



MEDICINA
USP



Biomarkers and genetic polymorphisms in NAFLD: what is the clinical utility?

Profa. Dra. Claudia PMS Oliveira

Livre Docente e Professora Associada do

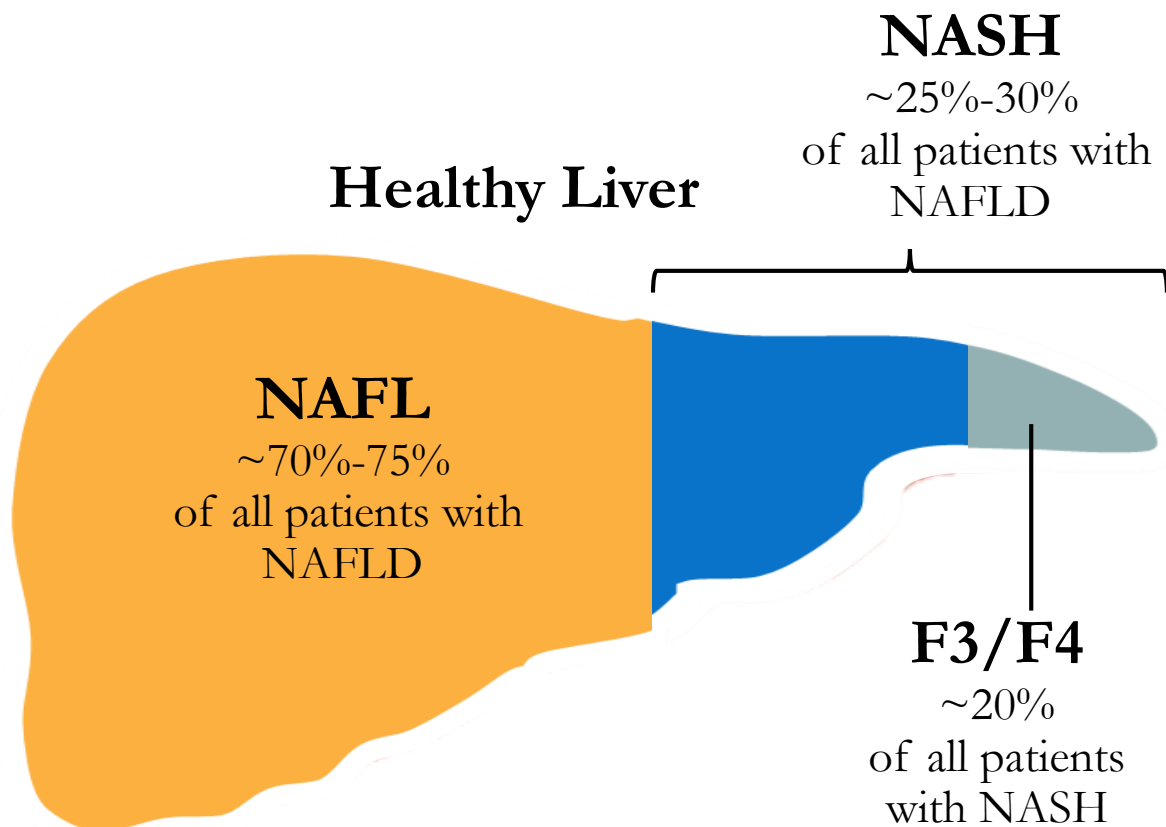
Departamento de Gastroenterologia

Faculdade de Medicina da Universidade de São Paulo

Disclosure of Conflict of Interest

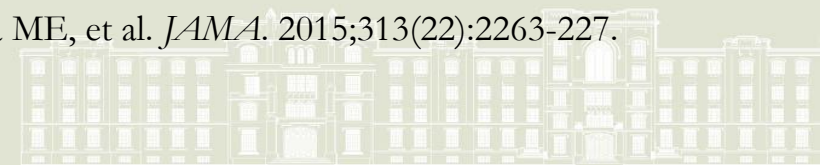
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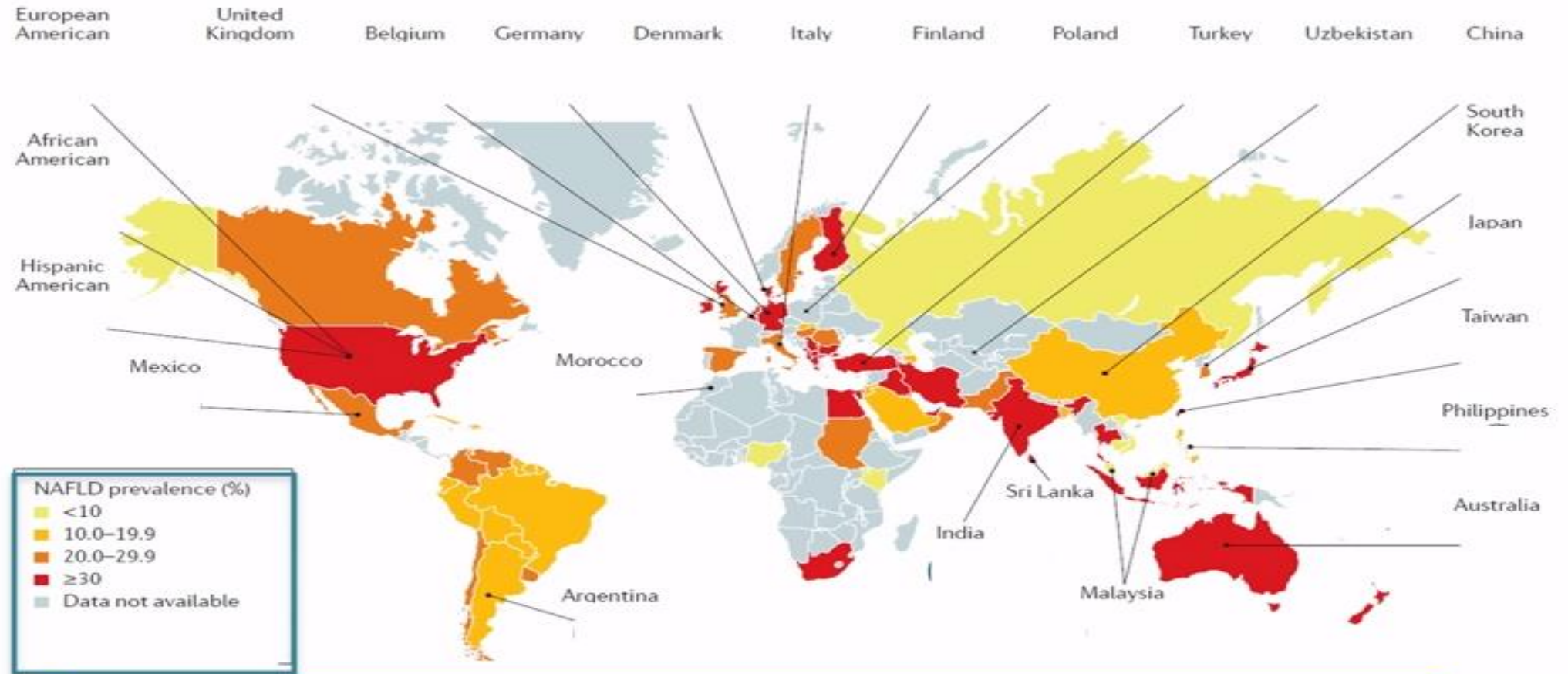


Advanced Fibrosis (F3/F4)

- Fibrogenesis
- Activation of stellate cells and replacement of hepatocytes by scar tissue
- Cirrhosis can ultimately progress to hepatocellular cancer, liver failure, liver transplant and death



Worldwide prevalence of NAFLD



GENERAL POPULATION
NASH in 10-20% of NAFLD
Advanced fibrosis/ cirrhosis in 10-15% of NASH



Nonalcoholic Steatohepatitis Is the Most Rapidly Growing Indication for Liver Transplantation in Patients With Hepatocellular Carcinoma in the U.S.

- Retrospective study 2002-2012 (OPTN/UNOS)
- 61.868 LT
- 10.061 HCC
- 2th cause of LT in US
- NASH - 2^o cause of HCC
- Hepatitis C increased 2x since 2002
- NASH increased 4x since 2002

Wong RJ, et al Gastroenterology 2015



HCC and NAFLD

Author	Year	n	Study population	Prevalence	
Bugianesi et al. [74]	2002	641	Patients with HCC in cirrhotic livers	23/641	3.6%
Hashimoto et al. [75]	2009	382	NASH population	34/382	8.9%
Takuma and Nouse [19]	2010	445	Patients curatively treated for HCC	11/445	2.5%
Hai et al. [76]	2006	481	Patients with resected HCC	2/418	0.5%
Maeda et al. [77]	2008	242	Patients with resected HCC	3/242	0.8%
Kawada et al. [78]	2009	1168	Patients with resected HCC	8/1168	0.7%
Malik et al. [79]	2009	98	Patients transplanted with NASH cirrhosis	17/98	17%
Chagas et al. [80]	2009	394	HCC detected by ultrasound	7/394	1.8%

Hepatocellular Carcinoma Management in Nonalcoholic Fatty Liver Disease Patients

Applicability of the BCLC Staging System

Luciana Kikuchi, MD, Claudia P. Oliveira,* Mario R. Alvares-da-Silva,†
Claudia M. Tani,* Marcio A. Diniz,* Jose T. Stefano,* Aline L. Chagas,*
Regiane S.S.M. Alencar,* Denise C.P. Vezozzo,* Gilmar R. Santos,*
Priscila B. Campos, Venancio AF. Alves,* Vlad Ratziu,‡ and Flair J. Carrilho**

From January 2010 until (to) April 2012, 42 patients with HCC acomited by (in the setting of) NASH were evaluated at the Department of Gastroenterology of São Paulo School of Medicine by *São Paulo Clínicas Liver Cancer Group*.(, São Paulo.)

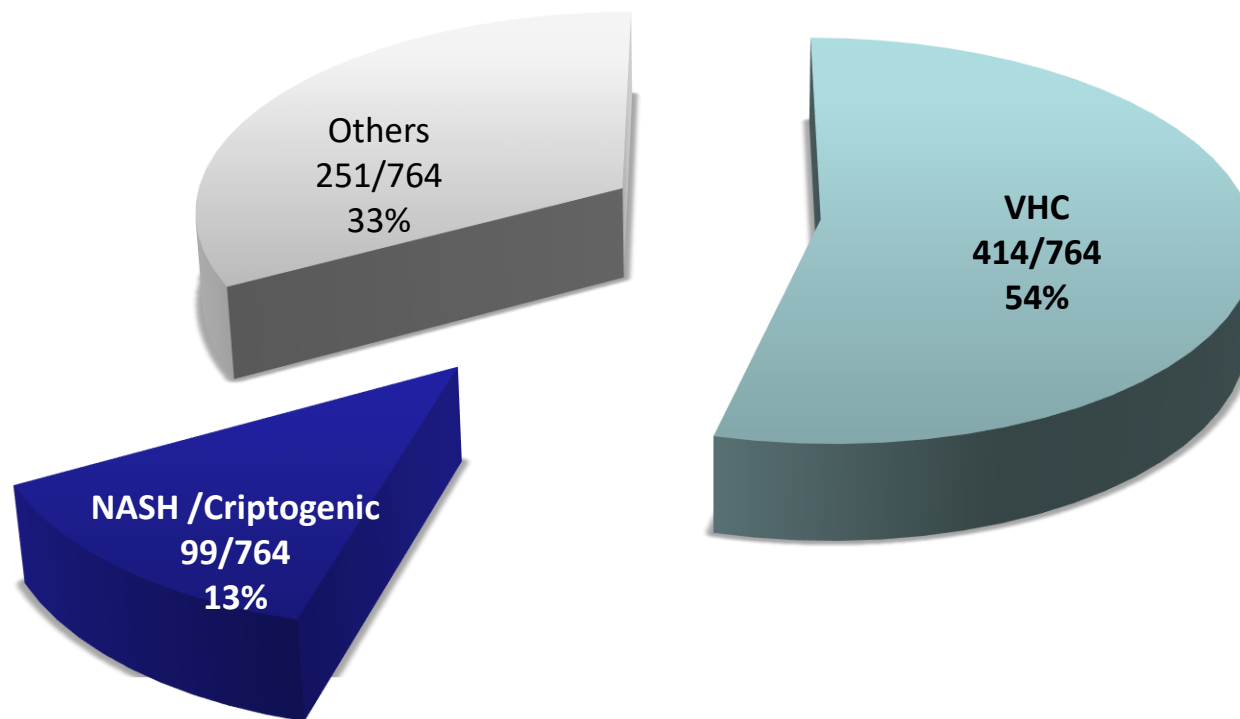
(Am J Clin Oncol 2014;1

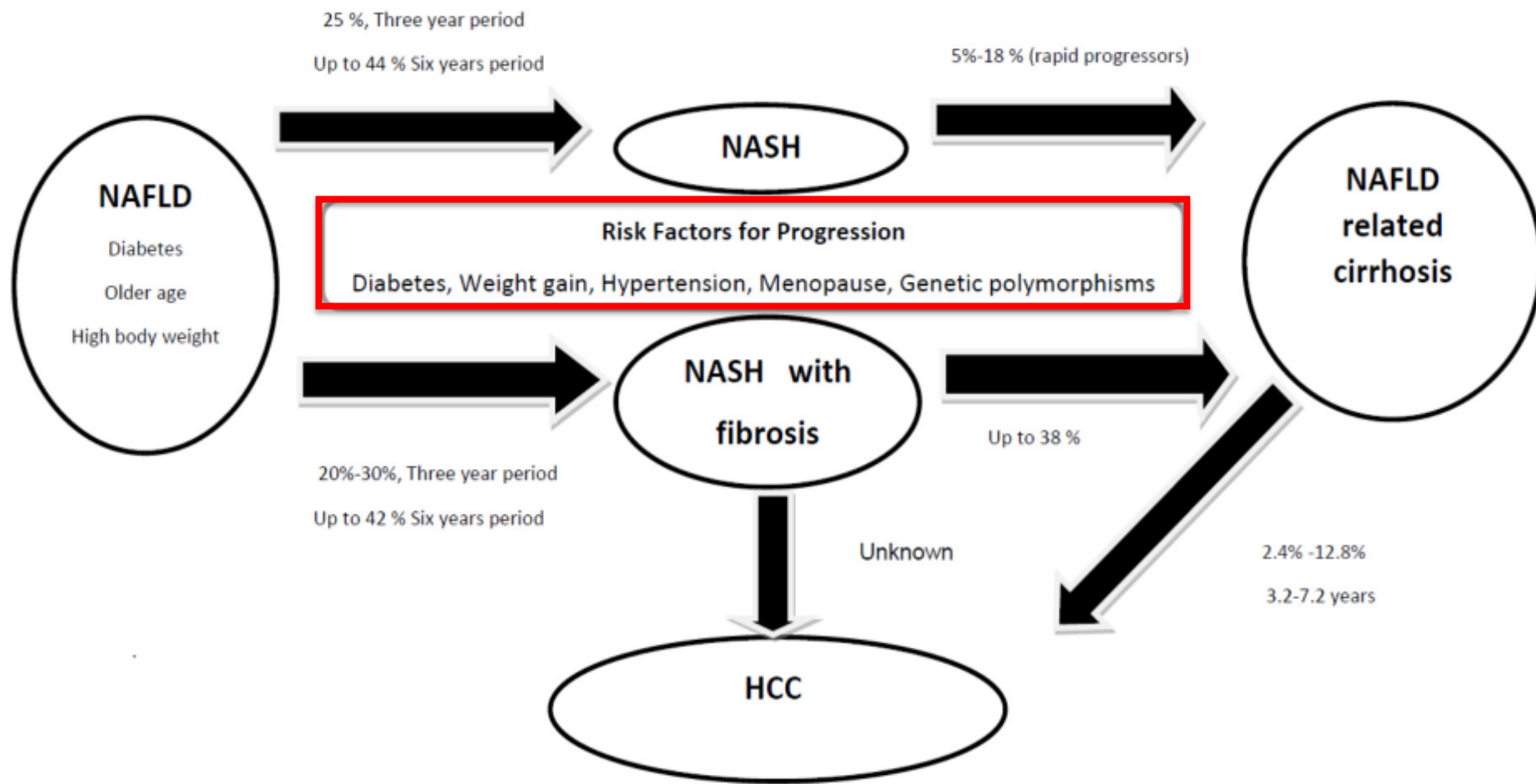


- *Hospital das Clínicas da FMUSP (Junho/2010 a Março/2017)*

- n = 764 cases

HCC







Genetics has the advantage that it can be assessed from the time of birth, well before the discriminative capacity emerges for the risk factors (e.g., hypertension or T2DM) used in clinical practice to predict NAFLD.



