Erasmus MC
Universitair Medisch Centrum Rotterdam

Genetische diagnostiek in de hepatologie

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21 juni 2017

Dutch Liver Week

Disclosures

None

Statement

Genetics play a pivotal role in hepatology, now and/or in the future

a. Agree

b. Somewhat agree

c. Neither agree nor disagree

d. Somewhat disagree

e. Disagree

Contents

- Basic principles of genetics
- Genetic studies
- Genetics in liver disease

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- **Basic principles of genetics**
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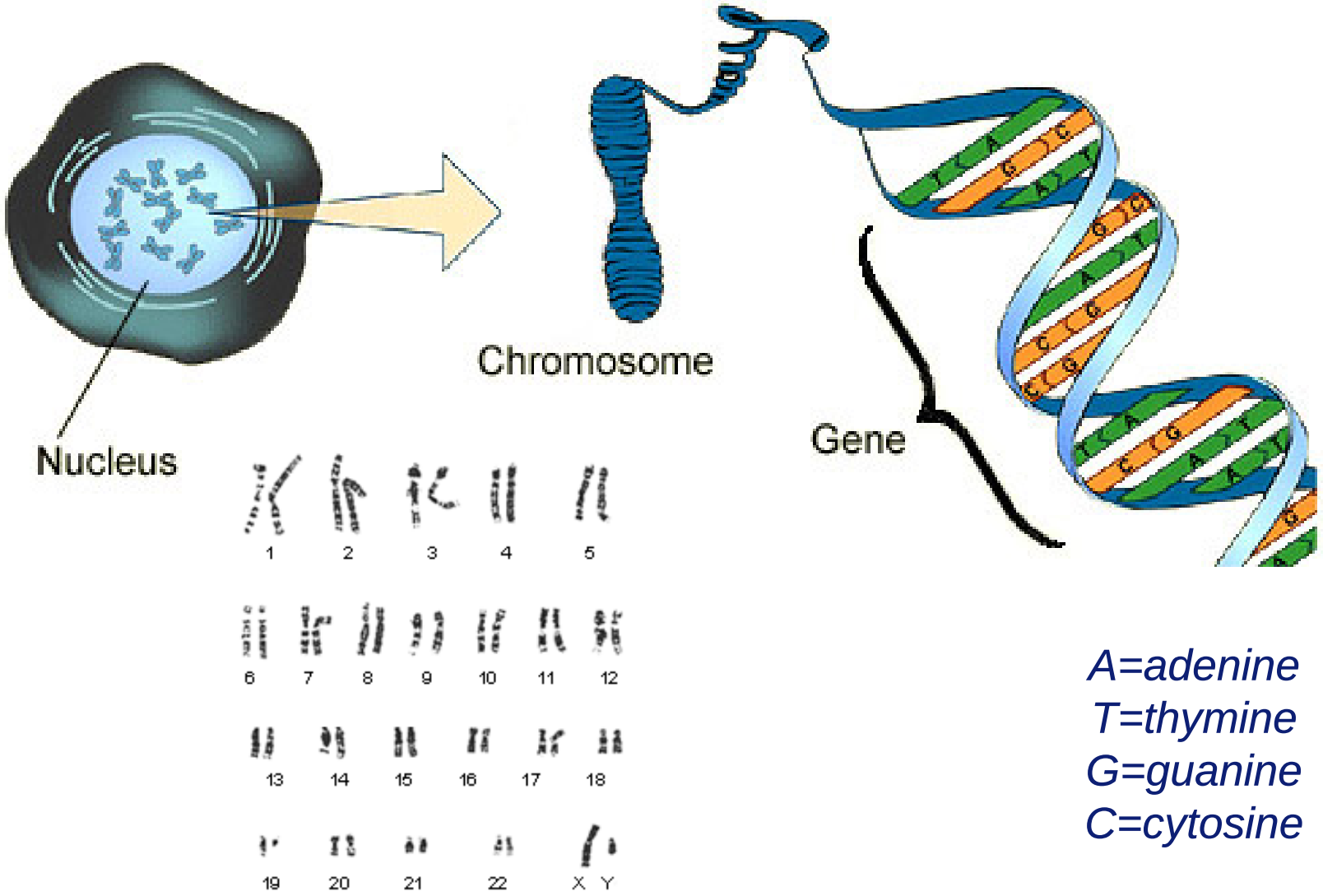
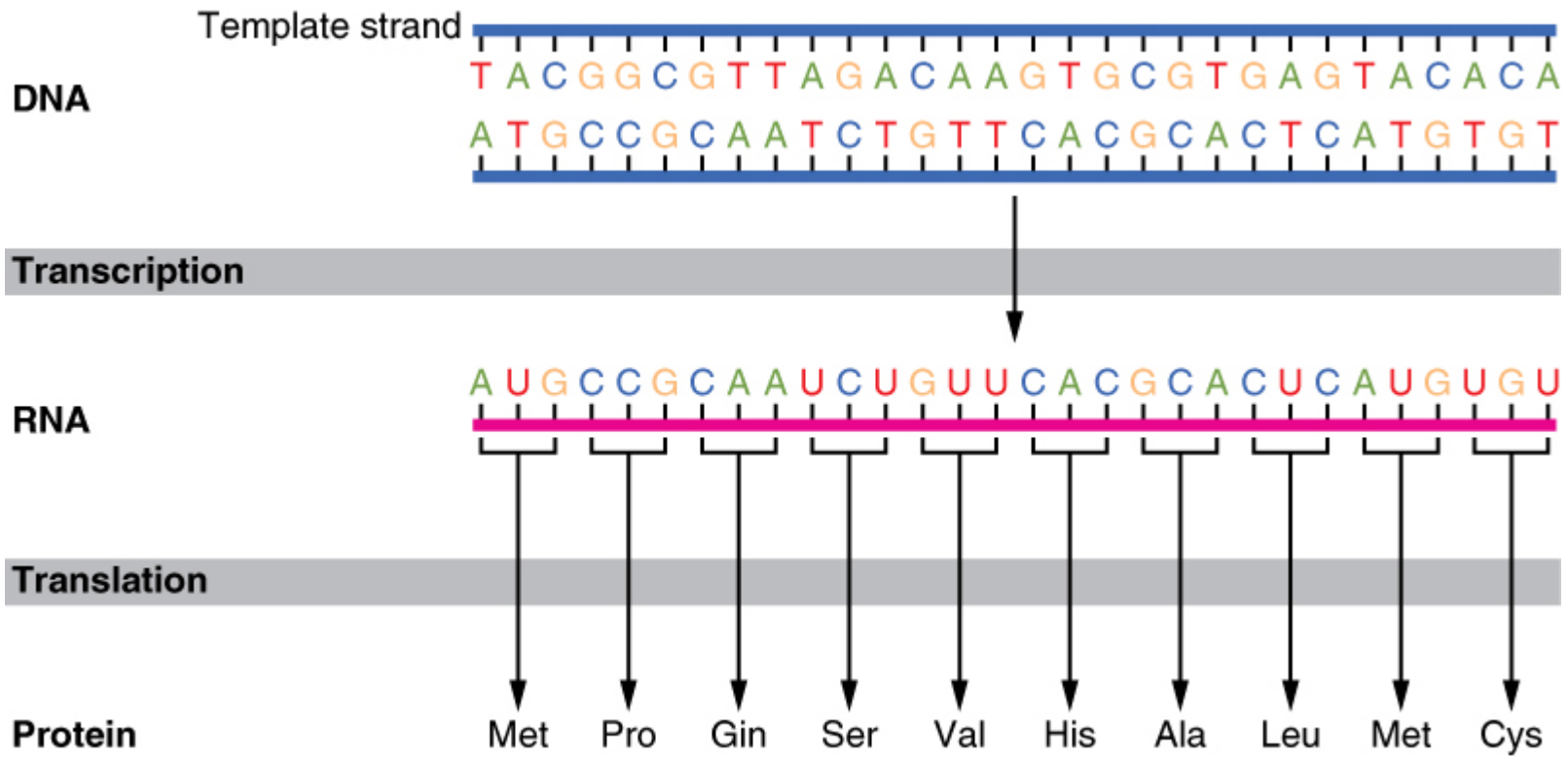
From Cell to Chromosome to DNA

