



# Genetic Epidemiology at the intersection between function and disease

**Florian Kronenberg**

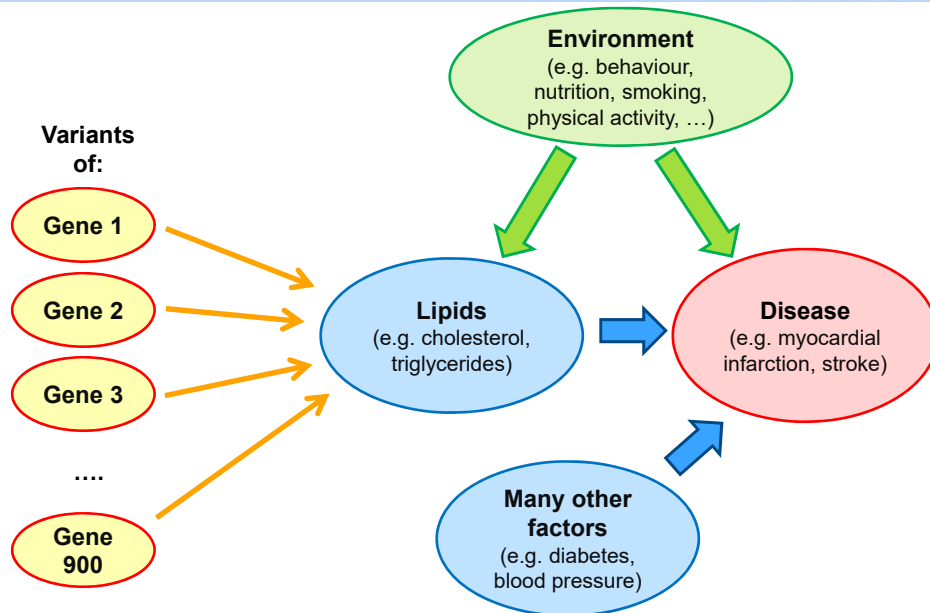
Institute of Genetic Epidemiology, Medical University of Innsbruck



## Overview

1. **Background**
2. Association studies
3. Genomewide association studies (GWAS)

## How is health and disease determined?



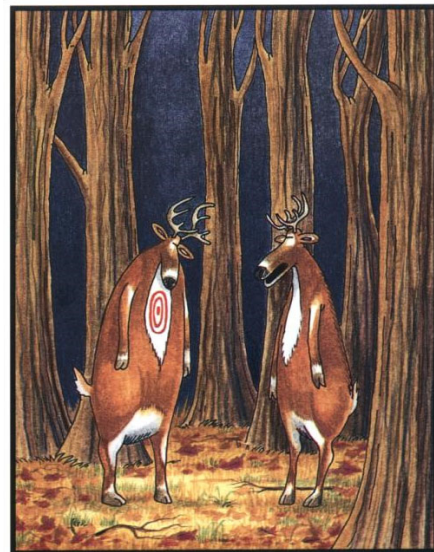
## Why are we interested in "new" genes?

By Victor A. McKusick, M.D., Baltimore, Maryland

*Ann. Int. Med.* 49:556-567, 1958

Study of genetic factors is important:

- (1) because potentially it will permit **recognition of genetic susceptibles**, for more effective application of preventive measures,



"Bummer of a birthmark, Hal."

## Why are we interested in "new" genes?

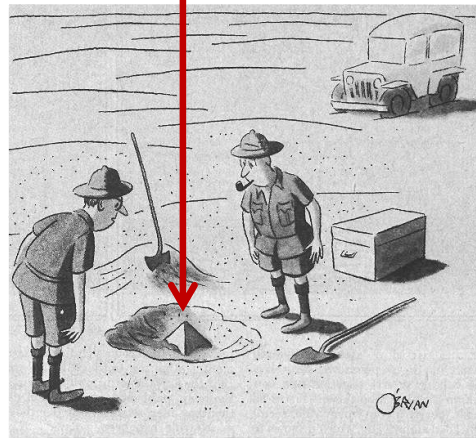
By Victor A. McKusick, M.D., Baltimore, Maryland

*Ann. Int. Med.* 49:556-567, 1958

Study of genetic factors is important:

- (2) because from our **understanding of the mechanism** whereby the gene or genes operate in these disorders can come preventive or therapeutic measures for breaking the chain leading to disease.

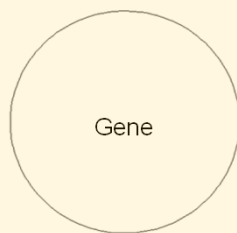
**Drug target?**



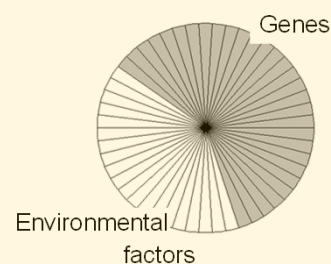
*"This could be the discovery of the century. Depending, of course, on how far down it goes."*

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## Monogenic and complex diseases



**Monogenic diseases:**  
e.g. Morbus Huntington



**Complex diseases:**  
e.g. Diabetes, myocardial infarction, overweight, cancer, ...

Environmental factors are e.g. smoking, physical activity, nutrition, education, sun exposition, ....

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## Knockout versus small changes by polymorphisms

### Knock-out



- Pronounced effects
- Animals: great models but not necessarily to extrapolate to humans
- Humans: often very rare cases

### Polymorphism



- Small effects
- Usually investigated in humans
- Real in vivo conditions
- Thousands of people can be studied easily
- Sample sizes of thousands are required

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## Genetic variability

GTGGTGTACATAAATGCGT



GTGGTGTACGTA AATGCGT

A) Single Nucleotide Polymorphism (SNP)

GTGGTGTACATAAATGCGT



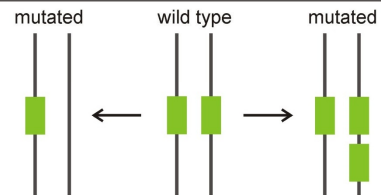
GTGGTGTAAAATGCGT

B) Indel aka. DIP

AGATGAGAGAGAGAGTCC

AGATGAGAGAGTCC

C) Short Tandem Repeat (STR)

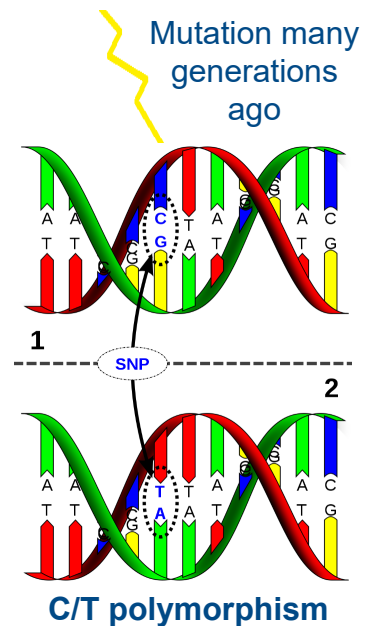


D) Copy Number Variation (CNV)  
Size >1 Kb

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## Single Nucleotide Polymorphisms (SNPs)