**N
A
F
L
D**



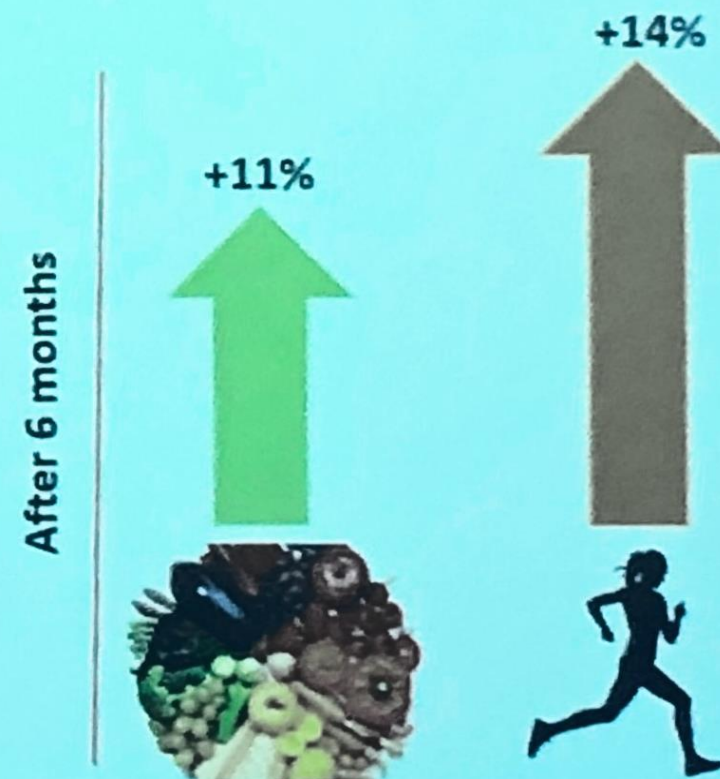
User perspectives

Eslam M, Sevilla, NASH EASL
meeting 2019

Primary Outcome Measures before and after Receipt of
Results of Genetic Testing (n=2037)

Outcome measure	Baseline score	Follow-up Score	P-value
Anxiety	35.2±9.6	34.6±10.0	0.80
Dietary fat intake	16.0±7.9	15.2±7.5	0.89
Exercise	28.6±23	28.6±22.9	0.61

Customers of 23andMe and Pathway Genomics
(n=1002) receive personal genomic testing



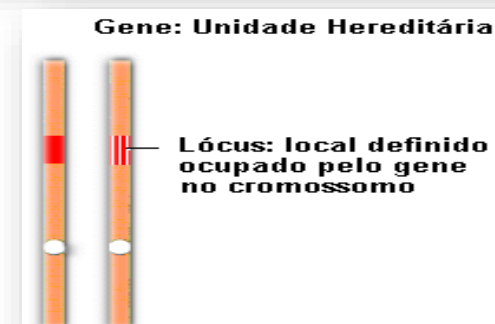
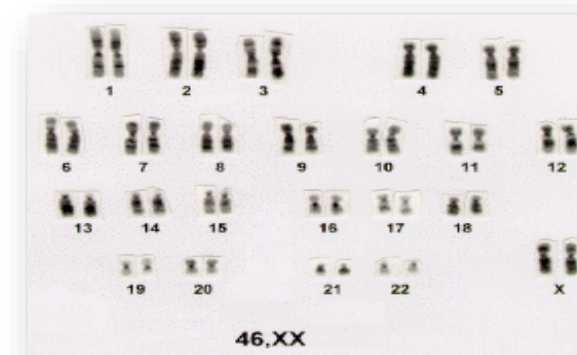
Genetic Polymorphisms and Micro RNAs

- **Genetics Markers Potential Clinical Utility**
- **Micro RNAs**



GENETIC POLYMORPHISMS

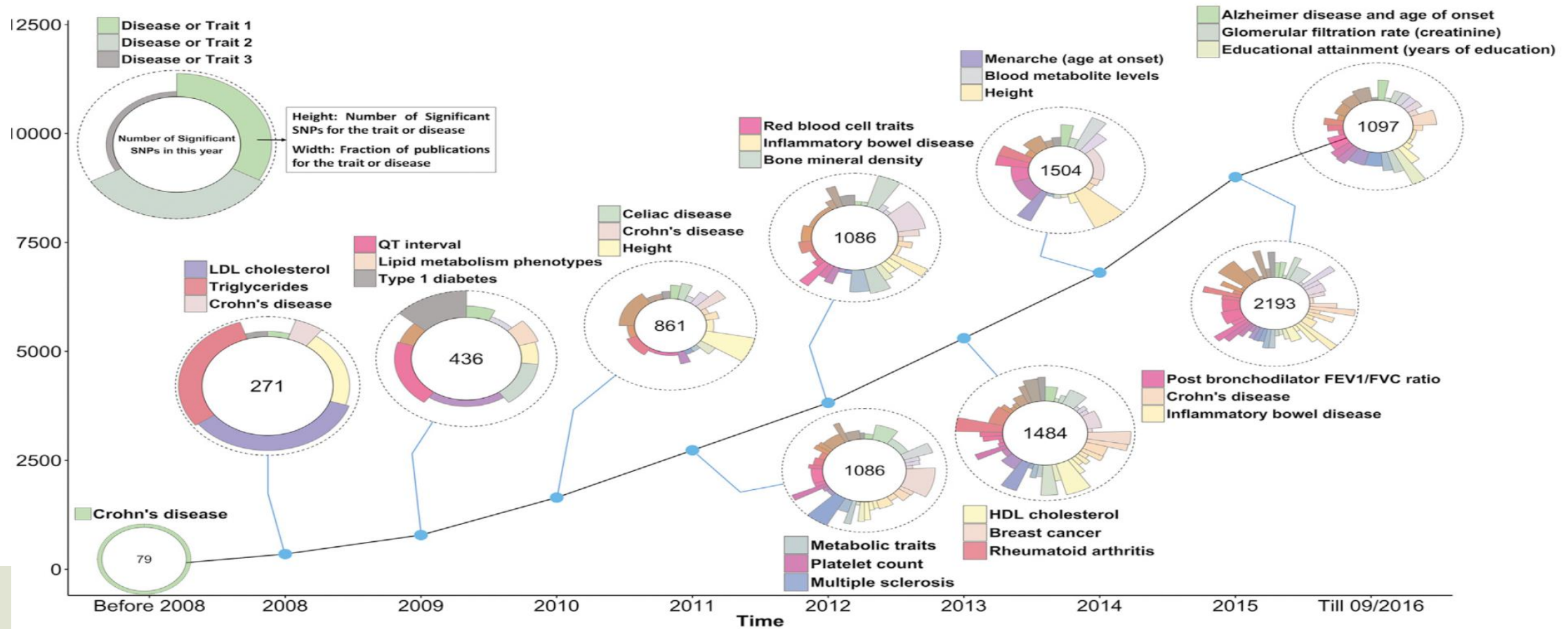
- Genetic variation found in less than 1% of the population
- Deletions, mutations, single base substitutions
- Contribution to Phenotypic traits (skin color, blood type) and Susceptibility to diseases
- Genetic markers
- Transmitted associated with other genes located in the chromosomal region close to them



10 Years of GWAS Discovery: Biology, Function, and Translation

Peter M. Visscher,^{1,2,*} Naomi R. Wray,^{1,2} Qian Zhang,¹ Pamela Sklar,³ Mark I. McCarthy,^{4,5,6} Matthew A. Brown,⁷ and Jian Yang^{1,2}

GWAS SNP- Trait Discovery Timeline



□

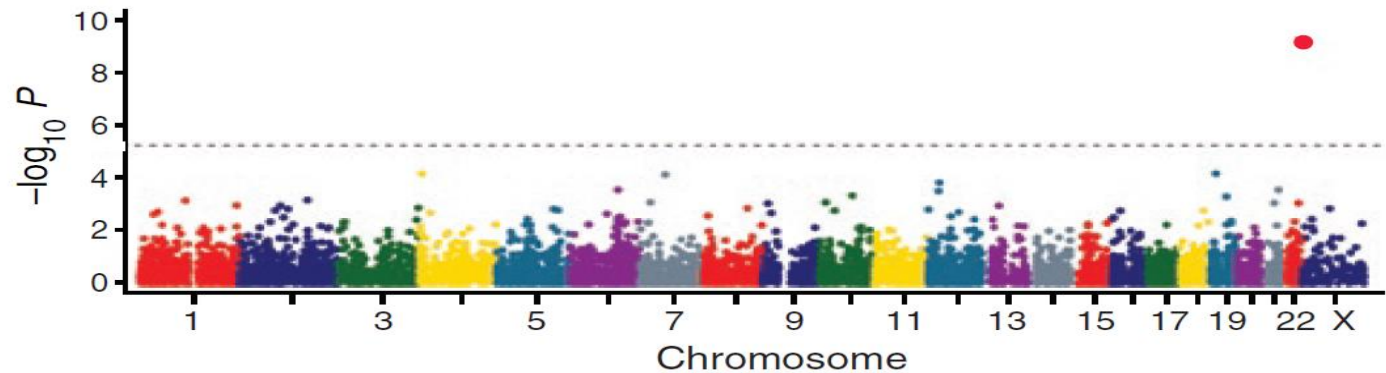
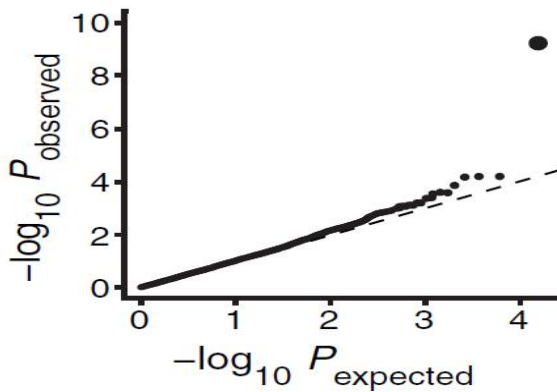
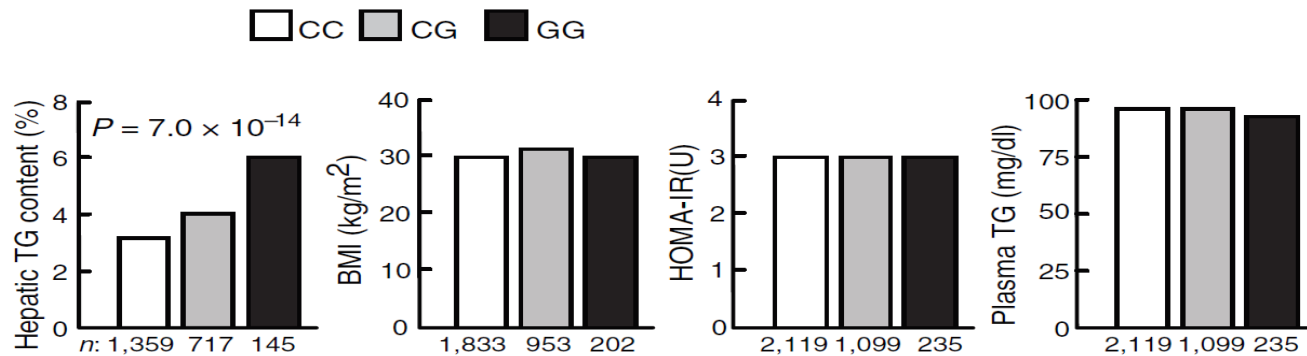
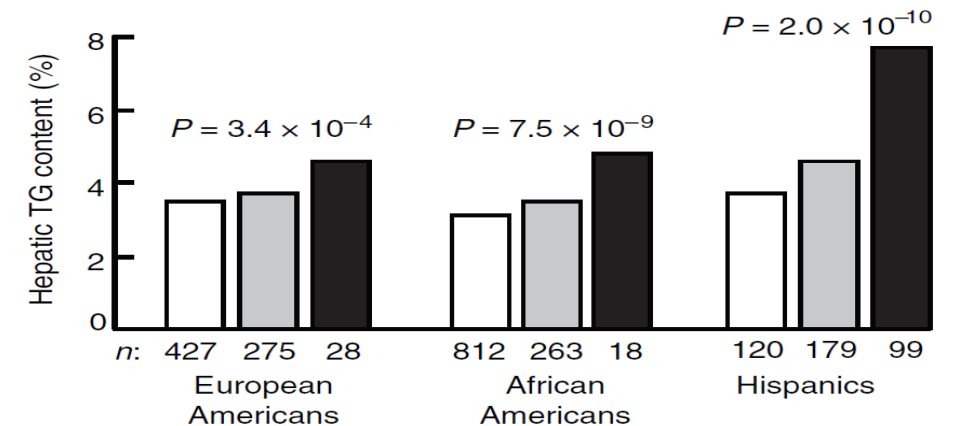


Figure 1 Genome-wide scan of liver triglyceride content measured by proton magnetic resonance imaging in the Dallas Heart Study ($n = 2,111$). (a) Quantile-quantile plot of P values. (b) Scatter plot of P values. The dashed line denotes the Bonferroni-corrected significance threshold ($P = 5.4 \times 10^{-6}$).

