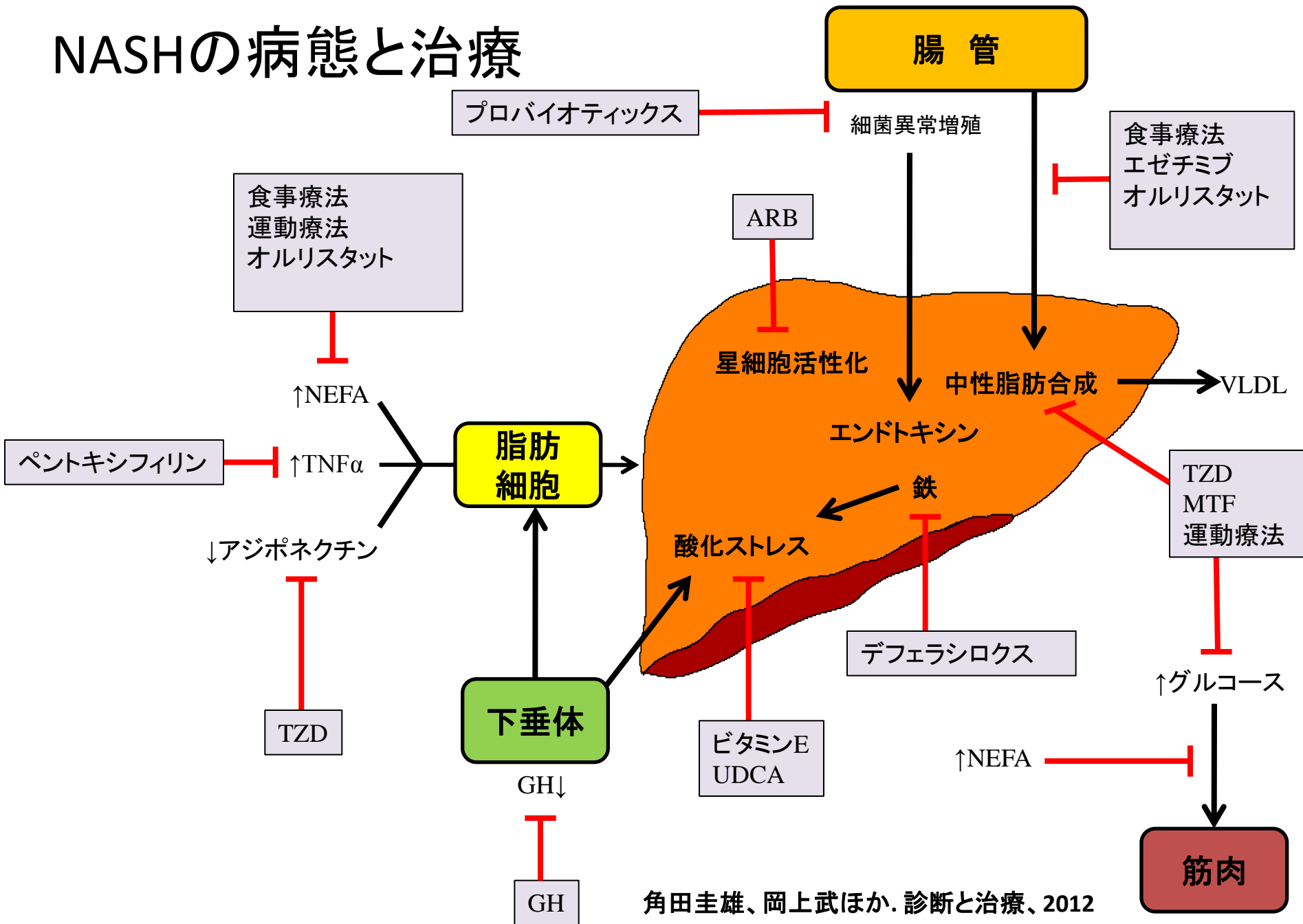
Sanyal AJ, et al. N Engl J Med 2010

PIVENS trial

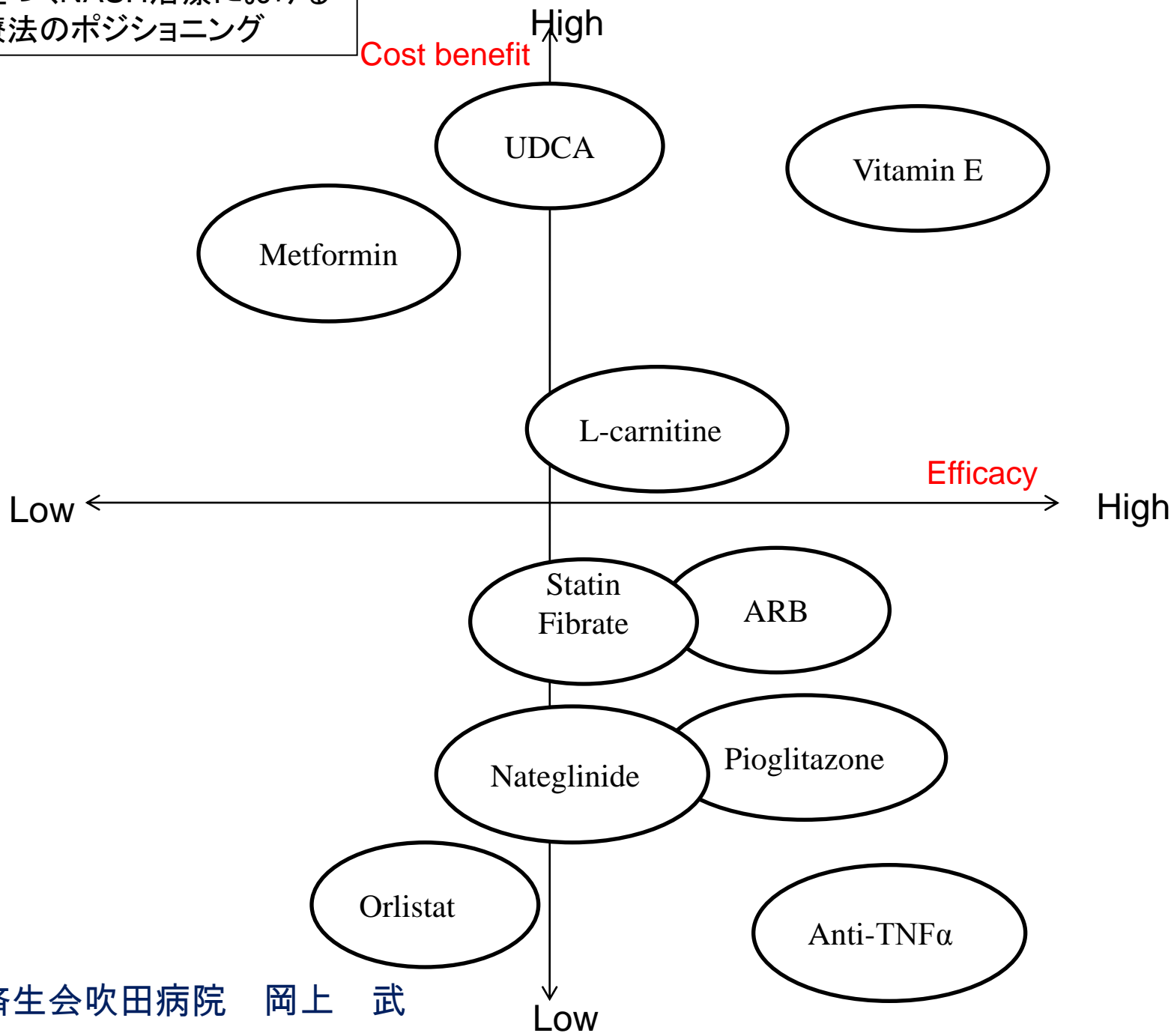
~ NASH治療における代表的大規模臨床試験 ~



NASHの病態と治療



EBMに基づくNASH治療における
各種治療法のポジショニング



NASH治療の課題

1. 治療効果の評価をどうするか

Sanyal AJ, et al. Endpoints and clinical trial design for nonalcoholic steatohepatitis . Hepatology 2011; 54: 344-353

2. NASHは遺伝的素因を含めheterogeneousな背景をもつ疾患である。

3. NASHの長期予後は十分明らかにされておらず、誰をいつどのように治療すればベネフィットがあるか

4. 求められる高いエビデンス

? vs. 食事運動療法

? vs. ビタミンE

5. 治療効果のモニター

炎症、線維化など組織学的所見を反映する最適なマーカーは

6. 予後や発がん抑制効果の検証

7. 単独薬物療法の限界

異なる作用機序の薬剤の組み合わせ

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