- Variations of single base pairs (bp) in the DNA sequence
- Heritable and stable.
- Account for 90% of the genetic variability
- Every 300 – 1000 bp
- At least 3 – 4 million SNPs per individual
- 10,000 – 11,000 non-synonymous SNPs per individual
- 700 million SNPs are described in databases



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## Single Nucleotide Polymorphism (SNP)

- **Coding SNPs within a gene**
  - synonymous exchanges: without influence on protein
  - non-synonymous exchanges: resulting in an AA exchange
- **SNPs within the regulatory regions:**
  - when and why a gene will be switched on or off
  - effect on quantity of protein production
- **SNPs within the untranslated regions**
  - with influence on mRNA stability
- **SNPs in intergenic regions**
  - functional consequences have to be evaluated

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## Some basics from epidemiology

### ■ Odds ratio

- ▶ Represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure.
- ▶ Values between 0 and infinite ( $\infty$ )
- ▶ 1.00 = same odds
- ▶ 1.50 = 50% higher odds
- ▶ 2.00 = 100% higher odds
- ▶ 0.50 = 50% lower odds

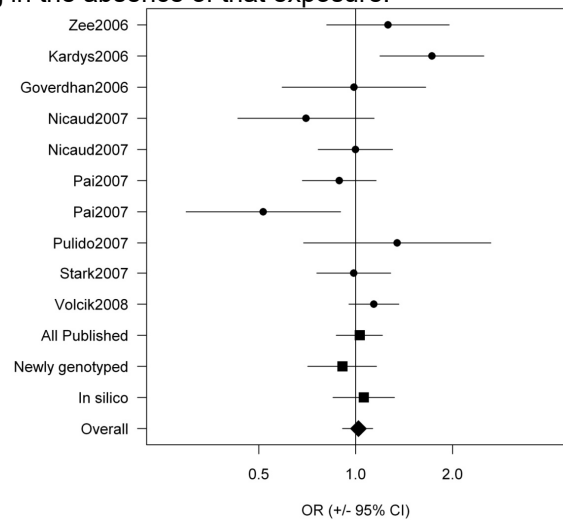
### ■ 95% confidence interval (CI)

### ■ Hazard ratio

- ▶ In case of prospective studies

### ■ Meta-analysis

- ▶ Combining data from more than one study



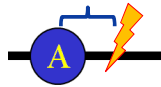
## Overview

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3. Genomewide association studies (GWAS)

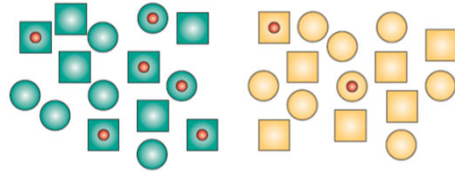


# Principle of association studies

- Qualitative analysis:**  
 Preferential association of an allele with a disease status

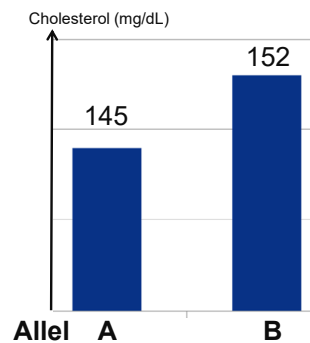


**Cases (e.g. diabetes)**    **Controls**



Allele ● 3 times more frequent in cases

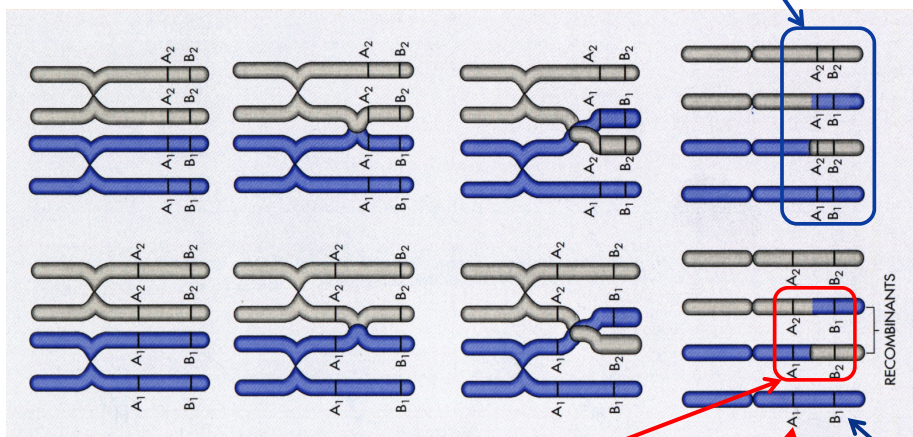
- Quantitative analysis:**  
 Carriers of a various alleles differ in the mean values of the investigated parameter (e.g. cholesterol level)



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**Remember: crossover and recombination during meiosis I**

No recombination of alleles of the gene loci A and B since crossover outside the region between A and B



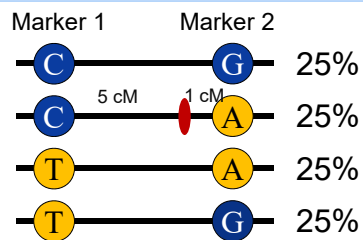
Recombination of alleles in the two gametes due to crossover

"Wanted" disease locus

Genotyped marker locus

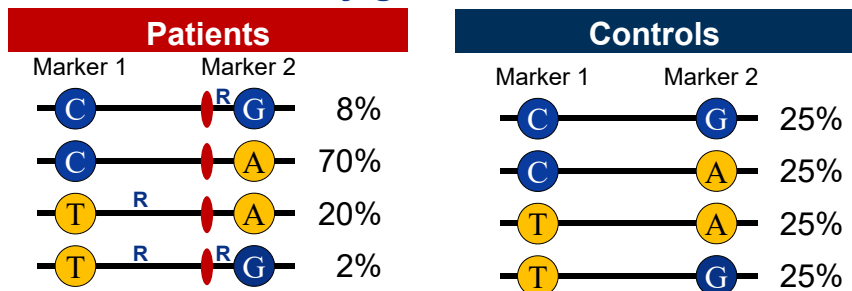
Jorde, Carey, Bamshad, White: Medical Genetics

## Basis of association: Linkage disequilibrium



	Patients	Controls
Allele 2A	90%	50%
Allele 1C	78%	50%

### After many generations



R ... Recombination

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## What does a significant genetic association mean?