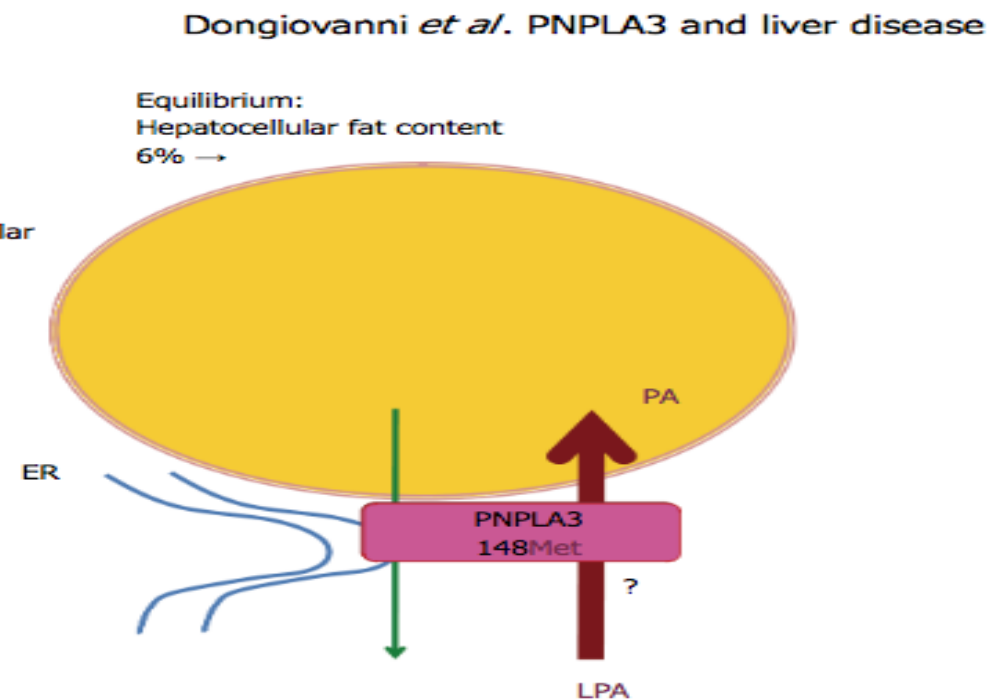
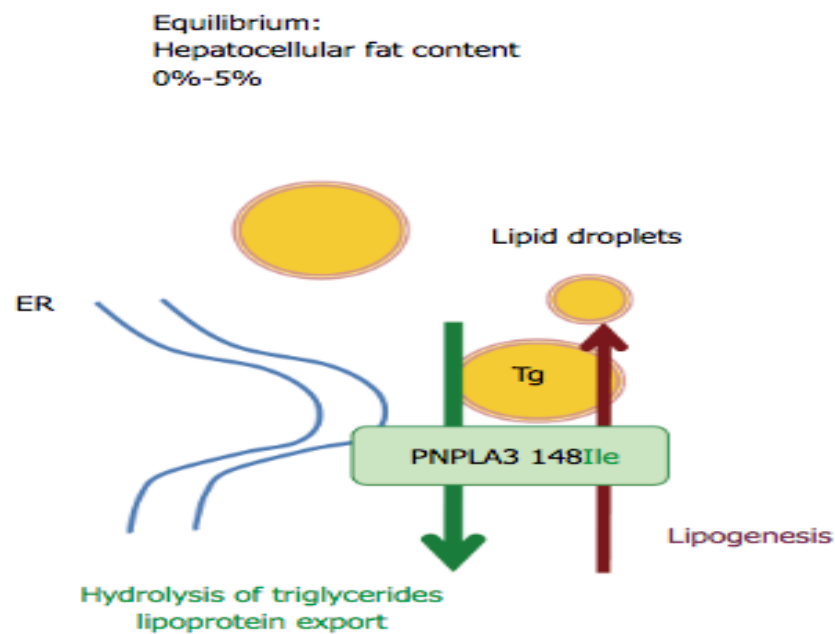
b



c



INFLUENCE OF PNPLA3 POLYMORPHISM IN NAFLD



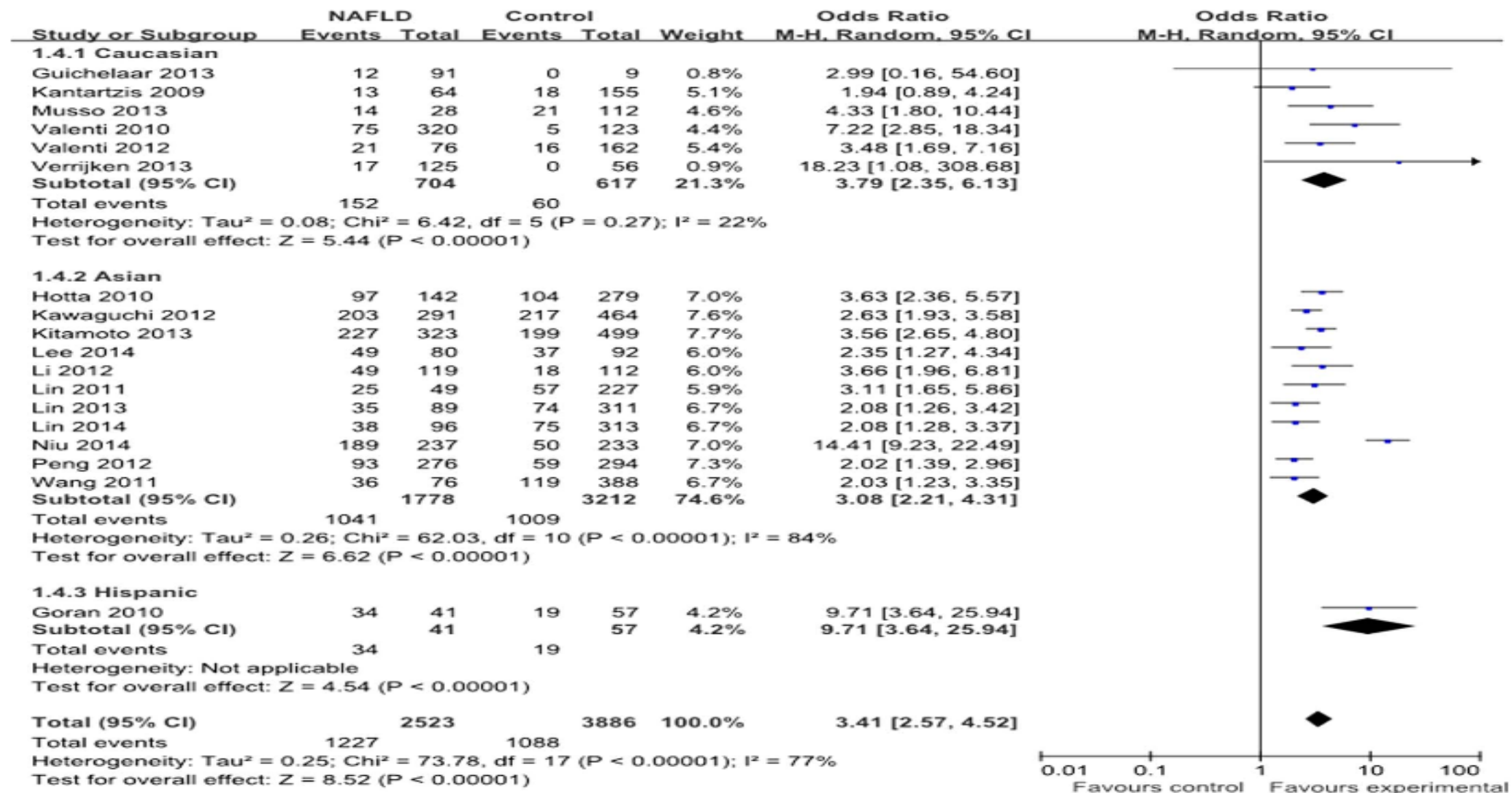
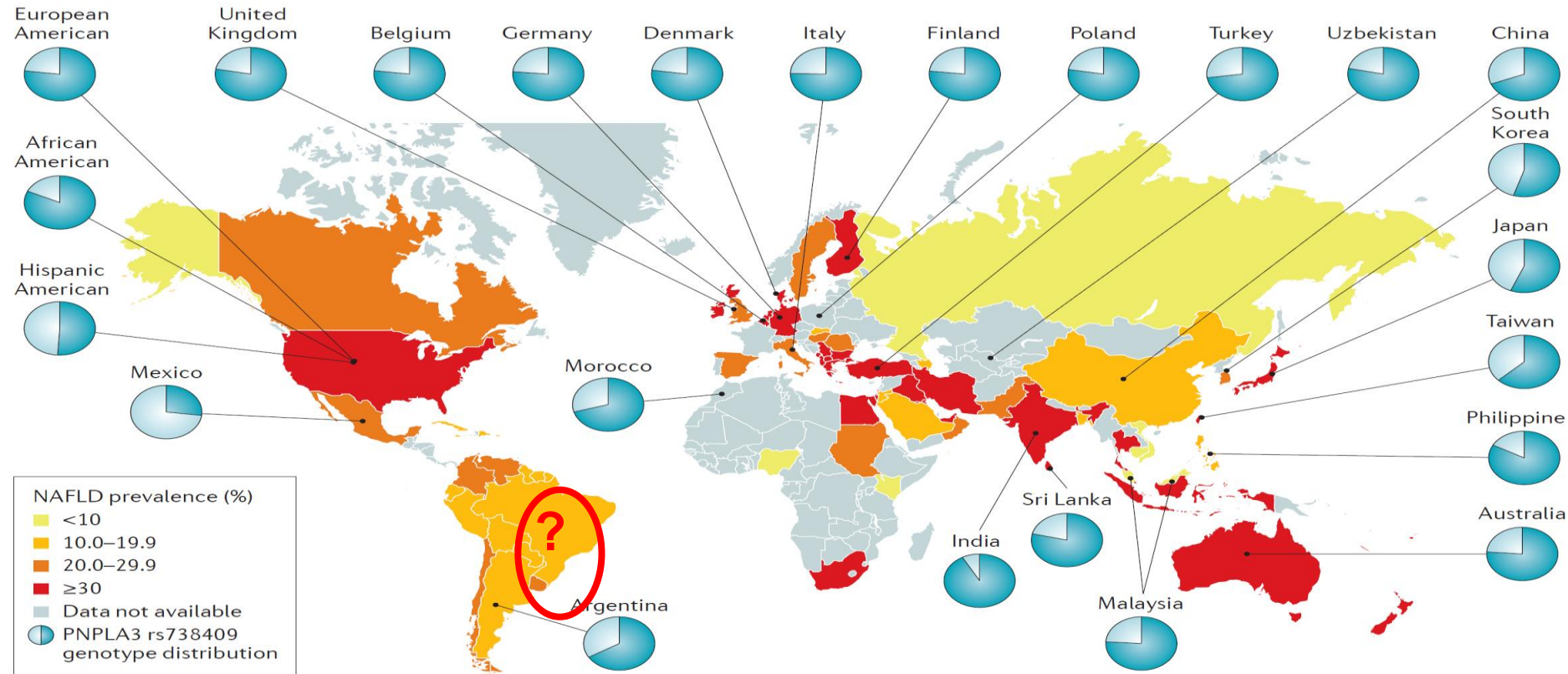


Figure 2 | Forest plot of NAFLD susceptibility associated with rs738409 polymorphism at additive model (GG vs CC).

GENETIC NAFLD/NASH NAFLD



Genetic Variants

PNPLA3
 TM6SF2
 MBOAT7
 GSKR
 FNDC5
 MERTK
 HSD17B13
 IFNL3
 NADPHOXIDASE
 MTP
 UCP3

Original article

Validation of PNPLA3 polymorphisms as risk factor for NAFLD and liver fibrosis in an admixed population



Daniel F. Mazo^{a,b,*}, Fernanda M. Malta^c, Jose Tadeu Stefano^a, Ana Paula M. Salles^c, Michele S. Gomes-Gouvea^c, Ana Catharina S. Natri^c, Jazon R. Almeida^b, Joao Renato R. Pinho^c, Flair J. Carrilho^a, Claudia P. Oliveira^a

Table 1

PNPLA3C>G polymorphisms frequencies in healthy subjects and patients with NAFLD.

	Genotype frequency		% (n)	Total %
	CC	CG	GG	
PNPLA3				
Healthy subjects (n = 134)	49.25 (66)	41.04 (55)	9.7 (13)	100
NAFLD (n = 248)	31.05 (77)	47.18 (117)	21.77 (54)	100



Results

		Genotype frequency % (n)			Total %
		CC	CG	GG	
PNPLA3	Control (n=13)	49.25 (66)	41.04 (55)	9.7 (13)	100
	NAFLD (n=24)	31.05 (77)	47.18 (117)	21.77 (54)	100
TM6SF2		CC	CT + TT		
	Control (n=13)	93.28 (125)	6.72 (9)		100
	NAFLD (n=248)	89.47 (221)	10.53 (26)		100

P=0.0044

P=0.0821

Razão de Chances (OR) para NAFLD

Análise ajustada para gênero e idade

PNPLA3	p-valor	OR	IC95%
CG x CC	0.0044	1.757	1.037-2.977
GG x CC	0.0044	3.296	1.504-7.225

Carriage of the *PNPLA3* rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma

Y.-L. Liu¹, G.L. Patman², J.B.S. Leathart¹, A.-C. Piguet³, A.D. Burt^{1,†}, J.-F. Dufour³, C.P. Day¹, A.K. Daly¹, H.L. Reeves^{2,*,‡}, Q.M. Anstee^{1,‡}

Table 1. Details of NAFLD-HCC and NAFLD cohorts.