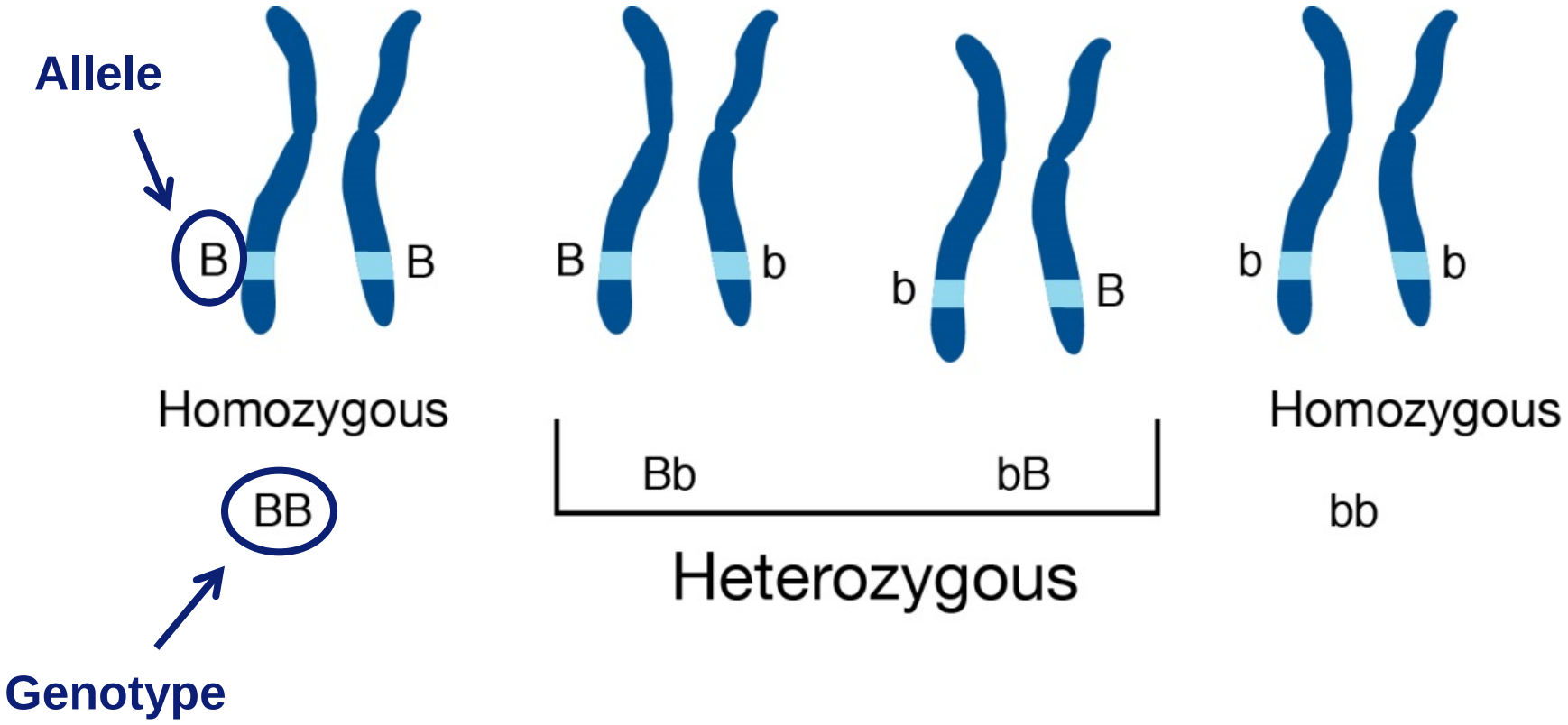


A=adenine
T=thymine
G=guanine
C=cytosine

From DNA to RNA to Protein

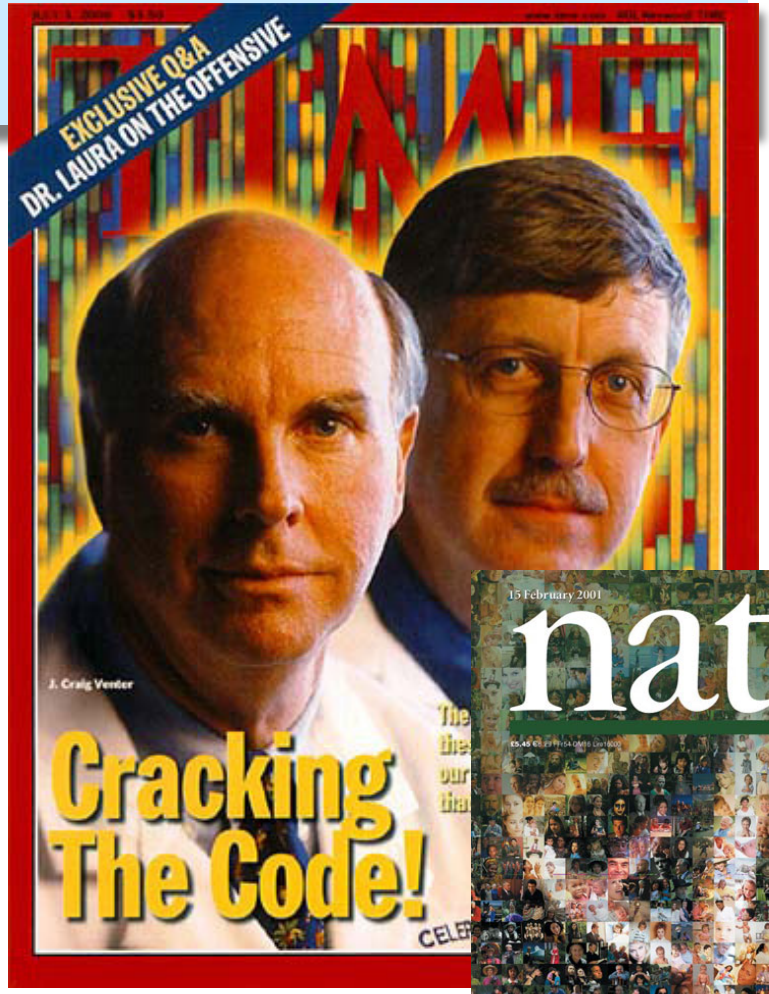


Alleles and genotypes



Human Genome Project

- “Human genome” presented in 2000
- Sequencing was finished in 2004
- Function is still not fully understood



What do we know?