### ■ Direct association

- ▶ The investigated genetic variant is indeed the causal disease-causing variant
- ▶ This was rarely the case in earlier times; improves nowadays by the dense map of markers we can investigate
- ▶ Optimum procedure: functional characterisation goes hand in hand

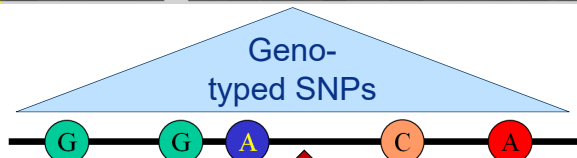
### ■ Indirect association

- ▶ The investigated genetic variant is in linkage disequilibrium with the causal variant

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## Indirect association



**Disease-causing variant  
we are searching**

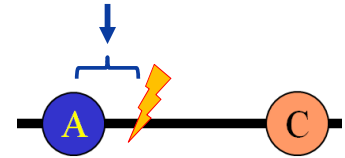
ACTAGAGCTACTACGAGGGACTAC...TACGAGCATCGACTA...GAGG  
TAGAGCTATA...TTCTAGGCTA...CTACGATCGATC...ACGTAG...

**High correlation**

The genotyped SNP is a marker of the „non-genotyped“  
disease-causing variant we are searching

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Due to the small distance there are  
rarely crossovers and  
recombinations during meiosis



**Allele A can be observed  
more frequently in the  
diseased group**

## What does a significant genetic association mean?

### ■ Direct association

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### ■ Indirect association

- ▶ The investigated genetic variant is in linkage disequilibrium with the causal variant

### ■ False-positive finding (spurious association)

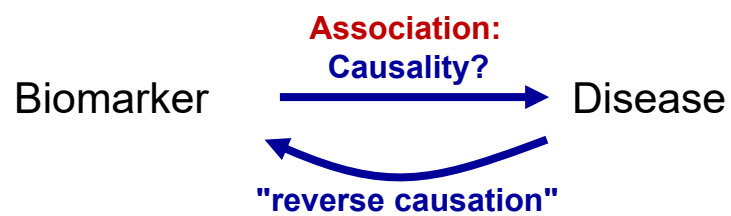
- ▶ Random finding (sample size!)
- ▶ Confounding: e.g. population stratification
- ▶ Often observed in small studies without replication

→ replicate, replicate, replicate!

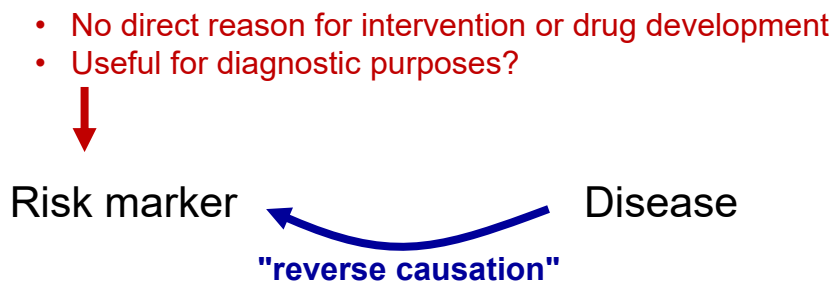
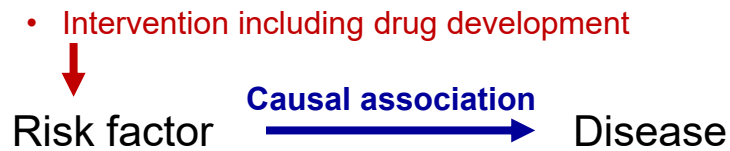
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## Mendelian Randomization study

## Biomarker for diseases: causality or consequence?



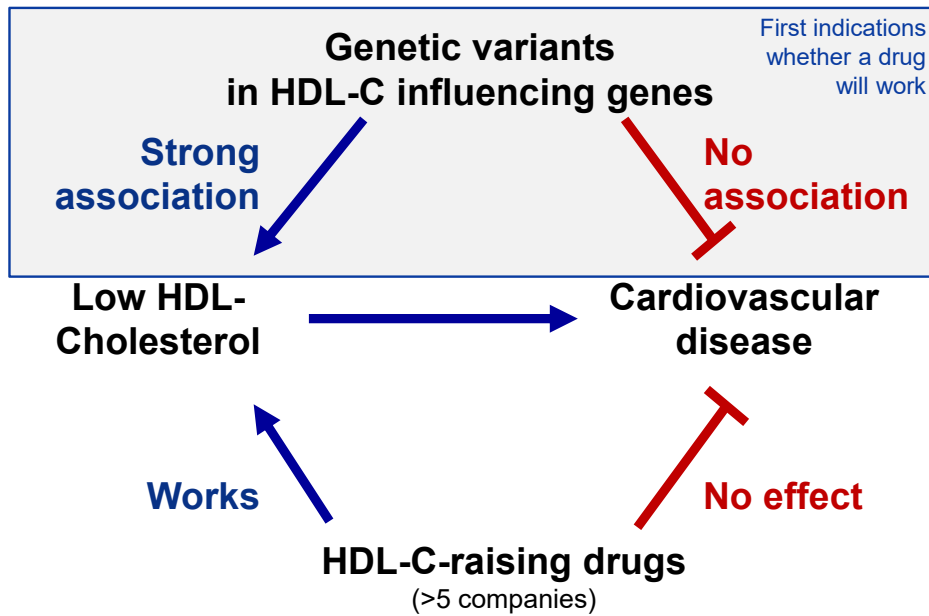
## Biomarker for diseases: causality or consequence?



## The big question for biomarkers

- You found an association with a disease
- **Risk factor or risk marker?**
- Classical epidemiological studies with prospective observation will last a long time and will not prove causality
- You have to decide now whether to go for drug development or not
- Worst case scenario: after 10-15 years of development the drug flops
- One reason might be that it is only a risk marker and not a risk factor.