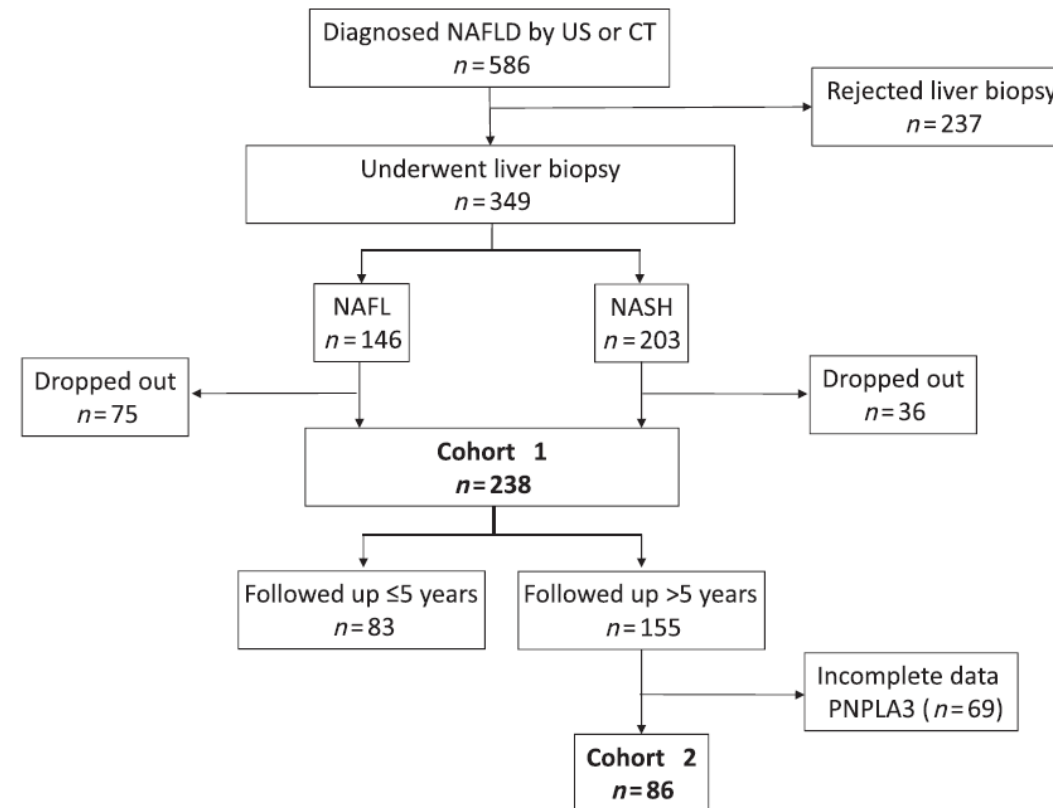
Phenotype	NAFLD-HCC cohort n = 100	NAFLD cohort n = 275	p value
<i>PNPLA3</i> rs738409 G-allele frequency	0.505	0.333	<0.0001
Age (Mean ± SD)	70.3 ± 8.0	50.9 ± 12.4	<0.0001
Sex, male (%)	82 (0.82)	161 (0.59)	<0.0001
BMI (Mean ± SD)	32.0 ± 6.6	34.4 ± 5.2	0.0003
Diabetes (%)	68 (0.68)	117 (0.43)	<0.0001
Cirrhosis (%)	67 (0.67)	26 (0.09)*	<0.0001

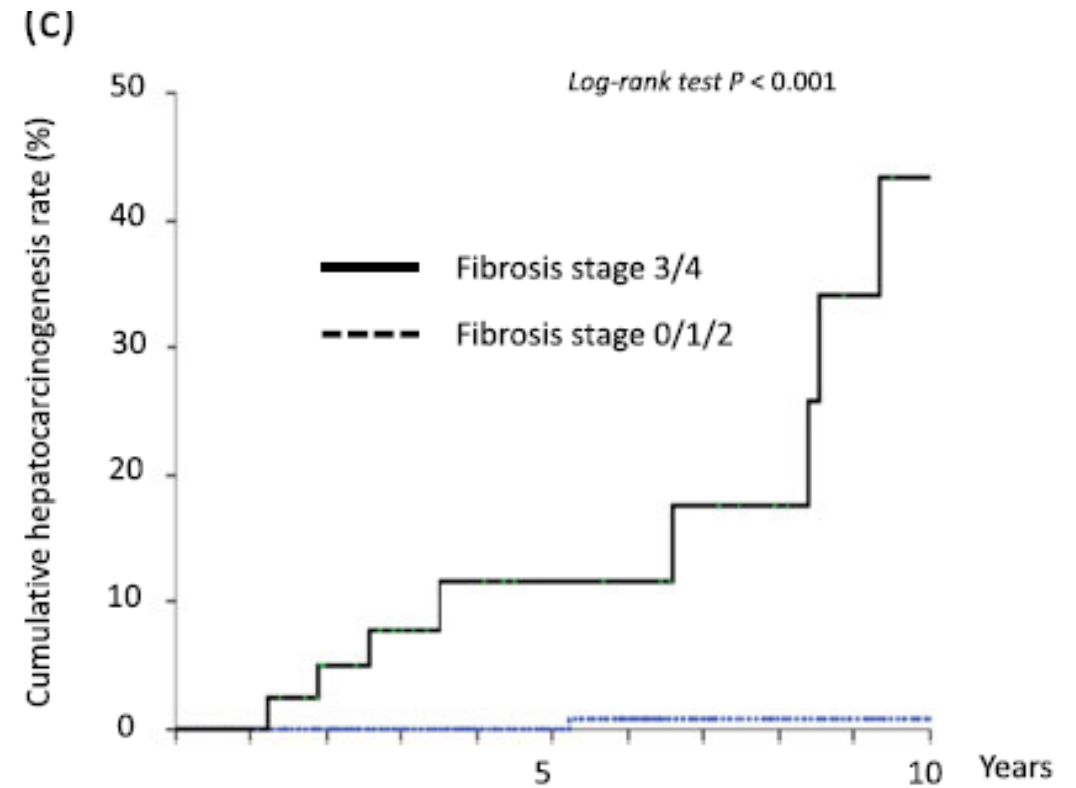
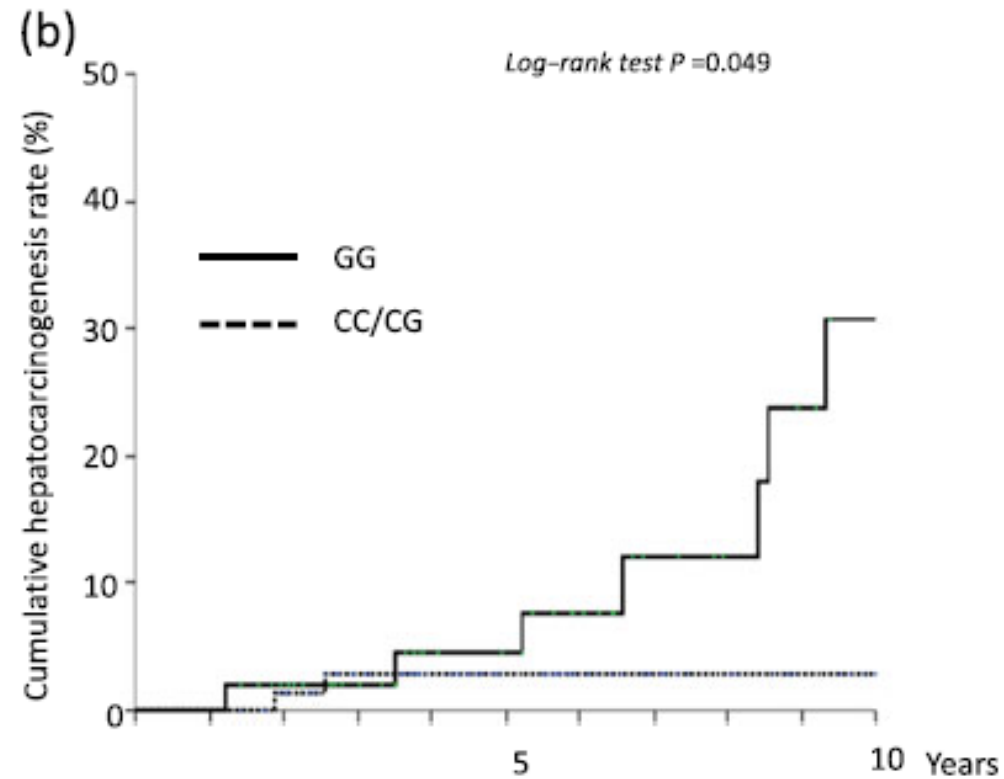
Table 4. Multivariate analysis of the effect of *PNPLA3* genotype on NAFLD-related HCC risk.

Variables	OR (95% CI)	p value
<i>PNPLA3</i> rs738409 genotype	2.26 (1.23-4.14)	0.0082
Age	1.24 (1.17-1.32)	<0.0001
Sex (Male)	11.11 (4.17-33.33)	<0.0001
BMI	0.94 (0.87-1.02)	0.148
Diabetes	2.33 (0.93-5.81)	0.070
Cirrhosis	9.37 (3.82-23.00)	<0.0001

Development of hepatocellular carcinoma in Japanese patients with biopsy-proven non-alcoholic fatty liver disease: Association between PNPLA3 genotype and hepatocarcinogenesis/fibrosis progression

Yuya Seko,¹ Yoshio Sumida,¹ Saiyu Tanaka,² Kojiro Mori,² Hiroyoshi Taketani,¹ Hiroshi Ishiba,¹ Tasuku Hara,¹ Akira Okajima,¹ Atsushi Umemura,¹ Taichiro Nishikawa,¹ Kanji Yamaguchi,¹ Michihisa Moriguchi,¹ Kazuyuki Kanemasa,² Kohichiroh Yasui,¹ Shunsuke Imai,³ Keiji Shimada³ and Yoshito Itoh¹

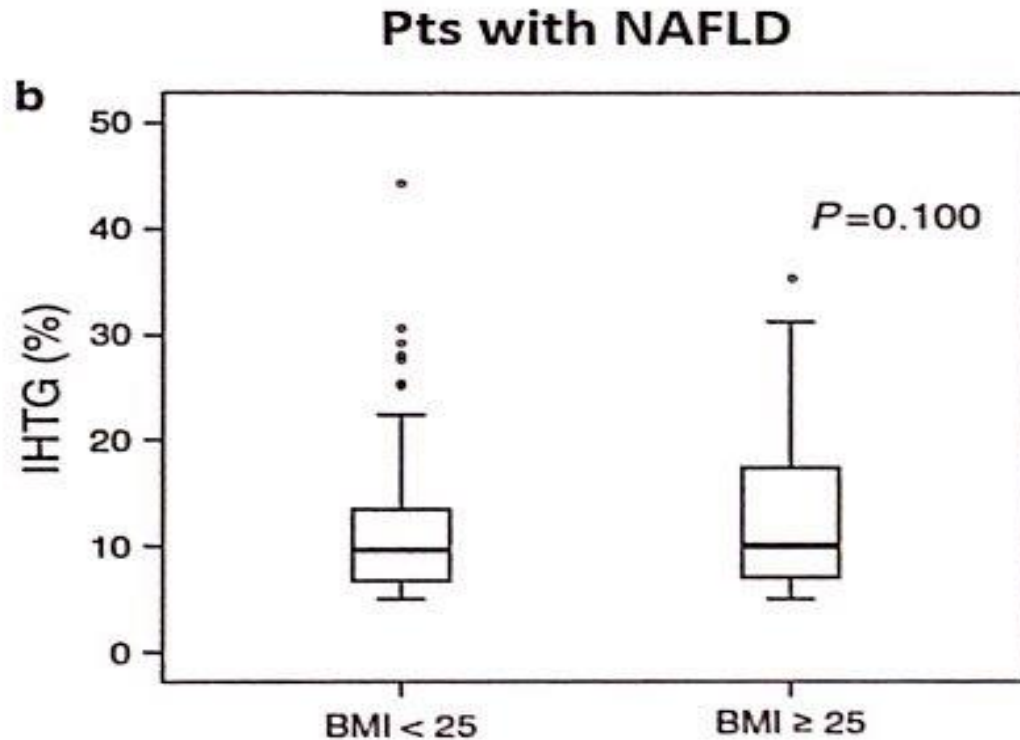




Determinants of non-obese fatty liver

911 subjects from the general Chinese population;
29% had fatty liver by MRI-PDFF

Prevalence of FL : 61% in obese; 19% in non-obese



Factors associated with FL in non-obese

Waist circumference/BMI
HOMA-IR/ HbA1c
Ferritin

PNPLA3 G allele

Non-obese FL

Obese FL

78%

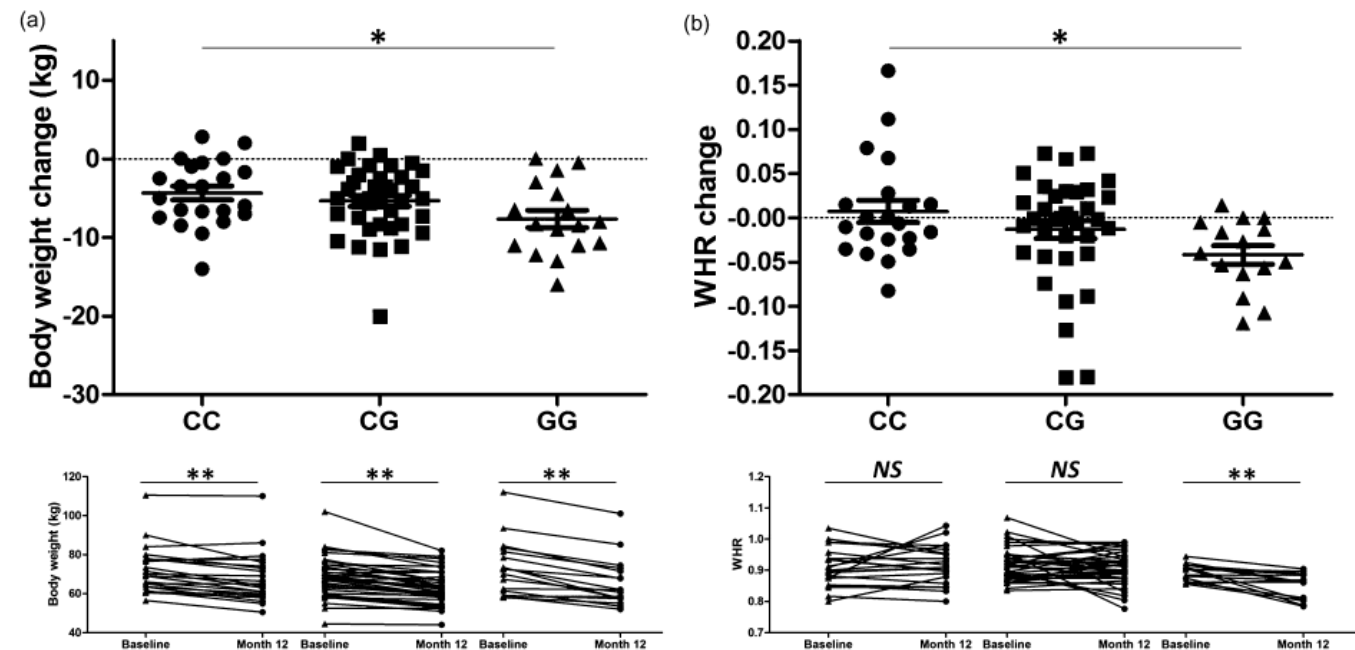
$P=0.001$

60%

***PNPLA3* gene polymorphism and response to lifestyle modification in patients with nonalcoholic fatty liver disease**

Jiayun Shen,^{*} Grace Lai-Hung Wong,^{*,†,‡} Henry Lik-Yuen Chan,^{*,†,‡} Ruth Suk-Mei Chan,^{*,§} Hoi-Yun Chan,^{*,†,‡} Winnie Chiu-Wing Chu,^{‡,¶} Bernice Ho-Ki Cheung,^{*,§} David Ka-Wai Yeung,^{**} Liz Sin Li,^{*,§} Mandy Man-Mei Sea,^{*,§} Jean Woo^{*,§} and Vincent Wai-Sun Wong^{*,†,‡}