- ~20,000 protein-coding genes = exome
  - 1.0-1.5% genome
  - 85% variability influencing disease

- Majority of non-coding DNA probably has function
  - Regulation gene expression
  - Organization chromosome architecture
  - Controlling epigenetic inheritance

Question

To what extent is your DNA sequence similar to that of the person sitting next to you?

a. 25%
b. 50%
c. 75%
d. 90%
e. 99.9%

What do we know?

Difference in genome between human individuals is ~0.1%

What do we know?

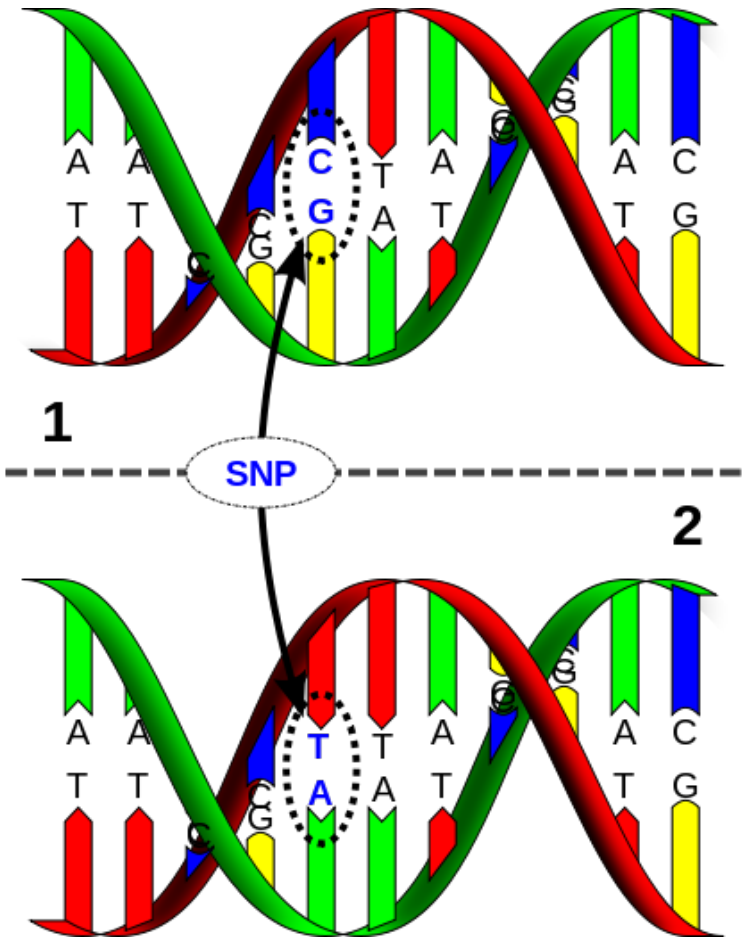
Difference in genome between human individuals is ~0.1%

Note:

- Difference between humans and chimpanzees/bonobos ~4%

Single Nucleotide Polymorphisms

- Single nucleotide polymorphisms (SNPs) are frequently occuring mutations in a single nucleotide
- Over 325 million SNPs in genome[1]
- Rs-number
- SNPs account for ~90% of all genetic variation
  - Mostly innocent



[1]NCBI. dbSNP Build 150 for human. Feb 2017.

What would happen without polymorphisms…

Contents

- Basic principles of genetics
- **Genetic studies**
- Genetics in liver disease

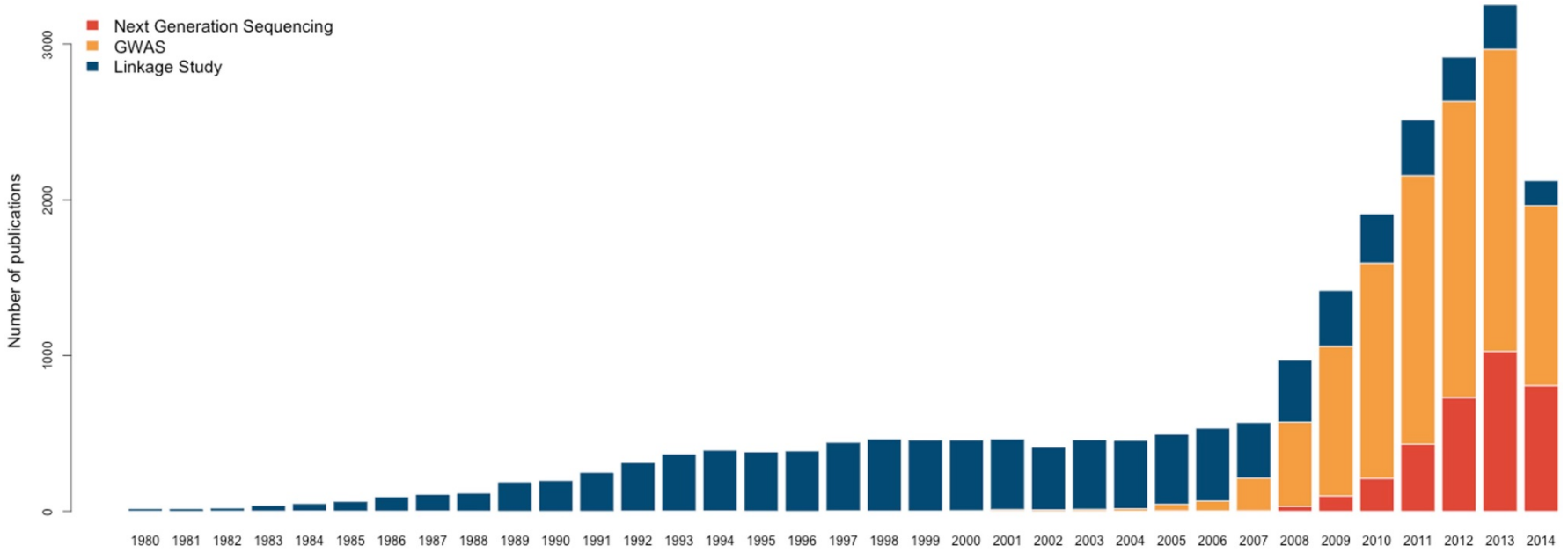
Why study genetics?