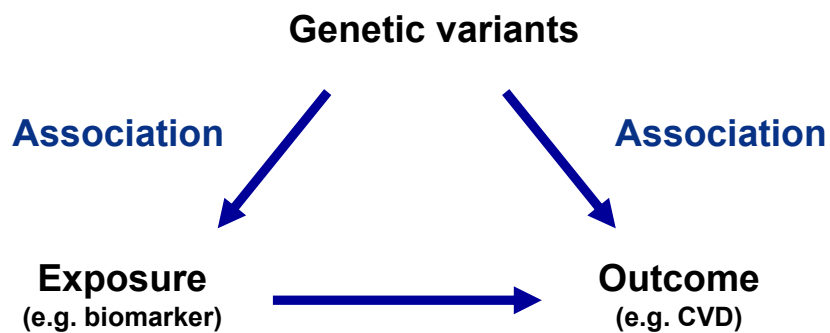
## Example of a flop: CETP inhibitors



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Kronenberg: Kidney Int. 89:747-9, 2016

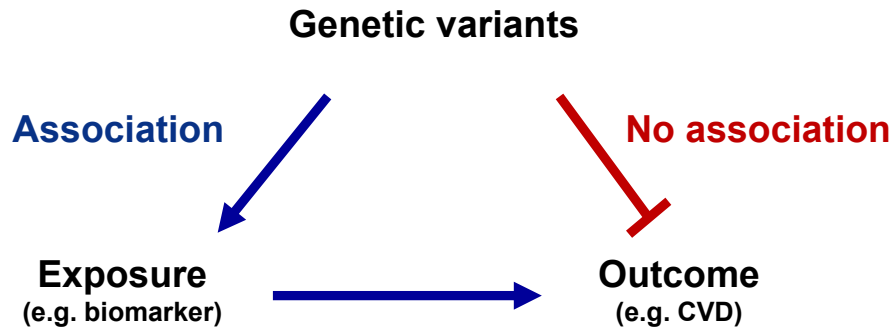
## Mendelian randomization approach



→ **Strong support for causality**

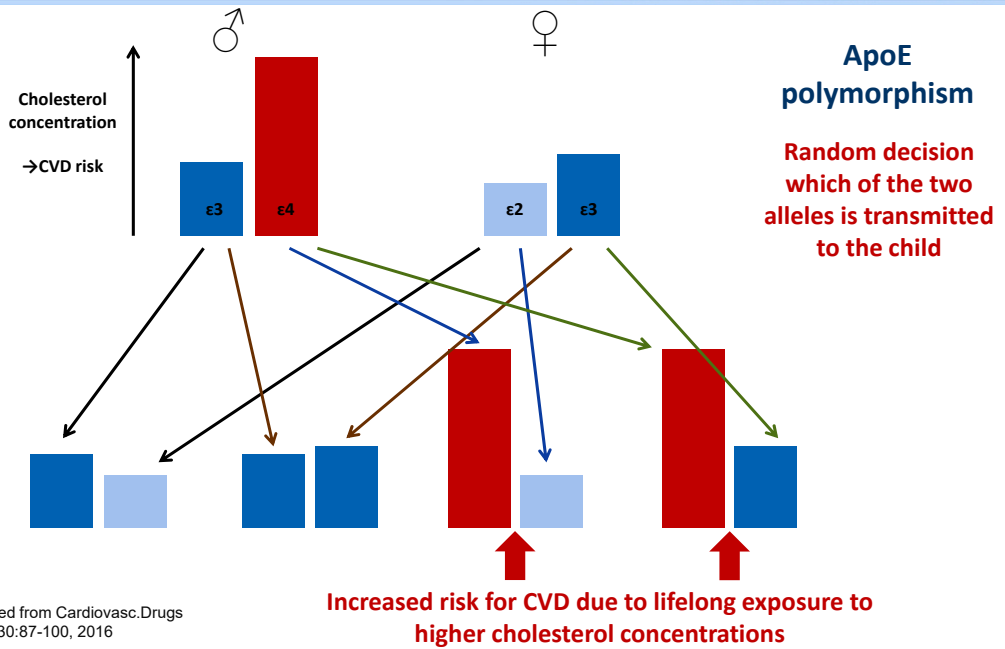
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## Mendelian randomization approach

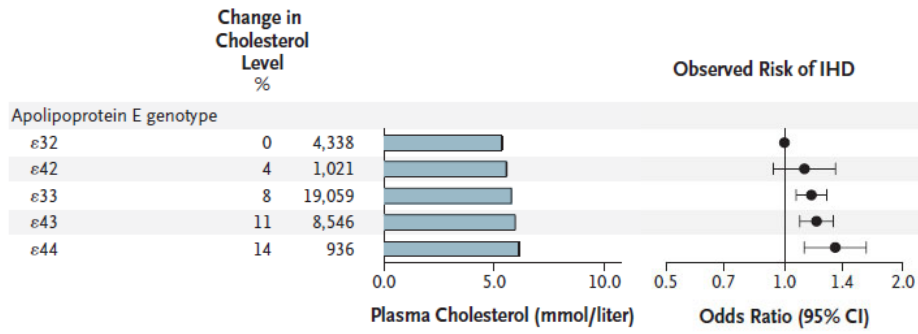


→ Causality is unlikely if the study is sufficiently powered

## Mendelian randomization at the time of conception



## ApoE, cholesterol and risk for CVD



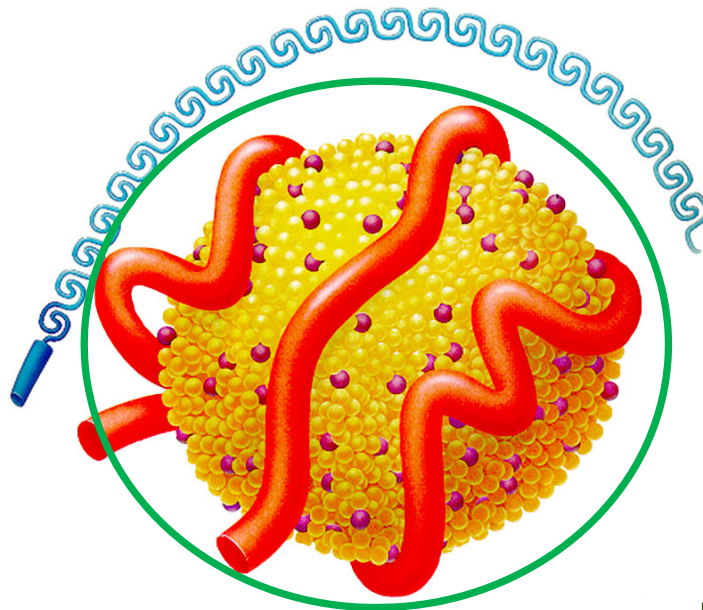
## Example: Lipoprotein(a) Lp(a)



## Experience of a young widow

- Husband 39 years of age
- Loses consciousness, cardiac arrest, revival not successful
  
- No classical risk factors
- Healthy lifestyle, physically active
- Health checkup on a yearly basis
  