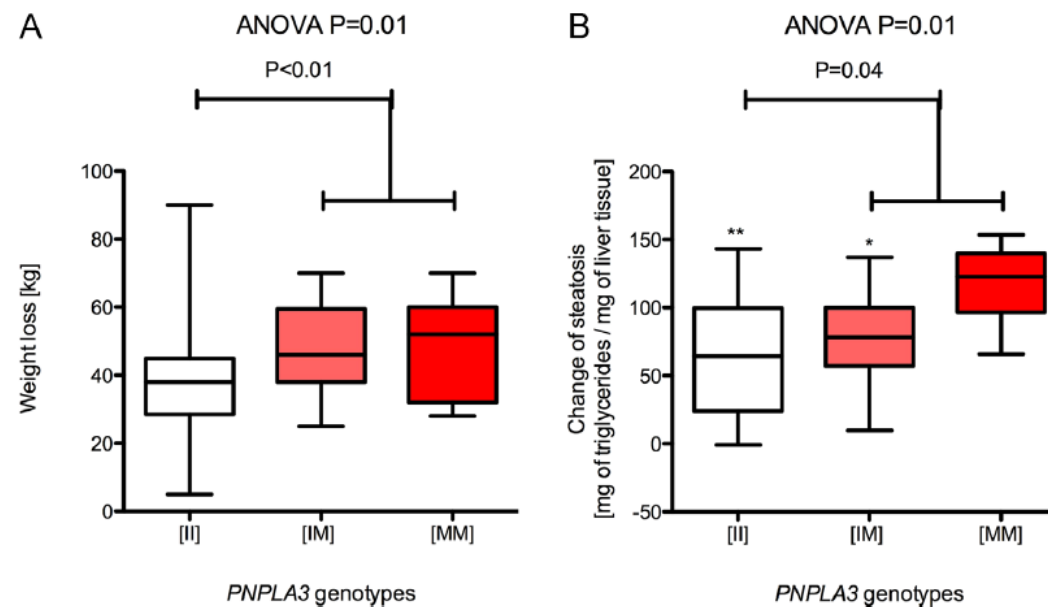
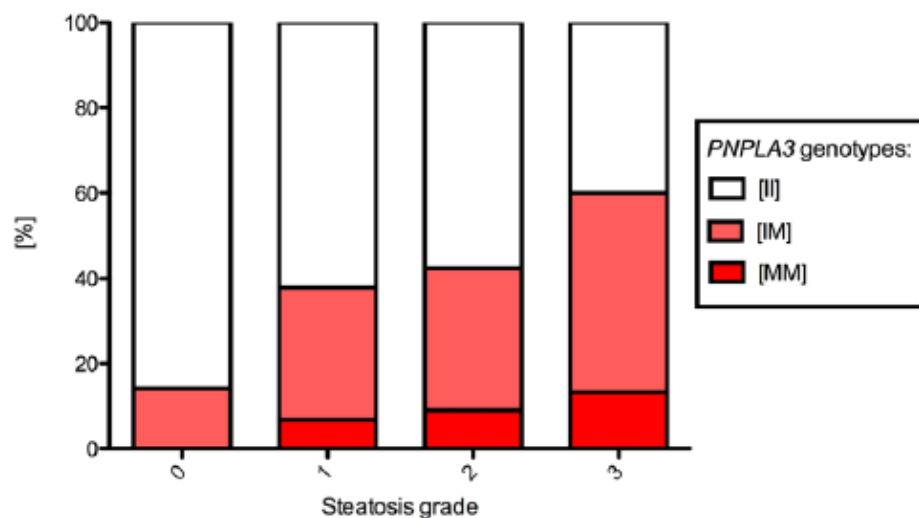
Conclusion: Although the *PNPLA3* GG confers increase risk of NAFLD, these patients are more sensitive to the beneficial effects of lifestyle modification



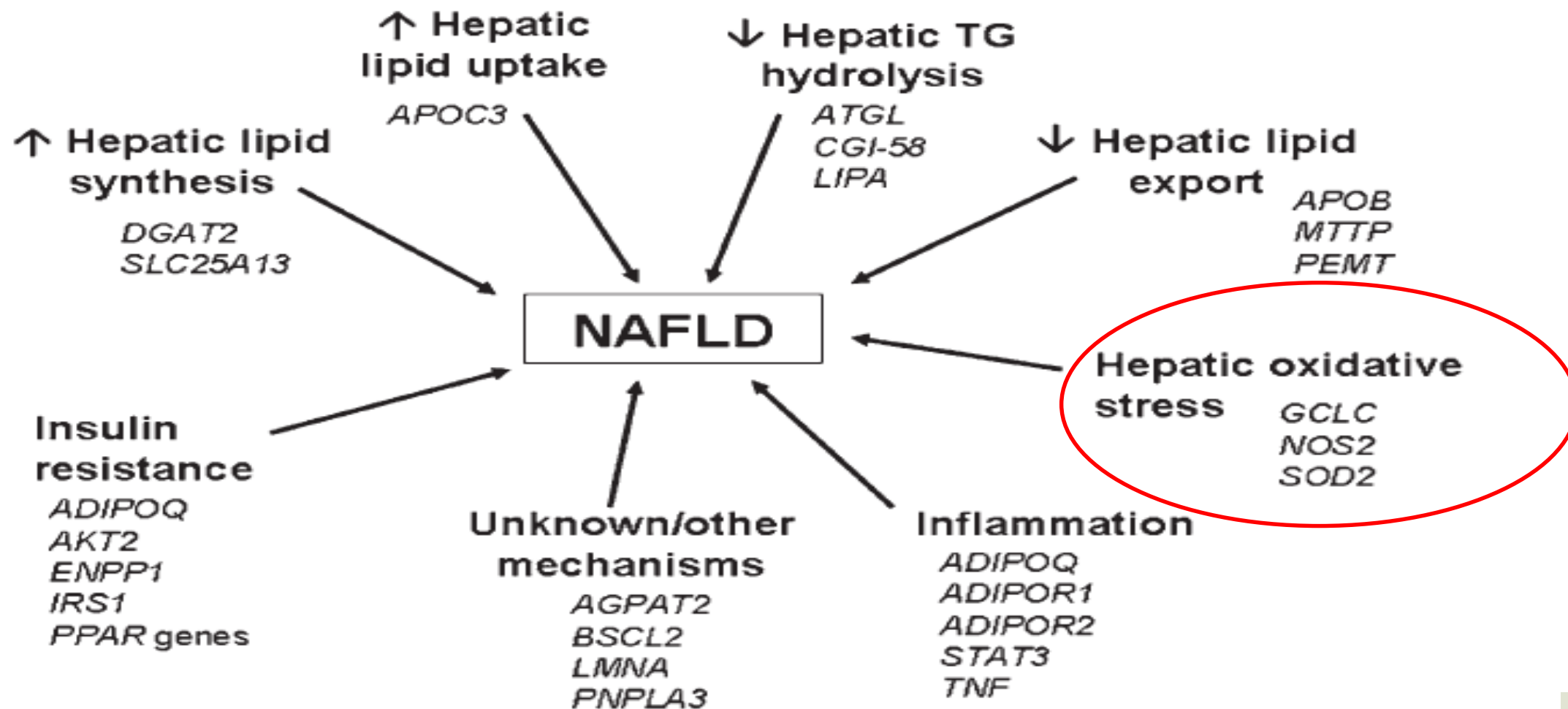
Original article

PNPLA3 p.I148M variant is associated with greater reduction of liver fat content after bariatric surgery

Marcin Krawczyk, M.D.^{a,b,*}, Raúl Jiménez-Agüero, M.D., Ph.D.^c,
José M. Alustiza, M.D., Ph.D.^{c,d}, José I. Emparanza, M.D., Ph.D.^e,
María J. Perugorria, Ph.D.^{c,f,g}, Luis Bujanda, M.D., Ph.D.^{c,f}, Frank Lammert, M.D., Ph.D.^a,
Jesús M. Banales, Ph.D.^{c,f,g,*}



Genes and Pathways involved in the Progression of NAFLD



HEPATOLOGY

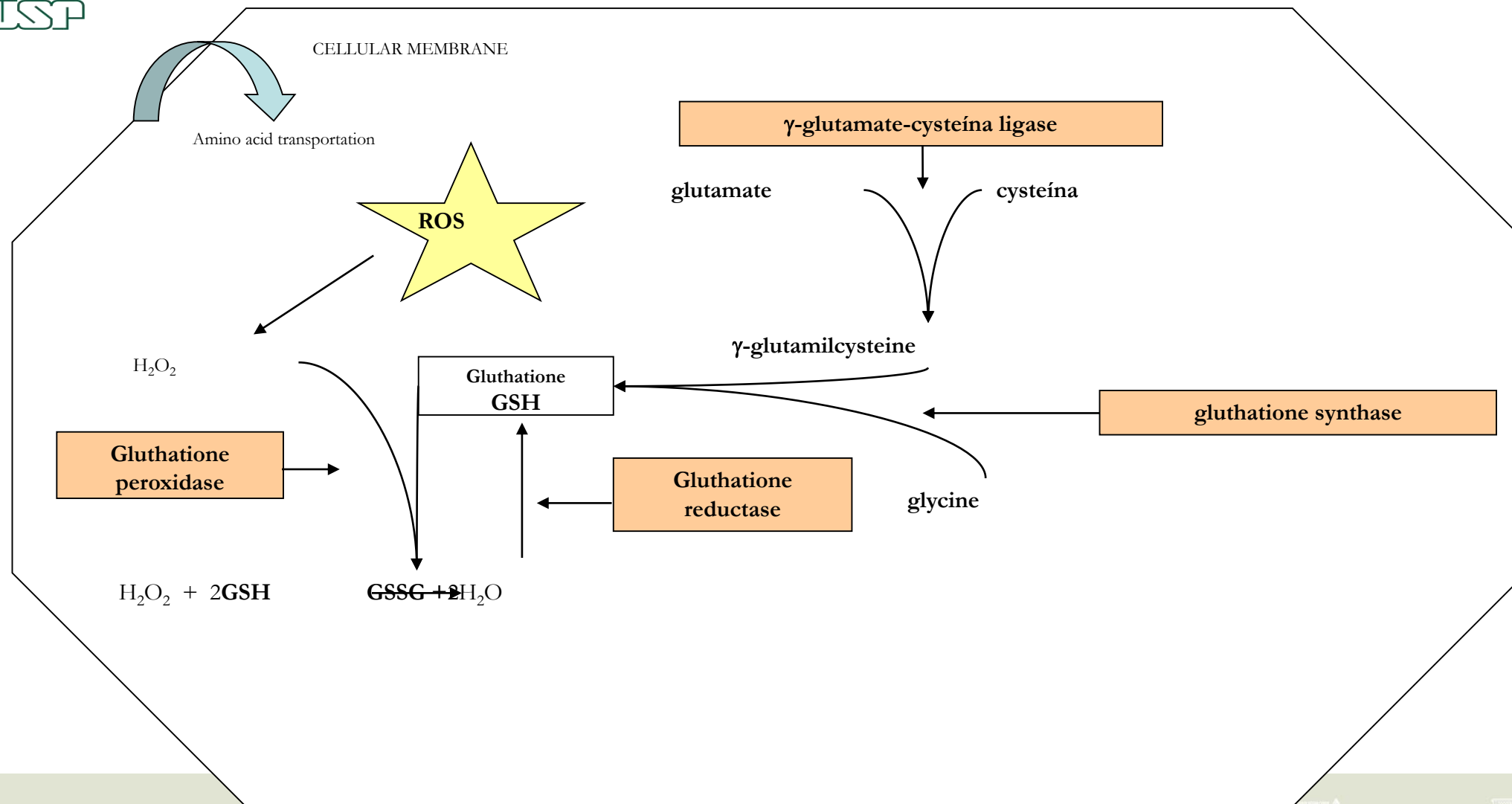
Association of polymorphisms of glutamate-cystein ligase and microsomal triglyceride transfer protein genes in non-alcoholic fatty liver disease

Claudia Pinto Marques Souza Oliveira,* José Tadeu Stefano,* Ana Mercedes Cavaleiro,[†] Maria Angela Henriques Zanella Fortes,[†] Suzana Maria Vieira,[†] Vicência Mara Rodrigues Lima,* Telma Eugenio Santos,* Virginia Nascimento Santos,[‡] Ana Lucia Farias de Azevedo Salgado,[‡] Édson Roberto Parise,[‡] Venâncio Avancini Ferreira Alves,[§] Flair José Carrilho* and Maria Lucia Corrêa-Giannella[†]

Departments of *Gastroenterology and [†]Pathology, University of Sao Paulo School of Medicine, [‡]Laboratory for Cellular and Molecular Endocrinology, Division of Endocrinology, University of Sao Paulo School of Medicine, and [§]Department of Gastroenterology, Federal University of Sao Paulo, Sao Paulo City, Sao Paulo, Brazil



Glutathione Metabolism



ROS - Espécies reativas de O_2 ; GHS - Glutathiona reduzida; GSSG - Glutathiona oxidada

Table 3: Binary logistic regression analysis assessing the independent association of the -129 C/T polymorphism of the GCLC gene and the presence of NASH.