- Understand pathophysiology of disease
- Finding new potential disease treatments
- Understand how DNA variation contributes to variation in
  - Risk of disease
    - Personalized medicine
  - Response to treatment
    - Pharmacogenetics

Genetic studies

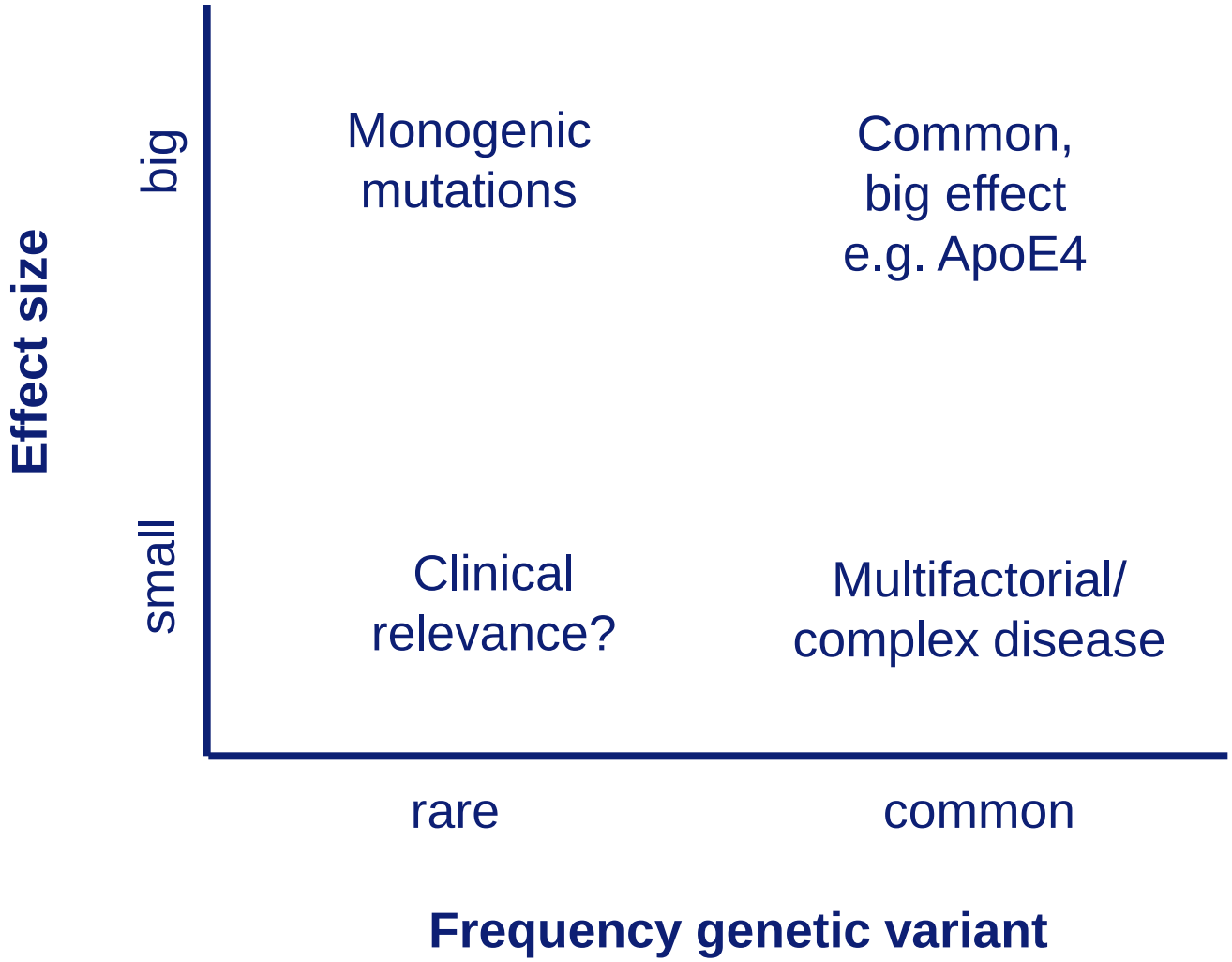
- Linkage studies
- Genetic association studies
- Genome wide association studies (GWAS)
- Next generation sequencing (NGS)
  - Selected genes
  - Exome sequencing
  - Whole genome sequencing

Increasing number of genetic studies over time



Wang, Front. Genet. 2015

Gene variants and effect on diseases



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Genetic discoveries in hepatology

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Year	Landmark genetic publication
1972	MHC associations in autoimmune hepatitis
1979	MHC associations in primary biliary cirrhosis
1982	MHC associations in primary sclerosing cholangitis
1989	*CFTR* in cystic fibrosis
1992/95	*UGT1A1* in Gilbert and Crigler-Najjar syndromes
1993	*ATP7B* in Wilson disease
1996	*HFE* in haemochromatosis (type 1)
1997	*ABCC2* in Dubin-Johnson syndrome
1997	*JAG1* in Alagille syndrome
1998	*ATP8B1* in PFIC (type 1)
1998	*ABCB11* in PFIC (type 2)
1998	*ABCB4* in PFIC (type 3)
2001	*SLC40A1* (ferroportin) in haemochromatosis (type 4)
2002	*TFR2* in haemochromatosis (type 3)
2003	*HAMP* in haemochromatosis (type 2B)
2004	*HJV* in haemochromatosis (type 2A)
2006	*NOTCH2* in Alagille syndrome
2007	*ABCG8* in gallstone disease (GWAS)
2008	*PNPLA3* in non-alcoholic fatty liver disease (GWAS)
2008	First GWAS on genetic factors associated with plasma liver enzyme activities
2009	First GWAS in primary biliary cirrhosis
2009	MHC associations in flucloxacillin DILI (GWAS)
2009	MHC associations in HBV clearance (GWAS)
2009	*IL28B* in HCV treatment response (GWAS)
2009	*IL28B* in spontaneous HCV clearance (GWAS)
2009	Genetic modifiers for *CFTR*-associated liver disease
2010	*PNPLA3* in alcoholic liver disease
2010	First GWAS in primary sclerosing cholangitis
2010	*ITPA* in ribavirin-induced anaemia (GWAS)
2010	First GWAS in hepatitis B-related hepatocellular carcinoma (GWAS)
2011	MHC associations in amoxicillin-clavulanate DILI (GWAS)
2012	*OATP1B1* and *OATP1B3* in Rotor syndrome
2012	First GWAS on liver fibrosis in chronic HCV infection
2014	First GWAS in autoimmune hepatitis
2014	*TJP2* in PFIC (type 4)
2014	MHC associations in hepatitis B vaccine response (GWAS)

Karlsen, J Hep, 2015

Hereditary hemochromatosis (HH)