- Autopsy: most severe heart disease
- **Very high Lp(a) concentrations**

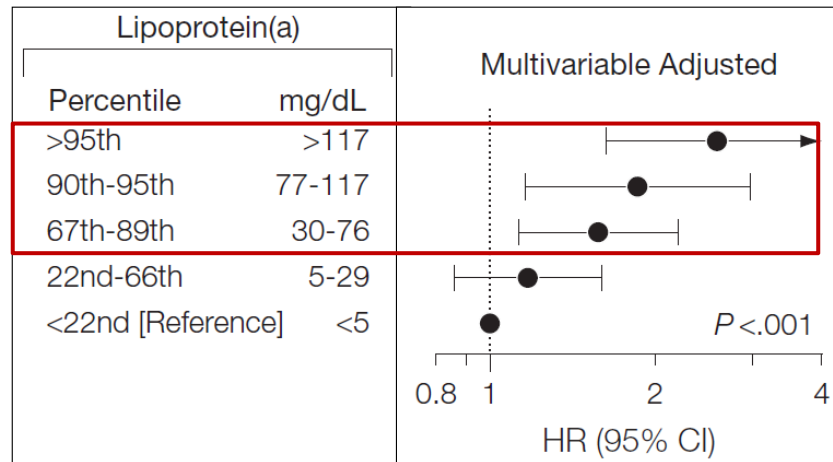
## Lp(a) – the mysterious brother of LDL



## Lp(a) and risk for myocardial infarction

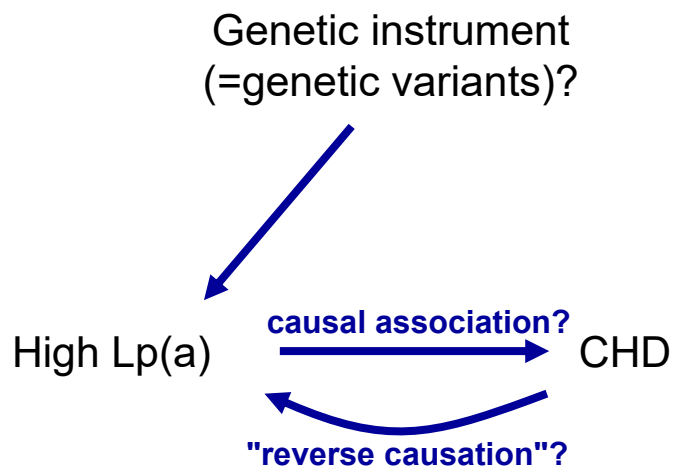
Results from the  
Copenhagen  
City Heart Study

**A third of the  
entire  
population!**

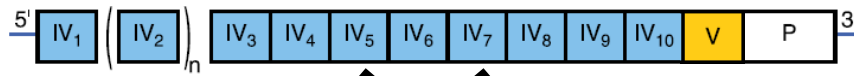


**Ist this association causal?**

## Lp(a) and CHD: truth or consequence?

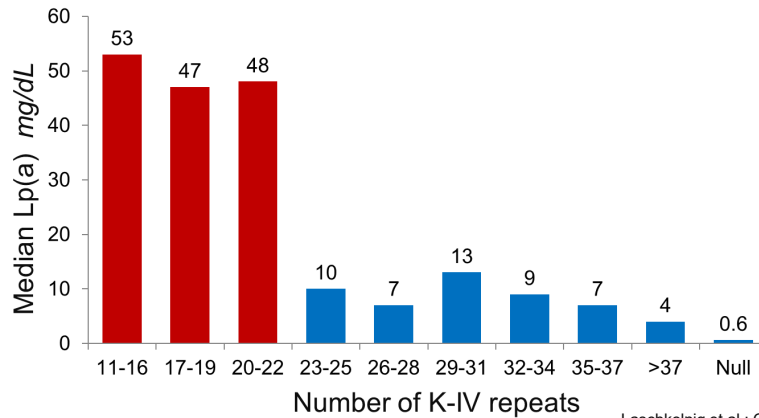


## Apolipoprotein(a) - Mr 300-800 kDa



**11-22 copies =  
small isoforms**

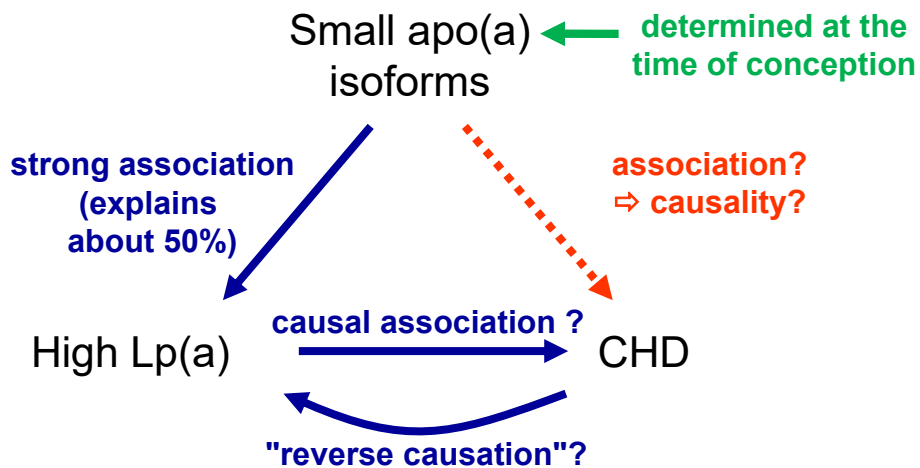
**>22 copies =  
large isoforms**



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Laschkolnig et al.: Cardiovasc Research 103: 28-36, 2014

## Lp(a) and CHD: Mendelian randomization

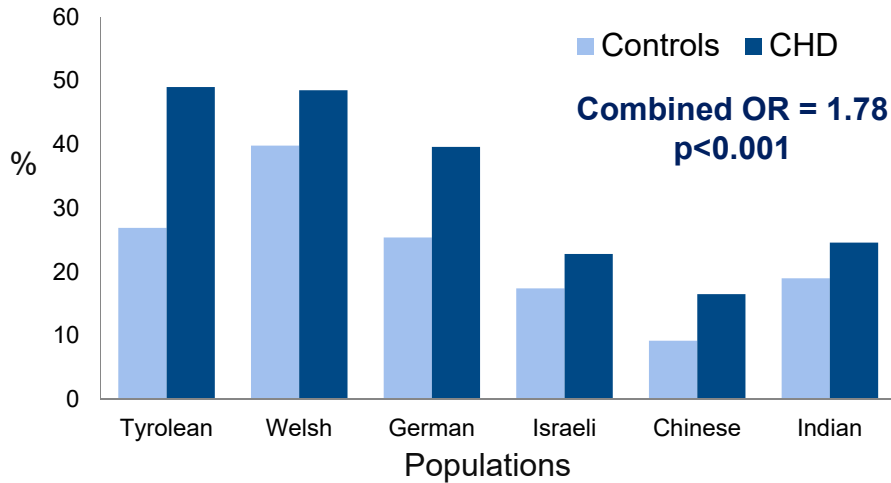


**Do carriers of small apo(a) isoforms  
more often have CHD?**

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## Apo(a) isoforms and risk for CHD