Variables	OR	95% CI	P value
Age	1.05	1.00 – 1.11	0.066
Type 2 Diabetes	5.35	1.62 – 17.65	0.006*
Fasting glucose	1.01	0.99 – 1.04	0.362
HOMA >2.5	2.5	0.66 – 9.53	0.180
AST	1.02	0.99 – 1.05	0.182
GGT	1.00	0.99 – 1.02	0.830
At least one T allele	12.14	2.01 – 73.35	0.007*



Contents lists available at [ScienceDirect](#)

Hepatobiliary & Pancreatic Diseases International

journal homepage: www.elsevier.com/locate/hbpd

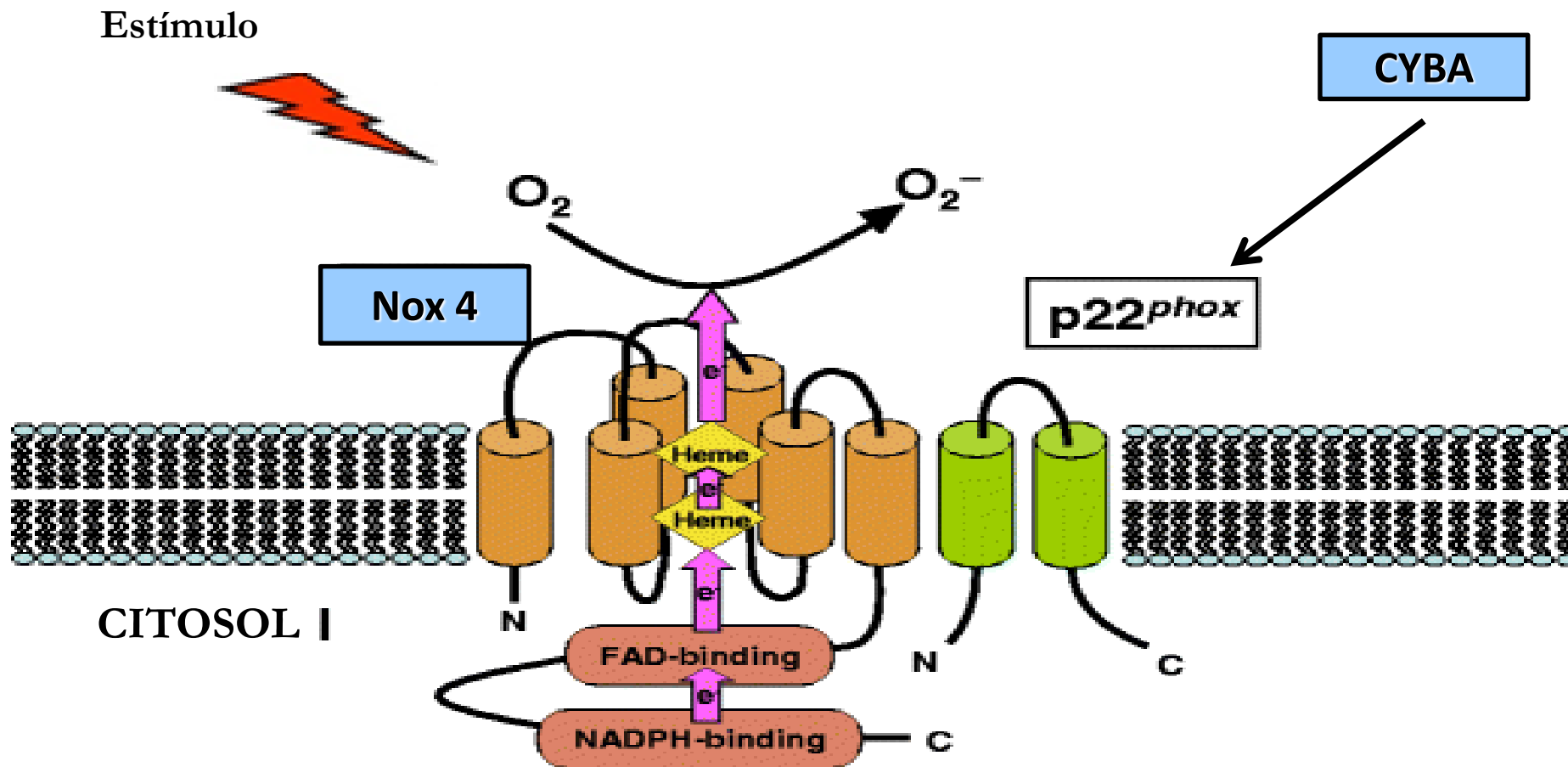
Original Article/Liver

Association between the CYBA and NOX4 genes of NADPH oxidase and its relationship with metabolic syndrome in non-alcoholic fatty liver disease in Brazilian population

Fabiola Rabelo^{a,b}, Jose Tadeu Stefano^b, Ana Mercedes Cavaleiro^c,
Rodrigo Vieira Costa Lima^{a,b}, Daniel Ferraz de Campos Mazo^b, Flair Jose Carrilho^{a,b},
Maria Lúcia Correa-Giannella^{c,d}, Claudia P. Oliveira^{a,b,*}



NADPH oxidase 4 System



Polymorphisms in CYBA gene according NAFL or NASH

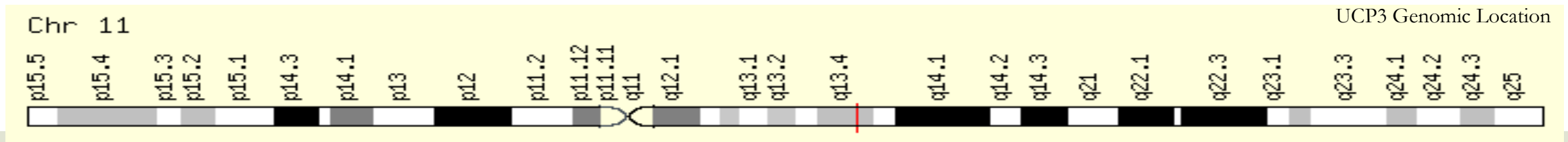
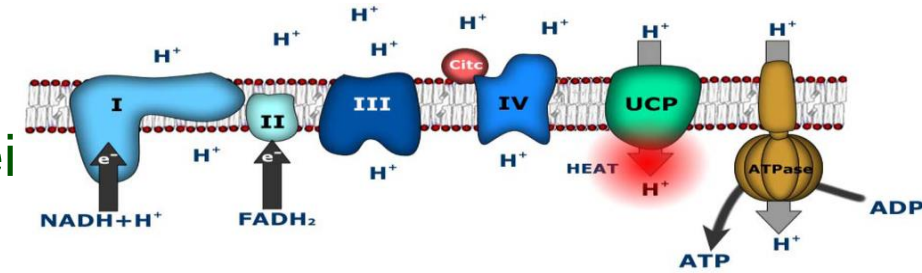
	NAFL	NASH	OR (IC 95%)	p-value
N	(n=47)	(n=141)		
*CYBA				
TT	0.907	0.822		
TA	0.093	0.156	5.06 (1.03 – 24.82)	0.0455
AA	0.000	0.022		

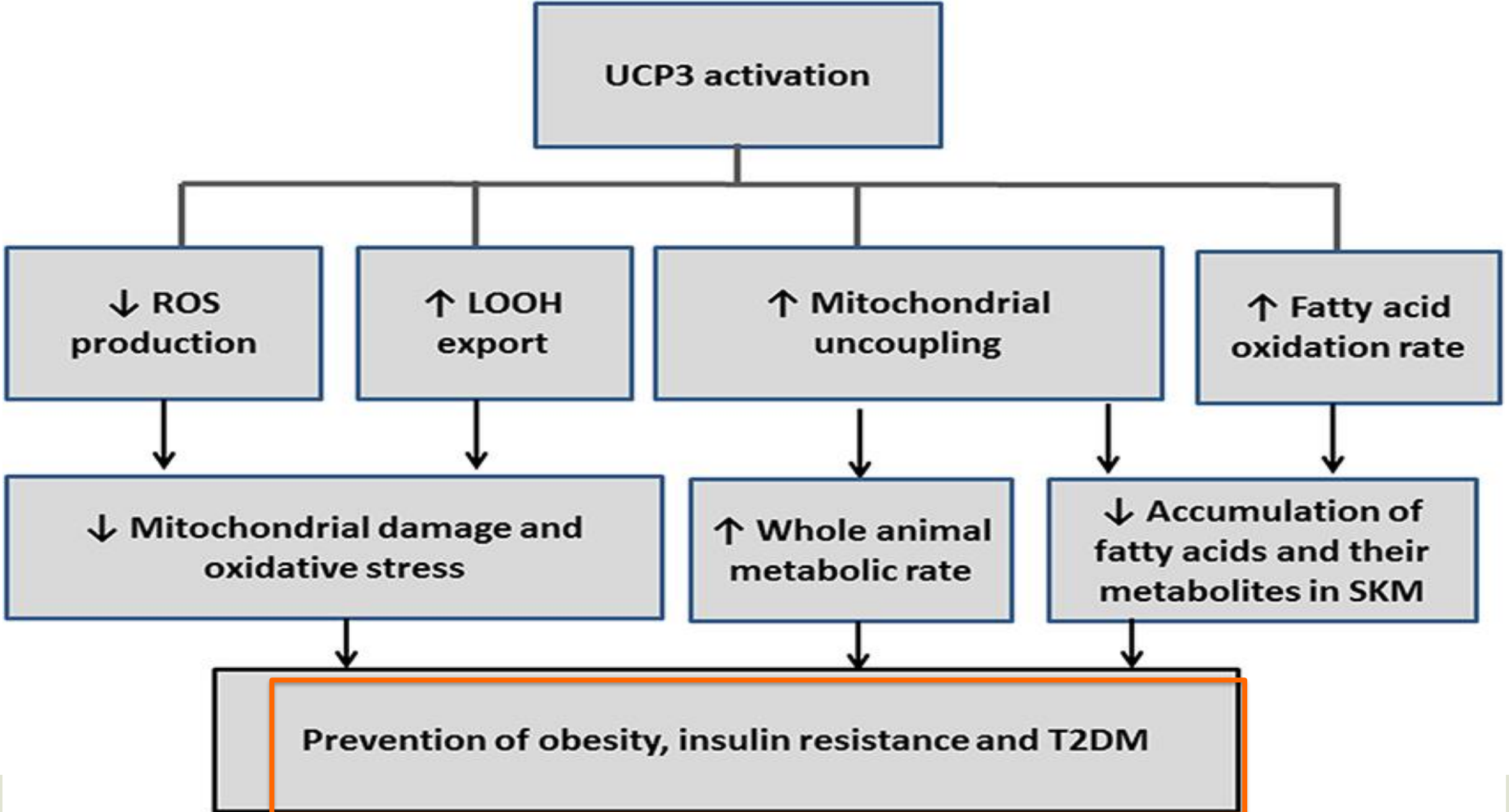


- **Mitochondrial Uncoupling Proteins (UCPs)**

- UCP1, UCP2, UCP3, UCP4, UCP5

- **Gene UCP3**
- Mitochondrial anionic carrier protein
- Located not cromossomo 11q13
- Expressão highly seletiva no skeletal muscle
- **Highest thermogenic site in humans**
- Alvo atrativo → studies of body weight regulation
- 57% homology with UCP1
- 73% homology with UCP2





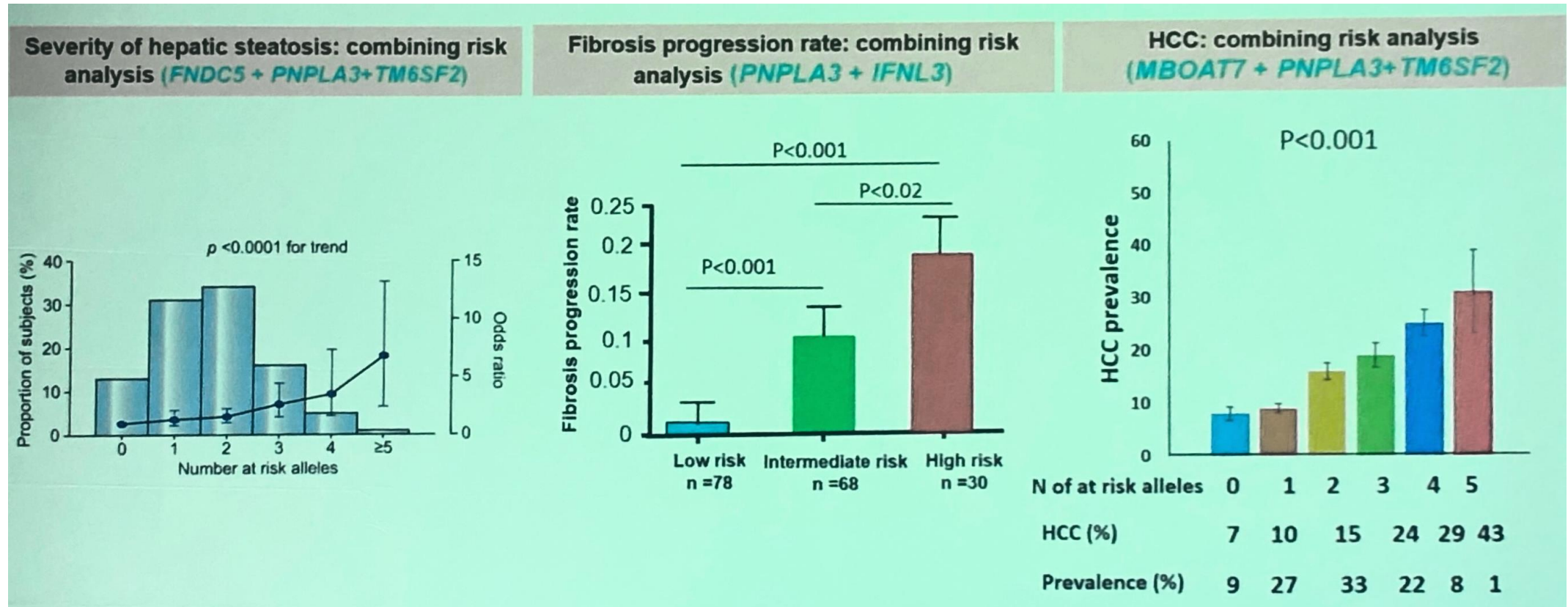
UCP3 polymorphisms protect against NASH and metabolic syndrome among Brazilian NAFLD patients

Table 4. Haplotype analysis of UCP3 gene polymorphisms according to the presence of NASH

Haplotype	Steatosis (n=20)	NASH (n=138)	OR (95% CI) for NASH	<i>P value</i>
CAA	0.058	0.016	0.27 (0.05 – 1.40)	0.09
CAG	0.250	0.348	1.60 (0.74 – 3.48)	0.23
CGA	0.091	0.136	1.57 (0.49 – 5.00)	0.44
CGG	0.075	0.052	0.67 (0.18 – 2.52)	0.55
TAA	0.036	0.000	0.01 (0.00 – 0.12)	0.002*
TAG	0.367	0.395	1.12 (0.56 – 2.27)	0.74
TGG	0.124	0.053	0.39 (1.13 – 1.20)	0.09