- HFE gene identified in 1996
- C282Y/C282Y mutation in ~80% of patients with HH

- Other mutations in HFE gene
  - H63D/H63D
  - C282Y/H63D
  - C282Y/wild type
  - H63D/wild type

- ~7% of patients with HH has mutation in other genes

Feder, Nat Genet, 1996, EASL guideline HFE hemochromatosis, J Hep, 2010, Hanson, A J Epidemiol, 2001

EASL guideline hemochromatosis

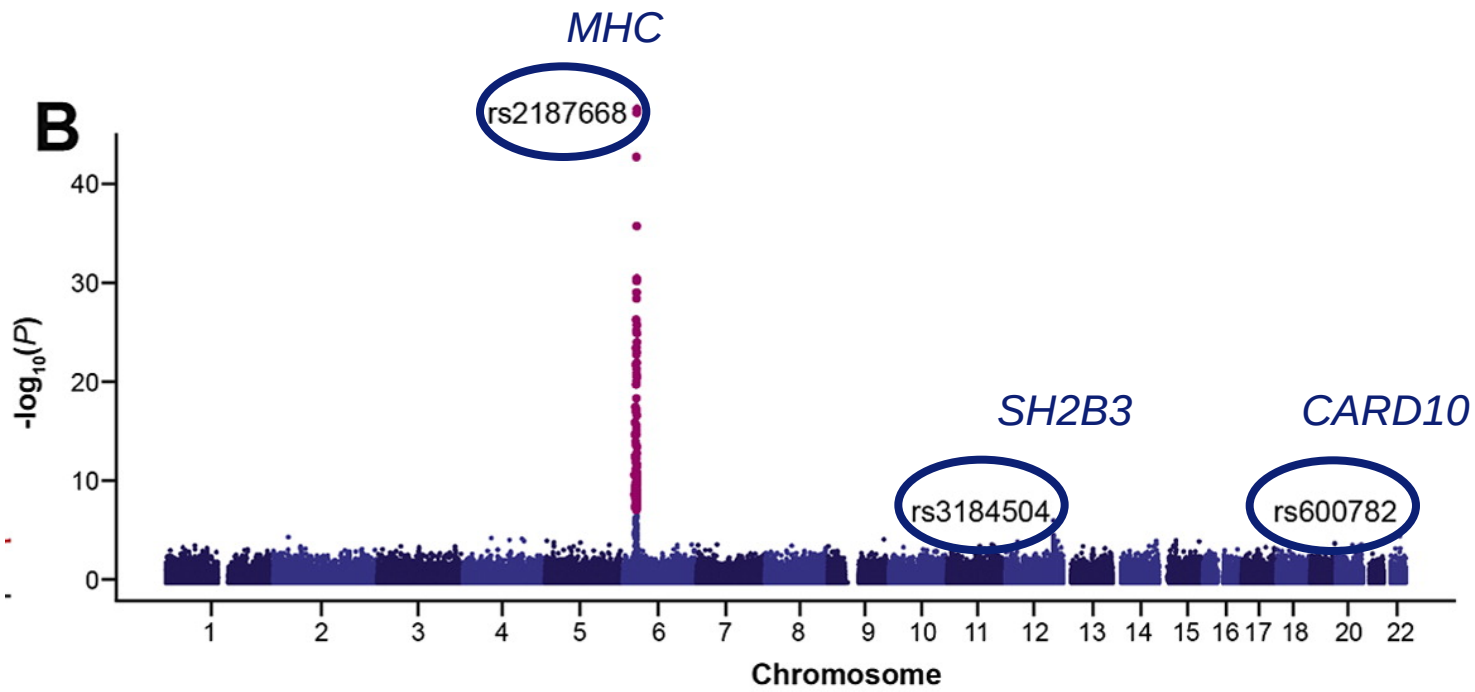
- *HFE testing for the C282Y and H63D polymorphism should be carried out in all patients with otherwise unexplained increased serum ferritin and increased transferrin saturation (1 B).*

- *In C282Y homozygote patients with increased iron stores, liver biopsy is no longer necessary to diagnose hemochromatosis. Liver biopsy could be offered to C282Y homozygous patients with serum ferritin above 1000 mg/L, elevated AST, hepatomegaly, or age over 40 years (1 C).*

EASL guideline HFE hemochromatosis, J Hep, 2010

GWAS in autoimmune hepatitis type 1

- 649 Dutch patients and 13,436 controls
- Replication in 451 German patients and 4103 controls