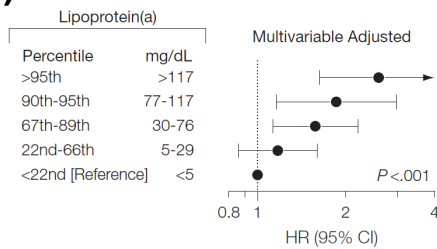
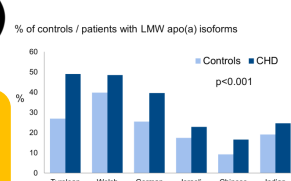
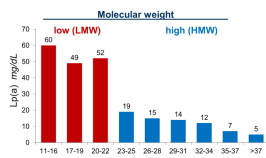
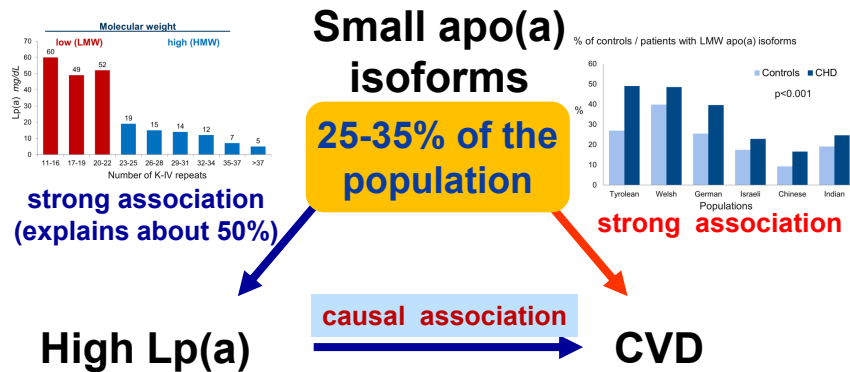
% of controls / patients with small apo(a) isoforms



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Sandholzer et al.: Arterioscler Thromb 12: 1214-26, 1992

## Lp(a) concentrations, apo(a) isoforms and CVD



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Cardiovasc. Drugs Ther.: 30:87-100, 2016

## Lipoprotein(a) in atherosclerotic cardiovascular disease and aortic stenosis: a European Atherosclerosis Society consensus statement

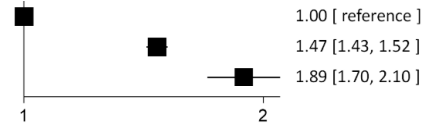
### Genetic variants associated with increased Lp(a) concentrations ...

#### ... are associated with increased cardiovascular risk

##### LPA genetic score

Number of Lp(a)-increasing variants *	No. participants	No. events	HR (95%CI)
0 (median Lp(a) 14 nmol/L)	358,464	20,610	1.00 [reference]
1 (median Lp(a) 146 nmol/L)	77,655	6,167	1.47 [1.43, 1.52]
2 (median Lp(a) 262 nmol/L)	4,249	410	1.89 [1.70, 2.10]

\* variants of rs10455872 and rs3798220 described by R. Clarke



### Lp(a) concentrations and apo(a) isoform as a risk factor

Having a small apo(a) isoform doubles the odds for CVD in 25-35% of the population

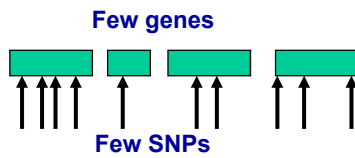
→ **strongest genetically determined risk factor for CVD**



"Bummer of a birthmark, Hal."

## Candidate gene approach vs. GWAS

### Candidate gene approach



### Association with phenotype

- Hypothesis-driven
- Biochemical or physiological *a priori* knowledge
- Few genes identified

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3. **Genomewide association studies (GWAS)**



## Candidate gene approach vs. GWAS

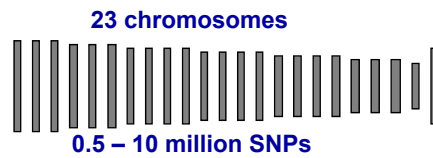
### Candidate gene approach



#### Association with phenotype

- Hypothesis-driven
- Biochemical or physiological *a priori* knowledge
- Few genes identified

### Genomewide association study GWAS



#### Association with phenotype

- **"Hypothesis-free"** (unbiased)
- No *a priori* knowledge
- New pathways
- Small effects detectable
- **Very large sample sizes required**

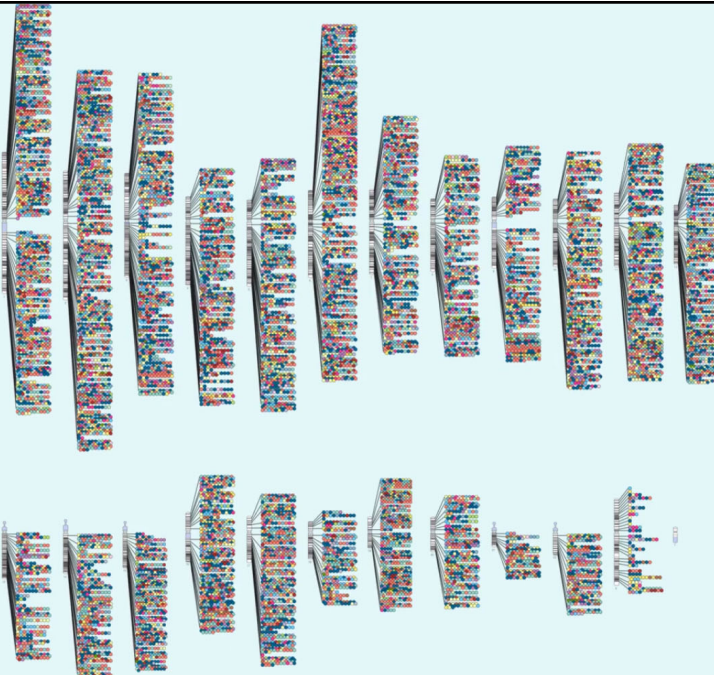
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**2018 Apr**

Associations: 69,885

Studies: 5,152

Papers: 3,378



[www.ebi.ac.uk/gwas](http://www.ebi.ac.uk/gwas)

## Gain in detected genes by GWAS

### Examples for metabolic traits

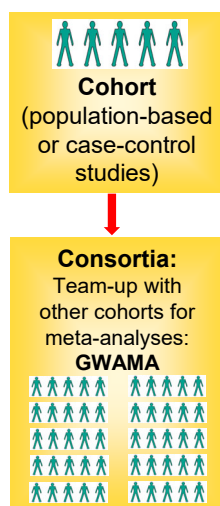
Disease	before 2007	2007 onward
Type 2 DM	3	50
Body mass index	1	30
Glucose or insulin	1	15
Fat distribution	0	20
Lipids	16	95
<b>Total</b>	<b>21</b>	<b>202</b>