Polygenic scores and NAFLD

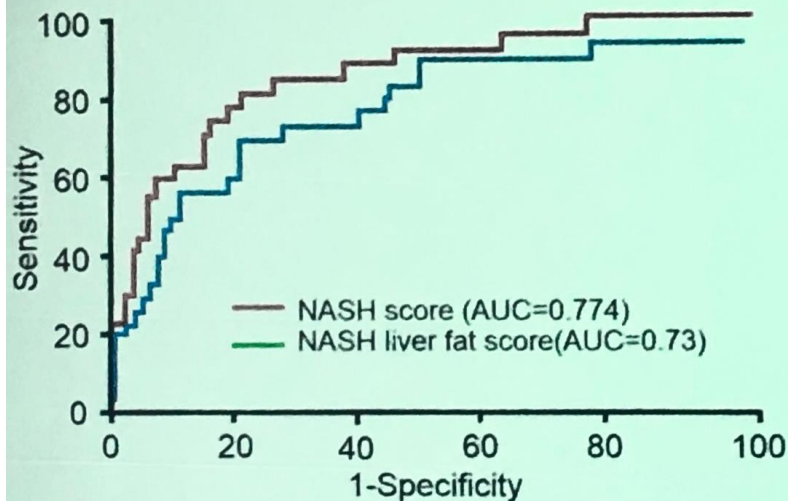
Eslam M, Sevilla, NASH EASL meeting 2019



Polygenic scores and NAFLD

NASH score: *PNPLA3* based score

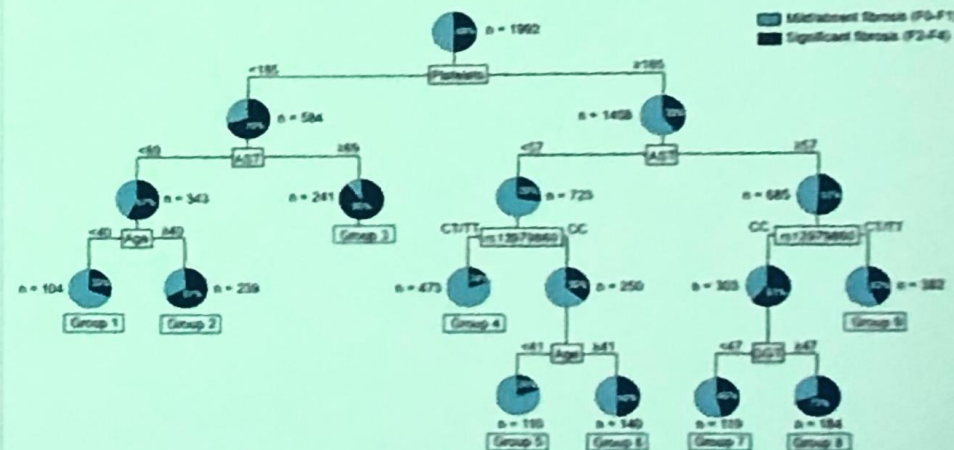
$-3.05 + 0.562 \times \text{PNPLA3 genotype (CC = 1/GC = 2/GG = 3)} - 0.0092 \times \text{fS-insulin (mU/L)} + 0.0023 \times \text{AST (IU/L)} + 0.0019 \times (\text{fS-insulin} \times \text{AST})$



Fibrogene: *IFNL3* based score

IFNL3 genotype, in addition to age, GGT, AST and platelets

NPV >0.96 for cirrhosis



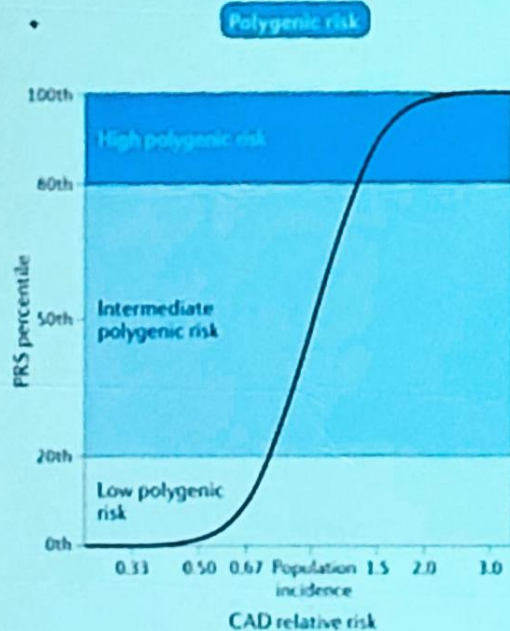
Genome-wide polygenic score (GPS)

Prediction of CAD

Initial dataset: 120,280 participants in the UK Biobank (phase 1).

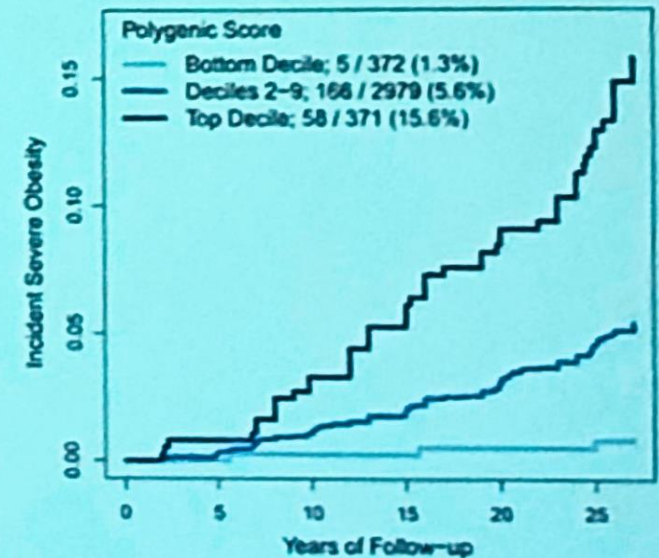
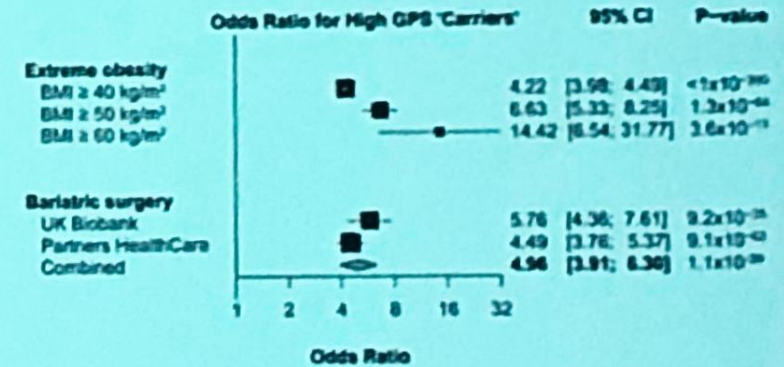
Testing dataset: 288,978 participants in the UK Biobank (phase 2).

Disease	Discovery GWAS (n)	Prevalence in validation dataset	Prevalence in testing dataset	Polymorphisms in GPS	Tuning parameter	AUC (95% CI) in validation dataset	AUC (95% CI) in testing dataset
CAD	60,801 cases; 123,504 controls	3.963/120,280 (3.4%)	8.676/288,978 (3.0%)	6,630,150	LDpred ($r^2 = 0.001$)	0.81 (0.80-0.81)	0.81 (0.81-0.81)



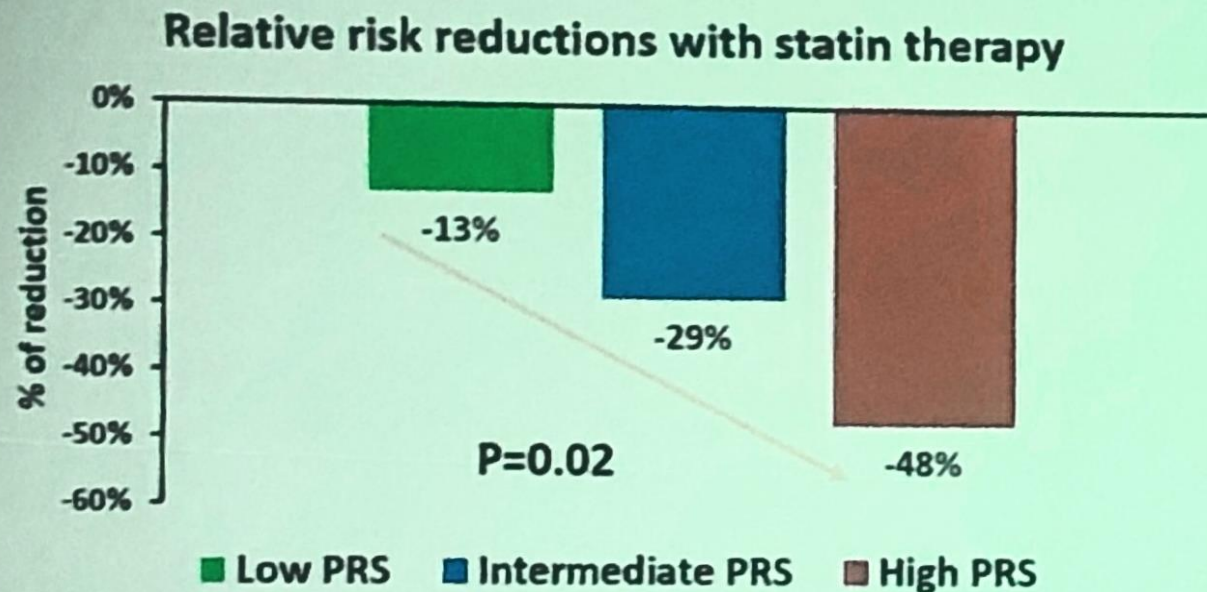
Prediction of Obesity

New polygenic predictor comprised of 2.1 million common variants
300,000 individuals ranging from middle age to birth



The utility of polygenic risk scores

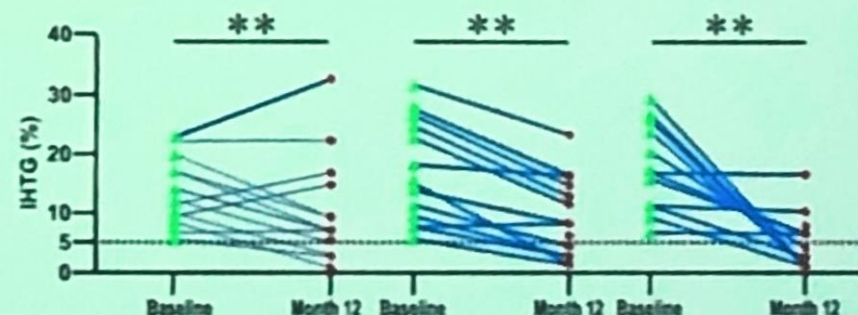
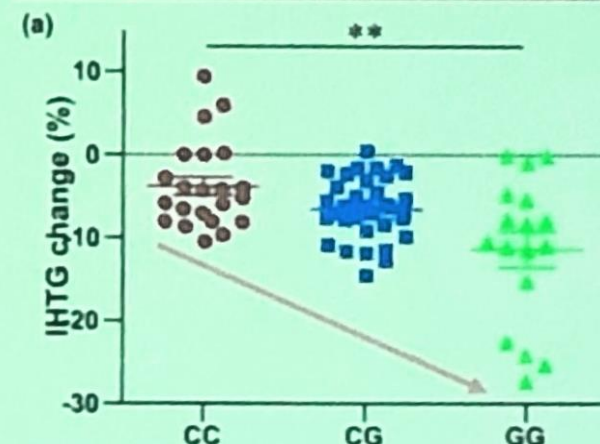
PRS-informed therapeutic intervention



- 48 421 individuals including from four randomised controlled trials of both primary prevention (JUPITER and ASCOT) and secondary prevention (CARE and PROVE IT-TIMI 22) with statin therapy
- PRS (27 genetic variants with incident or recurrent coronary heart disease)

Mega JL, et al. Lancet. 2015 Jun 6;385(9984):2264-2271.

PNPLA3 and Lifestyle Intervention

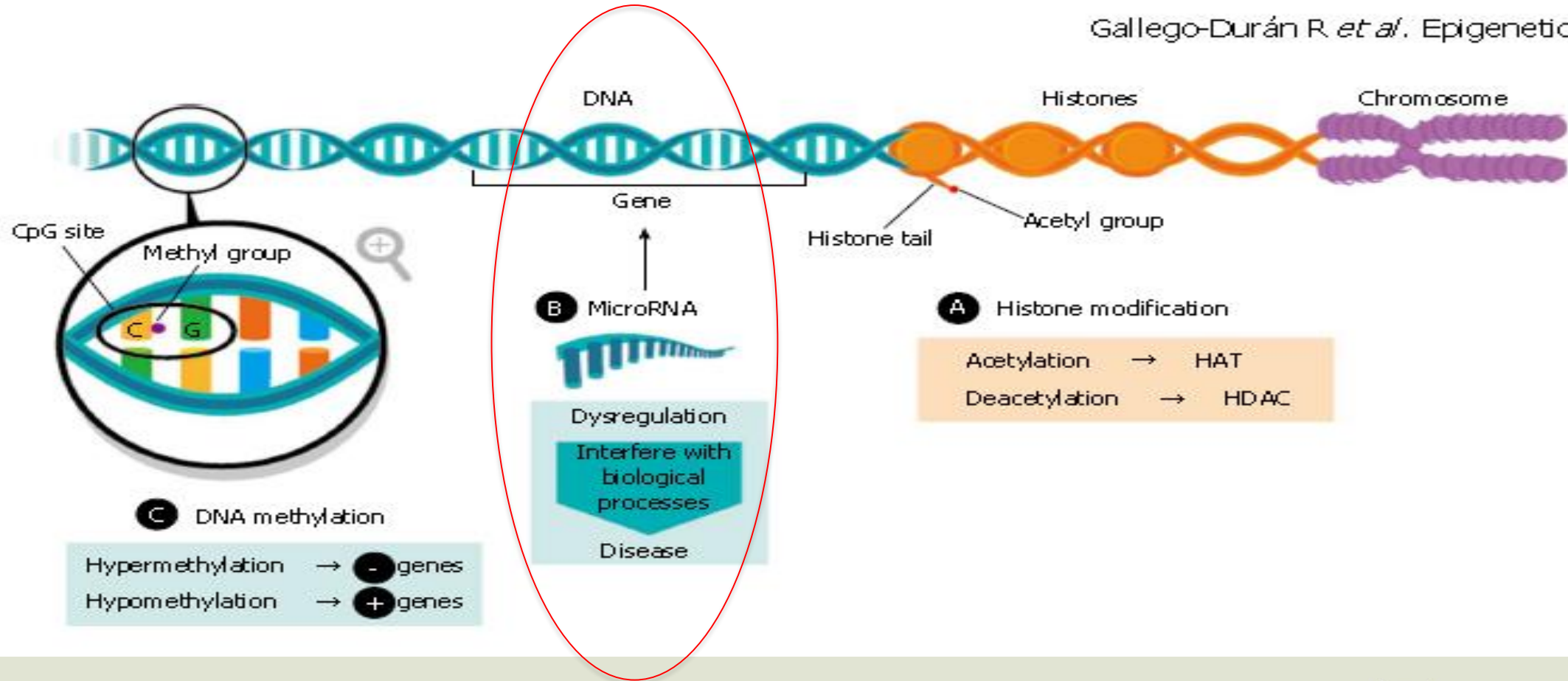


IHTG change: CC: $3.7 \pm 5.2\%$, CG: $6.5 \pm 3.6\%$ and GG: $11.3 \pm 8.8\%$ ($p=0.002$)

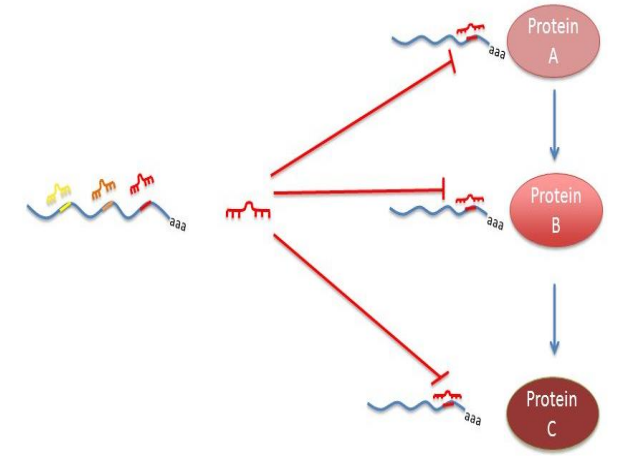
Shen et al, J Gastroenterol Hepatol. 2015;30(1):139-46.