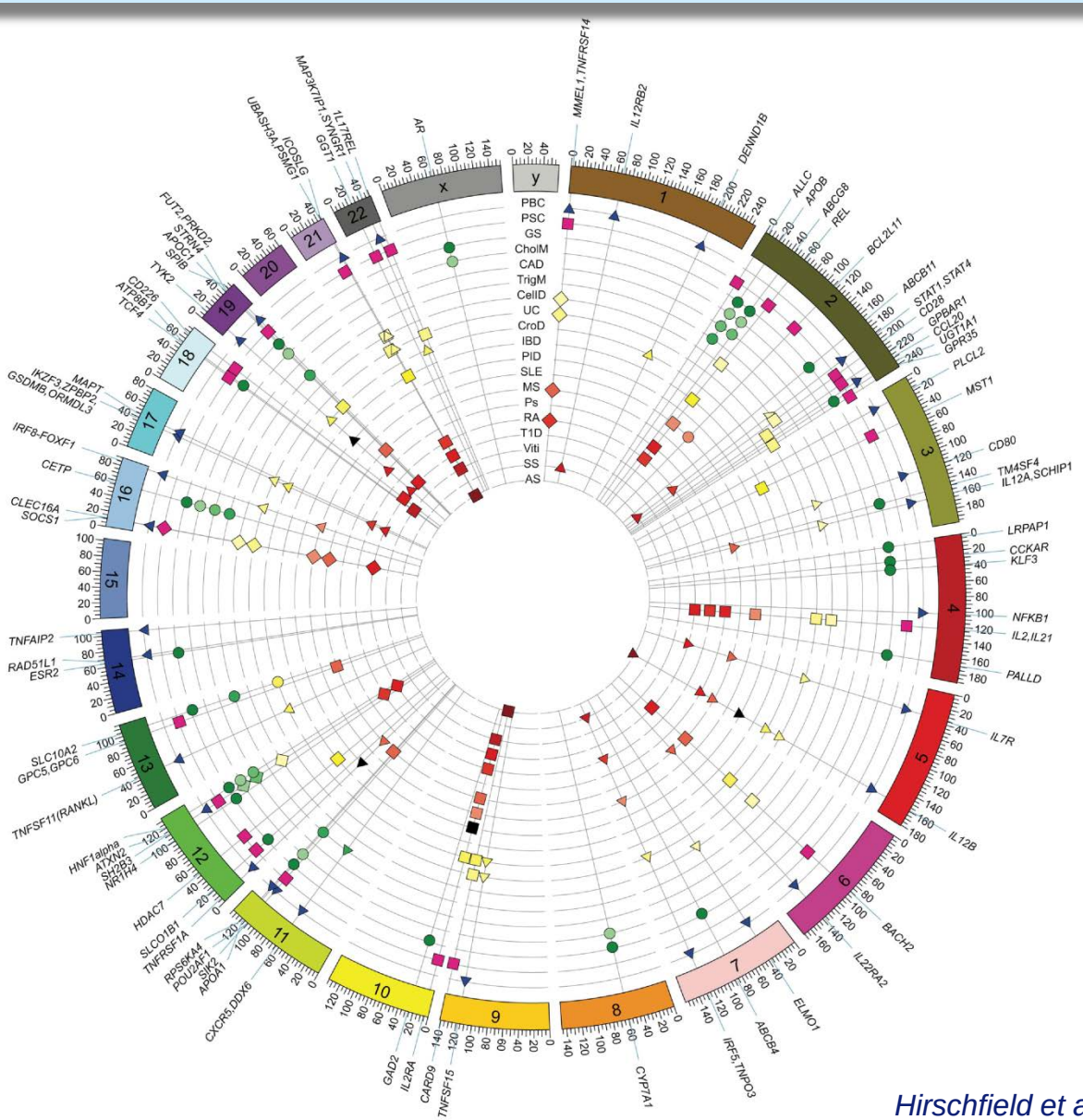
De Boer et al. Gastroenterology 2014

Genetics in cholestatic liver diseases

- GWAS have detected multiple risk alleles in *MHC (6p21)* in immune-related conditions
- Over 30 years ago, candidate gene variants in *MHC (6p21)* were already shown to be linked to PSC, PBC and AIH
  - Confirmed in GWAS in PBC and PSC
- Most new loci identified in GWAS are located in genes related to immune function

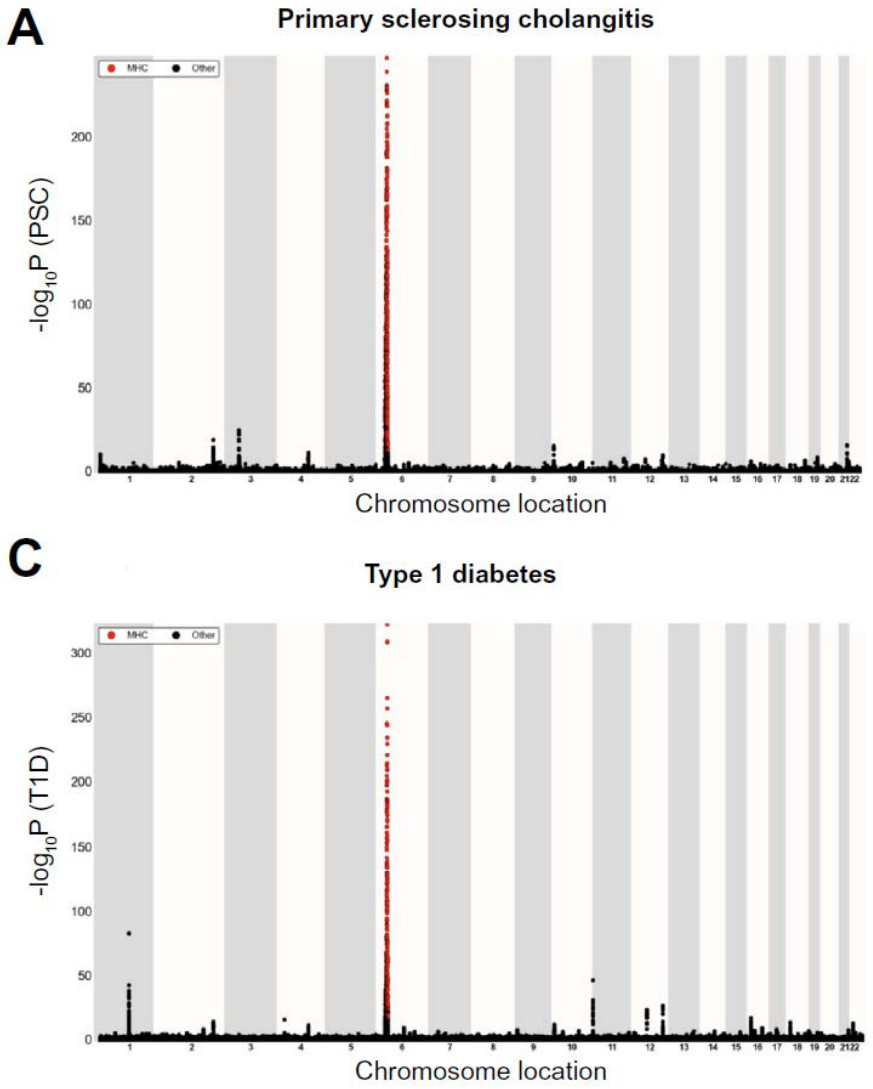
Hirschfield et al. 2013, Chapman et al. 1983, Melum et al. 2011, Hirschfield et al. 2009