### 7 examples of autoimmune diseases

Disease	before 2007	2007 onward
Ankylosis spondylitis	1	13
Rheumatoid arthritis	3	30
Systemic lupus eryth.	3	31
Type 1 DM	4	40
Multiple sclerosis	1	51
Crohn's disease	4	67
Ulcerative colitis	3	44
<b>Total</b>	<b>19</b>	<b>277</b>

Since 2012 the number of known genes has further increased by 5- to 10-fold

## Design and cost-performance ratio

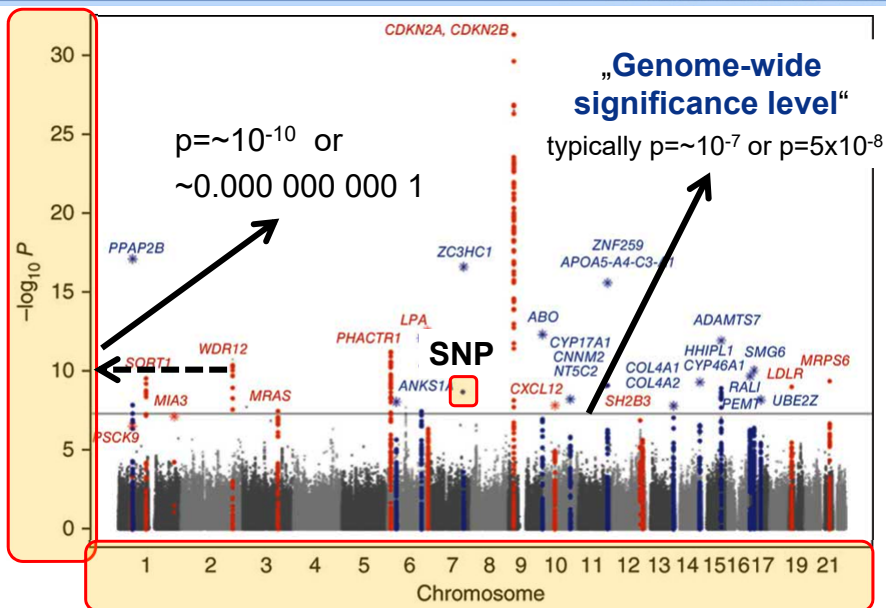


- Costs per array: 30-150 €
- You genotype only once and then do the GWAS for all phenotypes you have for the cohort

### Association with phenotypes (e.g.)

- BMI
  - Waist
  - Blood pressure
  - QT interval
  - Smoking
  - Lab values**
  - Lipids
  - Kidney function
  - CRP
  - Hemoglobin
  - ...
  - Diseases**
  - CAD
  - Stroke
  - Ankle-brachial-index
  - Cancer types
- Whatever has a genetic component and is measured**

## The Manhattan Plot



## Genome-wide association studies (GWAS)

- Study design
- Examples:
  - ▶ Lipids
  - ▶ Type 2 diabetes mellitus
  - ▶ Blood pressure
  - ▶ Kidney function
  - ▶ Addiction (smoking quantity)
  - ▶ BMI

## GWAS: Lipids

### ■ Consortium:

- ▶ Established during fall 2005
- ▶ Together with Helmholtz-Zentrum München
- ▶ 11 members studying various phenotypes

### ■ Innsbruck Group:

- ▶ Lipid metabolism
- ▶ HDL-C as a starting point
- ▶ Quantitative trait considered more powerful

### ■ Population and Genotyping:

- ▶ 1644 population-based subjects from KORA
- ▶ Affymetrix 500K SNP chip



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Iris Heid

Stefan Coassin

Eva Boes

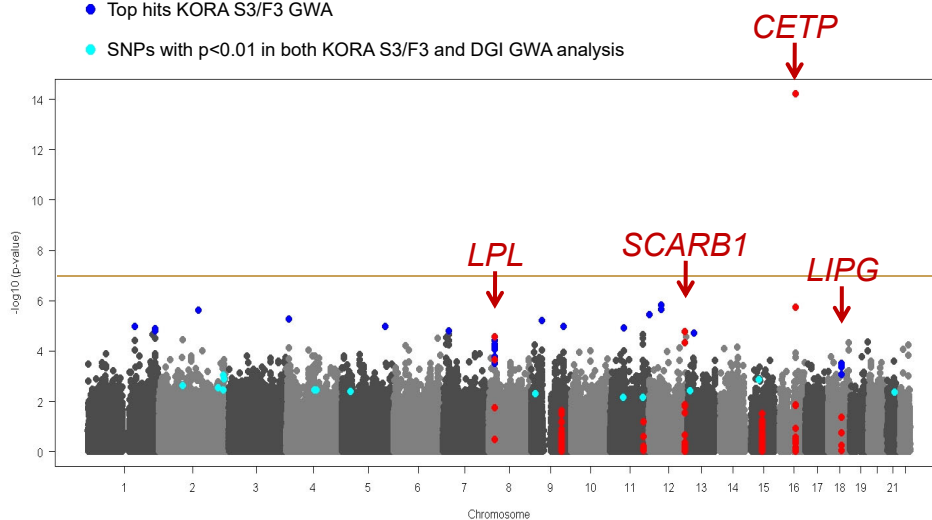
Barbara Kollerits

Anita Brandstätter

Claudia Lamina

## GWAS: HDL cholesterol

- SNPs in HDLC candidate genes  $\pm 15\text{kb}$
- Top hits KORA S3/F3 GWA
- SNPs with  $p < 0.01$  in both KORA S3/F3 and DGI GWA analysis



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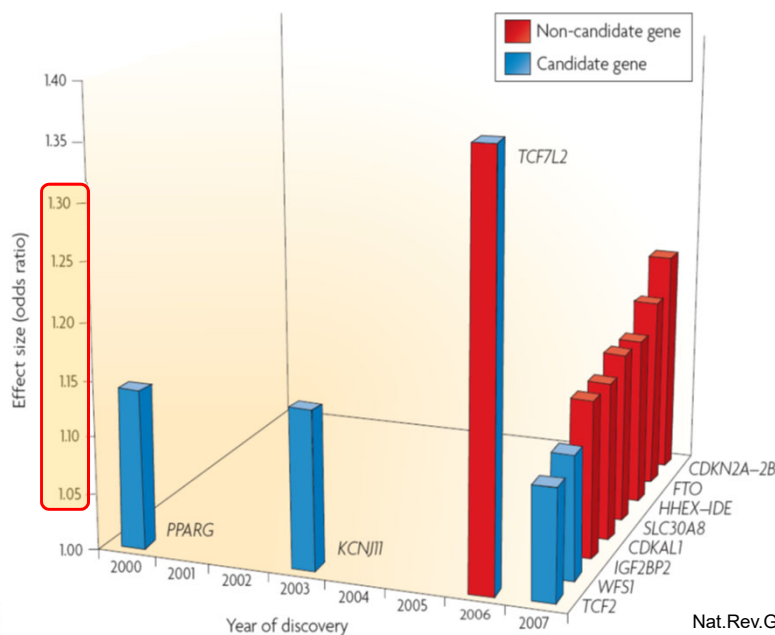
Heid et al.: Circ. Cardiovasc. Genet. 1:10-20, 2008