MicroRNAs - Epigenetic

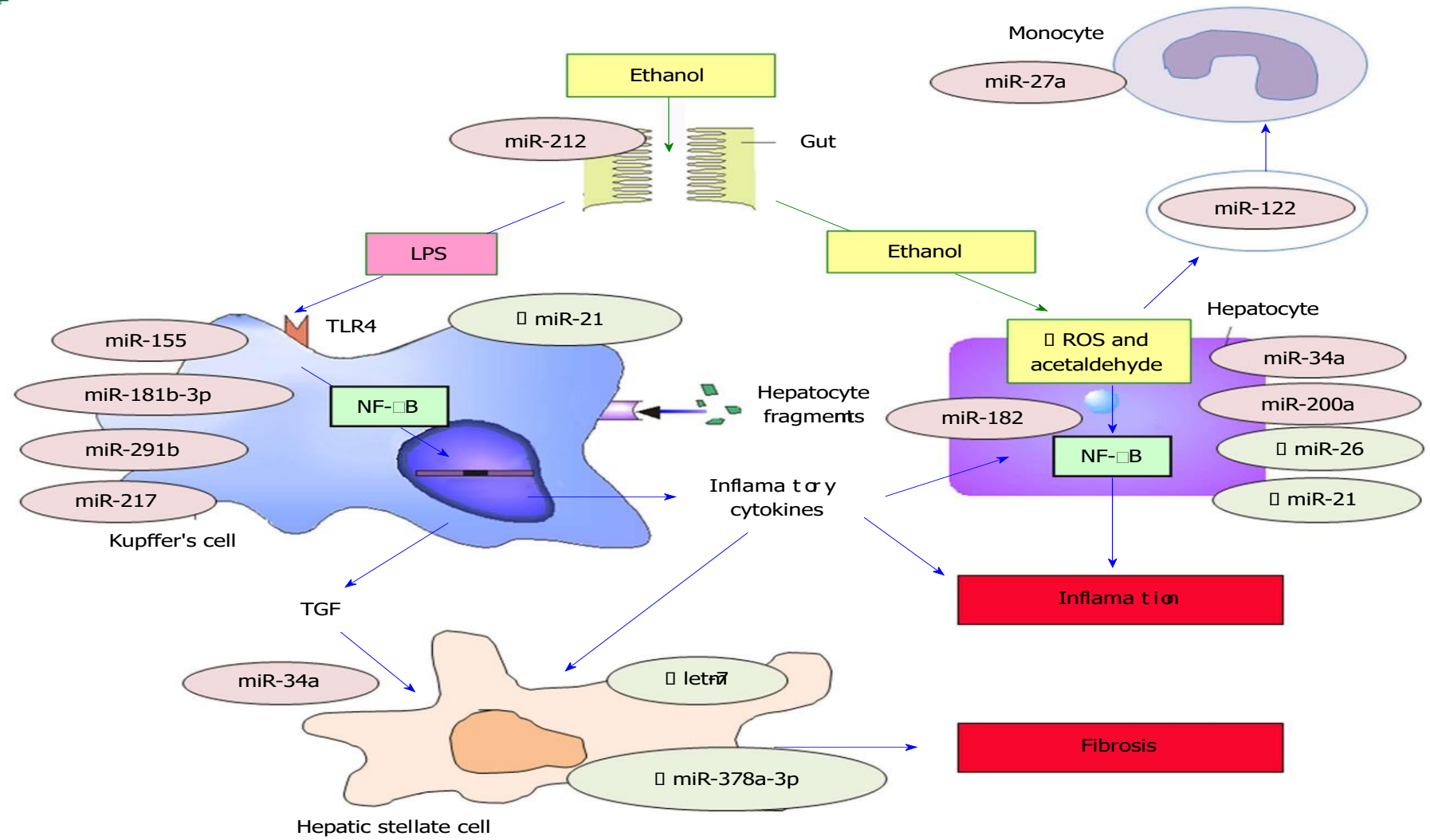
Gallego-Durán R. *et al.* Epigenetics in NAFLD



Introduction - MicroRNAs



- Non-coding RNA of 18-25 nucleotides
- They can interfere in all aspects of cellular activity
- They are stable in bodily fluids and are protected from degradation by RNAases.
- The serum levels of some miRNAs are altered under certain pathophysiological conditions, which makes them excellent biomarkers;



ASSOCIATION OF SERUM miRNAS AND POLYMORPHISM IN THE GENE PNPLA3 IN THE EVALUATION OF THE DHGNA PROGRAM: FROM THE ESTEATOS TO THE HEPATOCELLULAR CARCINOMA

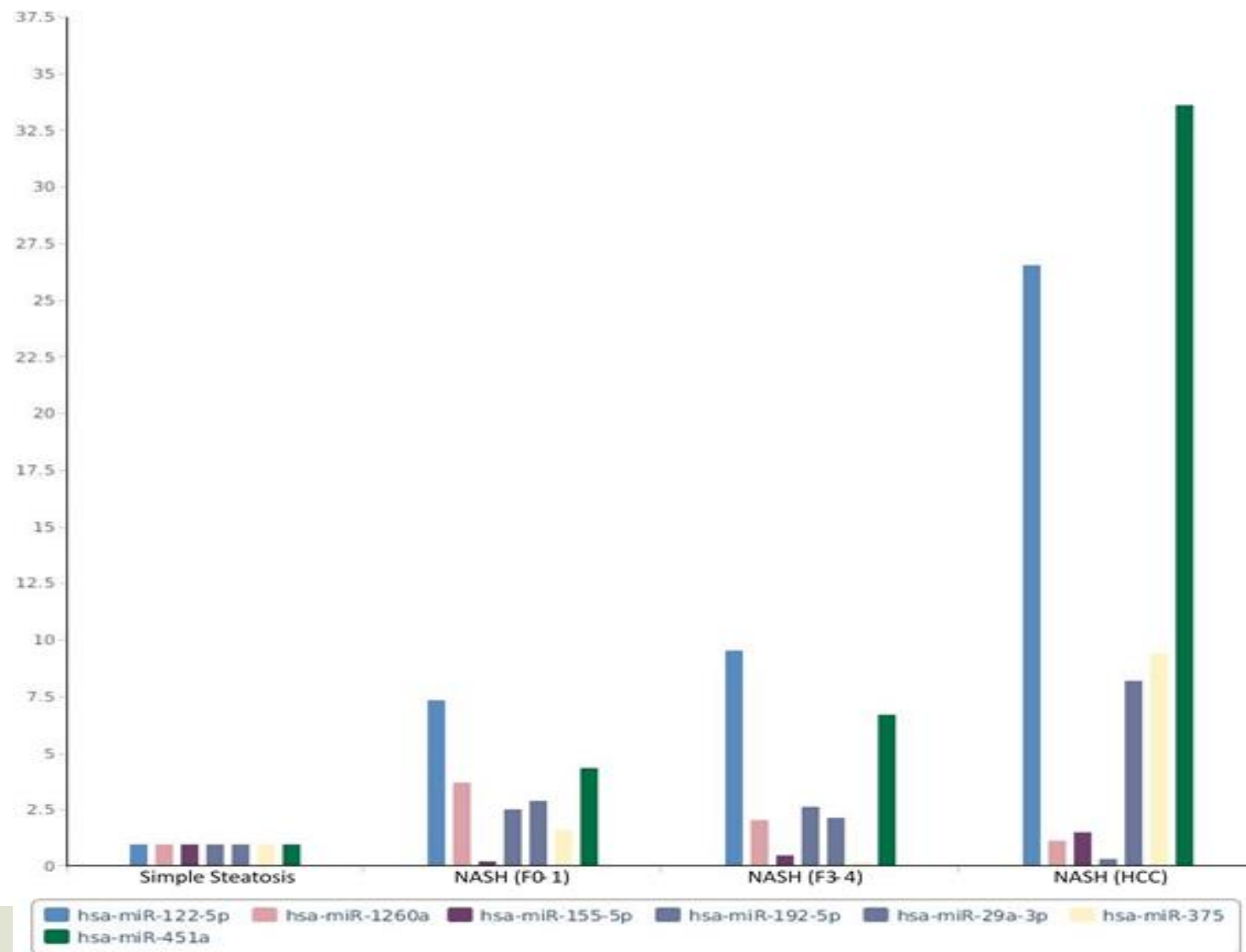
Fernanda Malta¹ (femalta@yahoo.com), Ana Paula M. Salles¹ (anamoreirasalles@gmail.com), Rodrigo V. Lima¹ (rodrigovieiracostalima@gmail.com), José Tadeu Stefano¹ (jstefano@usp.br), Venancio A. F. Alves² (venancio@uol.com.br), Flair José Carrilho¹ (fjcarril@usp.br), João Renato R. Pinho¹ (jrrpinho@usp.br), Claudia P. Oliveira¹ (cpm@usp.br)

¹ Departamento de Gastroenterologia (LIM-07) e ²Patologia (LIM-14), Faculdade de Medicina da Universidade de São, São Paulo, Brasil



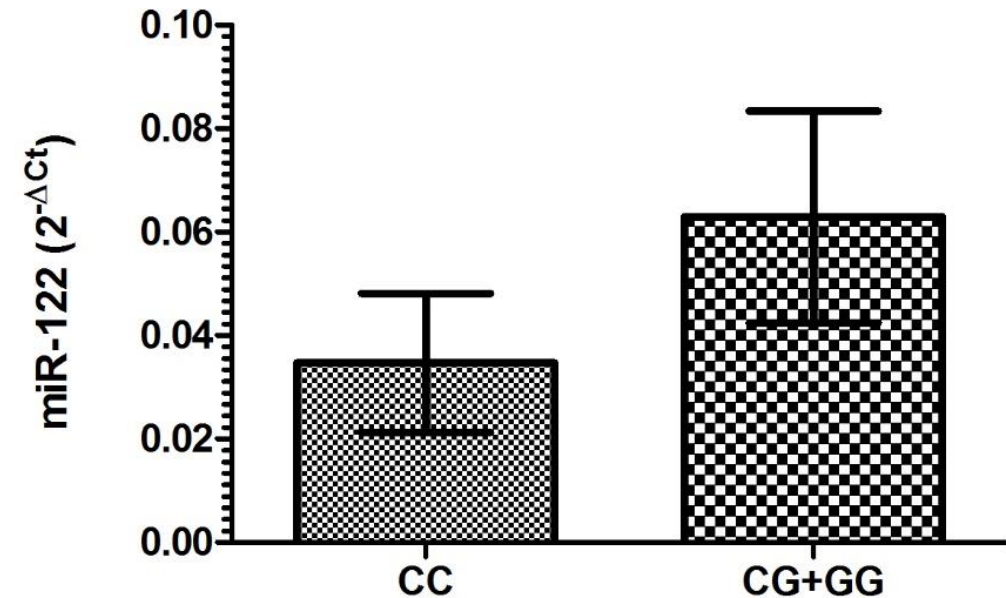
NASH patients presented a differentiated expression of the following miRNA

- ✓ miR-29a-3p
- ✓ miR-122-5p
- ✓ miR-155-5p
- ✓ miR-192-5p
- ✓ miR-375
- ✓ miR-451a
- ✓ miR-1260a



Patients with genotype CG + GG PNPLA3 presented a higher serum level of miR-122-5p in relation to patients with CC ($p = 0.023$)

- Modulates the expression of genes involved in the hepatic metabolism of lipoproteins and cholesterol;



Precision medicine: the near future

Clinical-based categories

Obese non diabetics



Diabetics



Obese and diabetics



Integrated data

Phenotyping
liver vs CVD
biomarkers
imaging
Physiology
Fat distribution
IR, beta cell
Genetic susceptibility
Metabolomics profile
Blood vs tissue
Gut microbiota
Behaviour

Data driven categories

Cluster 1



Cluster 2



Cluster 3



Cluster 4



Cluster 5



Follow
up and
Therapy

Take Home Messages

Genetic variation found in less than 1% of the population is called polymorphisms

The polymorphism of PNPLA3 increases the risk of developing NAFLD, NASH, higher levels of fibrosis and hepatocellular carcinoma. If embragp can respond to weight loss

Other polymorphisms related to oxidative stress increase the risk of developing NASH, fibrosis and metabolic syndrome

Polygenetic risk score are likely to be very useful diagnostic tool to predict phenotype



Take Home Messages

The microRNAs are epigenetic alterations, which can interfere in all aspects of cellular activity

They are stable in bodily fluids and are protected from degradation by RNAases.

The serum levels of some miRNAs are altered under certain pathophysiological conditions

Excellent biomarkers



University of São Paulo School of Medicine Brazil



Thank you!

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