Genetics in cholestatic liver diseases



Hirschfield et al. Gastroenterology 2013

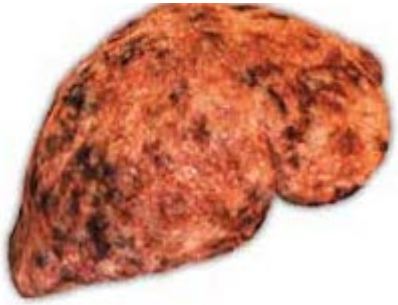
Genetic architecture in PSC and DM I



Karlsen, J Hep, 2015

Genetics in fatty liver disease

- Multifactorial pathogenesis
  - Alcohol, diet, exercise, genes…

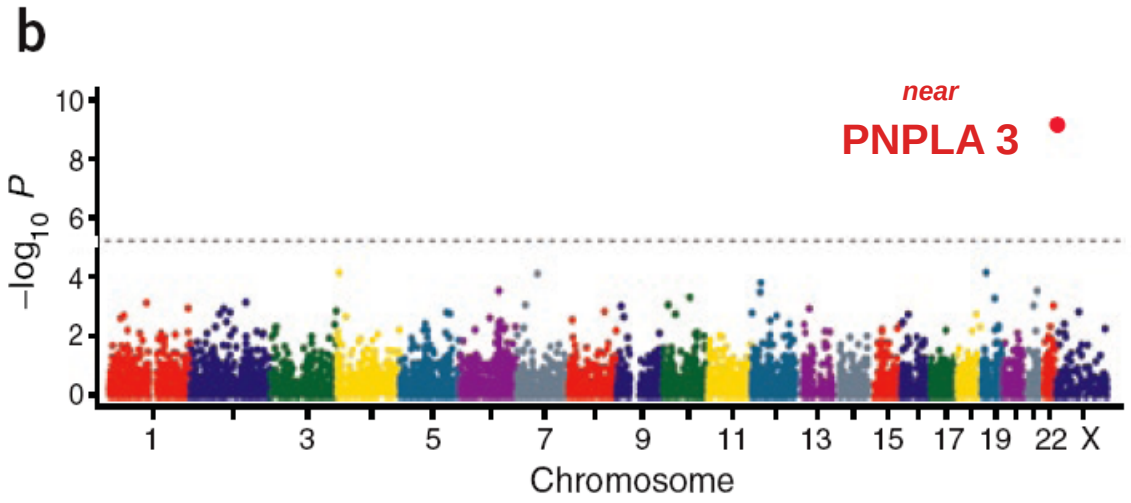


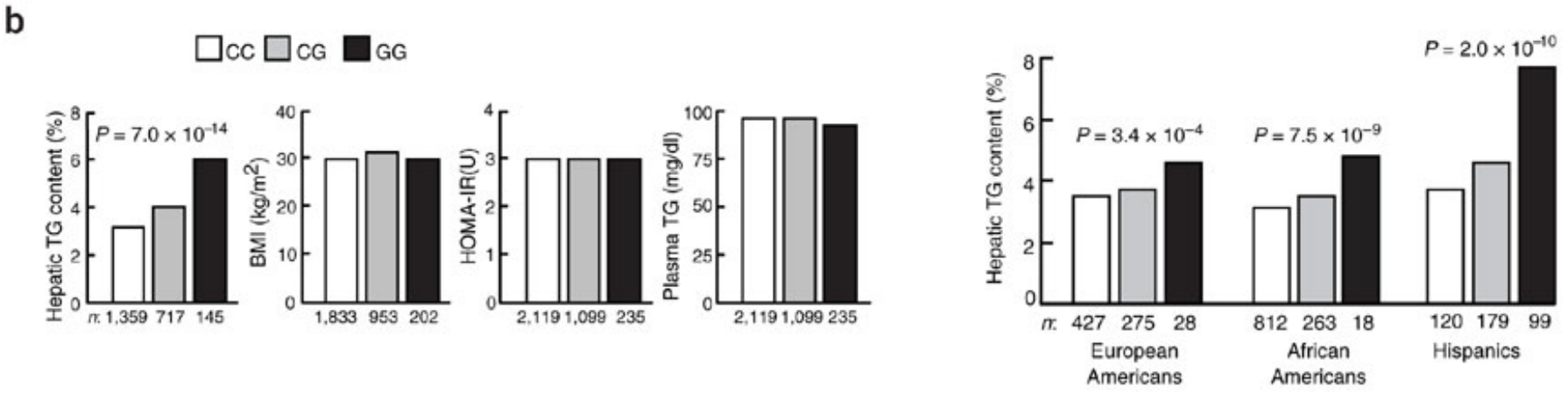
- GWAS on NAFLD risk in 2008
  - Steatosis assessed by MR spectroscopy
  - US-based population
  - 2,111 individuals from different ethnic backgrounds
  - G allele of rs738409 was associated with liver fat content
    - Irrespective of ancestry, BMI, DM or alcohol consumption

Romeo, Nature Genetics, 2008

Genetic variation in *PNPLA3* confers susceptibility to nonalcoholic fatty liver disease

Stefano Romeo[1,8], Julia Kozlitina[2,3,8], Chao Xing[1,2], Alexander Pertsemlidis[1], David Cox[4], Len A Pennacchio[5], Eric Boerwinkle[6], Jonathan C Cohen[1] & Helen H Hobbs[1,7]

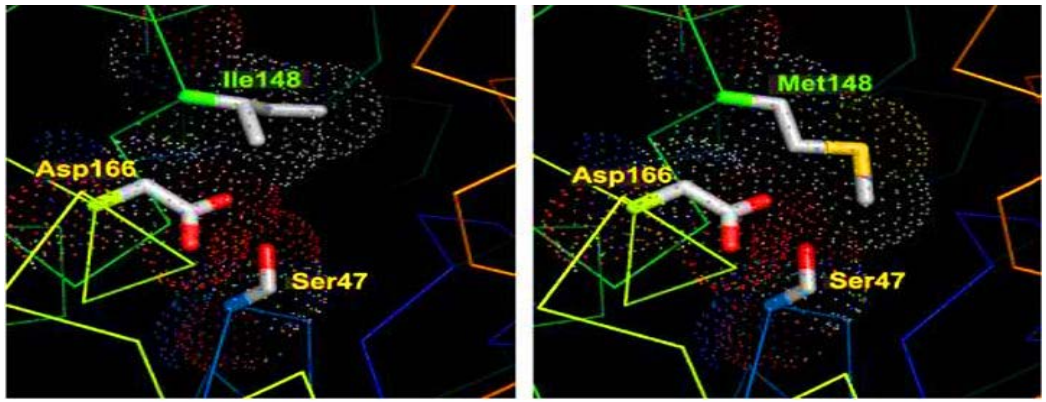




Romeo, Nature Genetics, 2008

What is PNPLA3?

- Patatin-like phospholipase A3 or adiponutrin
- rs738409 C → G results in amino acid substitution I 148 M
- Risk allele (G) frequency 21-28% in Europe
- One of the most robustly replicated common genetic risk factors for chronic liver diseases
- Modulator of hepatic lipogenesis, triglyceride hydrolase and deposition in the liver



Romeo, 2008, Zimmer and Lammert, 2014

PNPLA3 in NAFLD

PNPLA3 increases

- Liver fat content by 70%
- Necroinflammatory scores
- ALT levels
- Risk of NASH 3-fold
- Risk of developing fibrosis and cirrhosis
- Risk of developing HCC 12-fold

Krawczyk 2015, Liu 2014 , Sookoian and Pirola, Hepatology 2011, Ge 2009, Krawczyk 2013

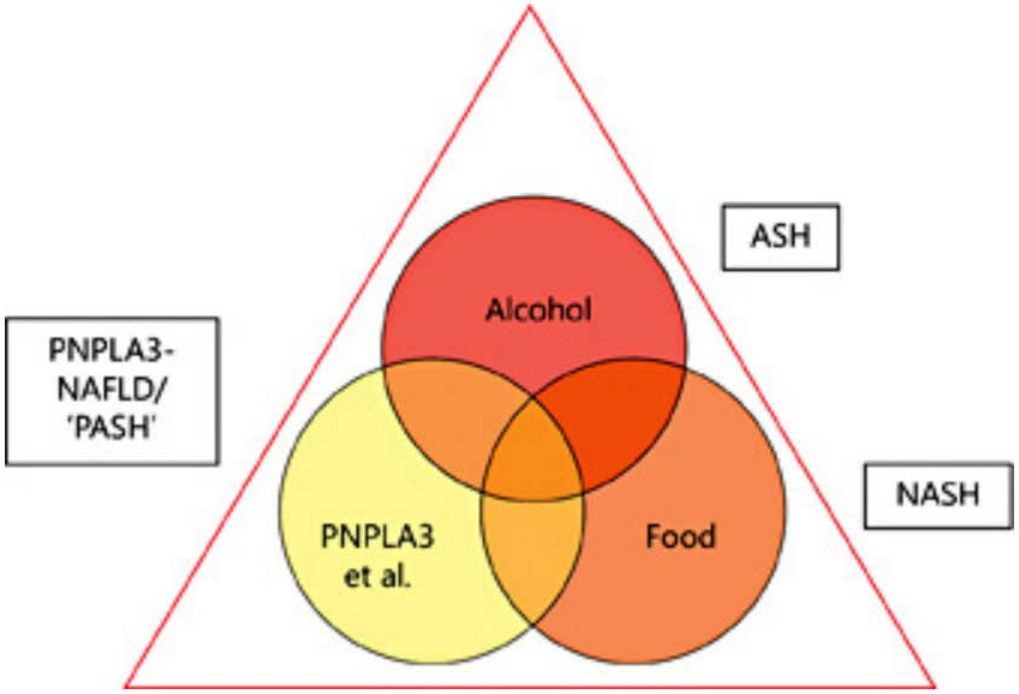
PNPLA3 in other liver diseases

- Major genetic risk factor in alcoholic liver disease
  - Accelerated progression to cirrhosis
  - Increases risk of HCC
  - Increases medium-term mortality
- In HCV, it increases
  - Fat content
  - Fibrosis progression
  - Risk of HCC

Atkinson 2017, Buch 2015, Stickel 2011, Tian 2010, Trepo 2011, Guyot 2013, Nischalke 2011, Burza 2012, Corradini 2011,Falletti 2011, Valenti 2011, Cai 2011, Clark 2012, Trepo 2012

PASH

- PNPLA3-associated steatohepatitis (PASH)
- PNPLA3 as major driver of disease progression, combined with alcohol and/or Western diet or without



Krawczyk 2013, Zimmer 2014, Lammert 2016, Karlsen 2015

PNPLA3 in clinical practice

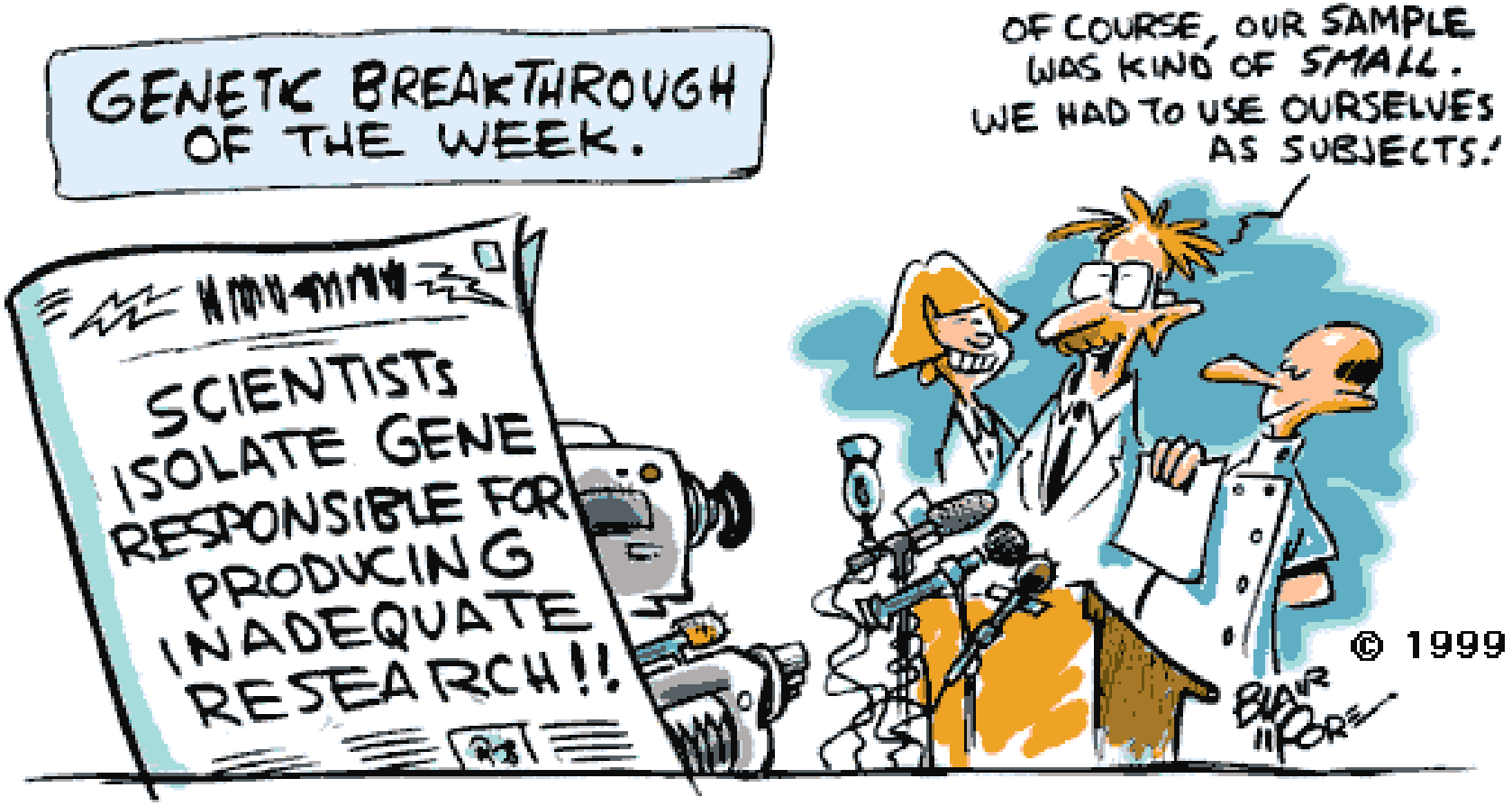
- EASL/EASD/EASO guideline NAFLD
  - *Carriers of the PNPLA3 I148M […] variants have a higher liver fat content and increased risk of NASH. […] Genotyping may be considered in selected patients and clinical studies but is not recommended routinely (B2).*

- Future
  - Risk stratification for tailored HCC surveillance in NAFLD?
  - Pharmacological treatment of PASH in the absence of alcohol/metabolic syndrome?

EASL/EASD/EASO clinical practice guidelines for the management of NAFLD, 2016

Conclusion

- Increasing interest and discoveries in genetics in liver disease
- Genetics play a role in pathophysiology, diagnosis, treatment and risk stratification
- Bridging the gap between genomic research and clinical practice
- First steps towards personalized medicine and new therapeutic interventions
- To be continued…


GENETIC BREAKTHROUGH OF THE WEEK.
SCIENTISTS ISOLATE GENE RESPONSIBLE FOR PRODUCING INADEQUATE RESEARCH!!
OF COURSE, OUR SAMPLE WAS KIND OF SMALL. WE HAD TO USE OURSELVES AS SUBJECTS!
© 1999
BLAIR MORE

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