## GWAS on Lipids (TC, HDLC, LDLC, TG): next steps

- **First own GWAS** (*Circ. Cardiovasc. Genet.* 2008)
  - ▶ 1644 probands: found nothing new
- **Engage Consortium:** (*Nature Genetics* 2009)
  - ▶ 22,000 probands: **22 genes found associated**
- **Global Lipids Genetics Consortium:** (*Nature* 2010)
  - ▶ >100,000 probands: **95 genes found associated**
- **Global Lipids Genetics Consortium:** (*Nature Genetics* 2013)
  - ▶ >188,000 probands: **roughly 155 genes found associated**
- **Global Lipids Genetics Consortium:** (*Nature* 2021)
  - ▶ 1,65 million probands: **>900 Gene**

**Functional characterisation for most of the genes has to be done**

## GWAS: Type 2 diabetes mellitus

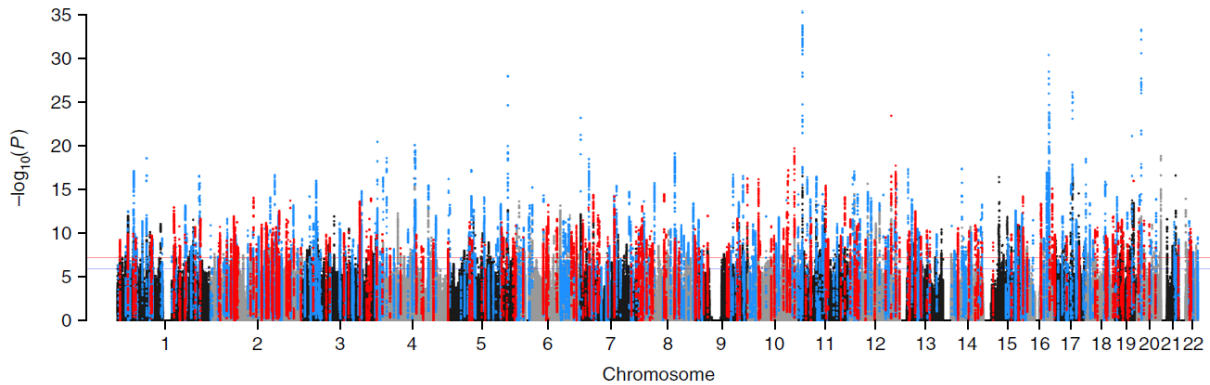


**2017:**  
≈ 86 genes

**2019:**  
≈ 250 genes

## GWAS and blood pressure traits

- Phenotypes: systolic and diastolic BP, pulse pressure
- > 1 million study participants
- 901 genetic loci in total (535 novel)



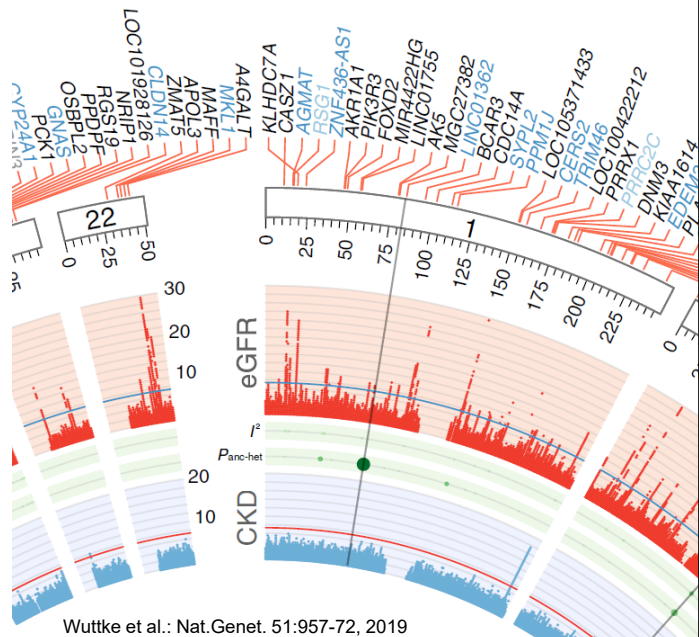
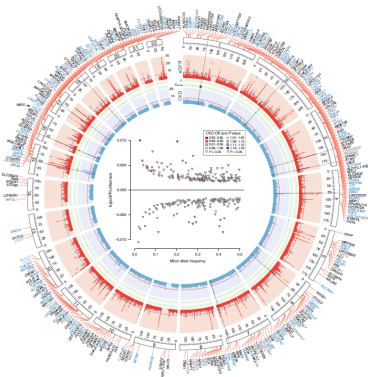
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Evangelou et al.: Nature Genet. 50:1412-25, 2018

## GWAS on kidney function

### ■ CKDGen consortium

- ▶ 1,046,070 persons
- ▶ 264 associated loci (166 new)
- ▶ Circos plot
- ▶ comprehensive priority list of molecular targets for translational research

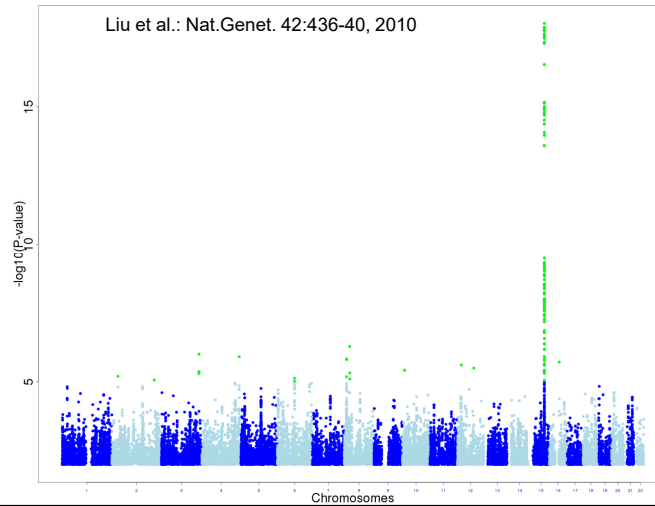


Wuttke et al.: Nat.Genet. 51:957-72, 2019



## GWAS on smoking (quantity)

- ▶ Addiction research
- ▶ >40,000 persons
- ▶ Neuronal nicotinic acetylcholine receptor subunits
- ▶ Same region was found for lung cancer, COPD, lung function and PAD



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## Genes for body mass index and overweight

### Own behavior (lifestyle)



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### Genetics

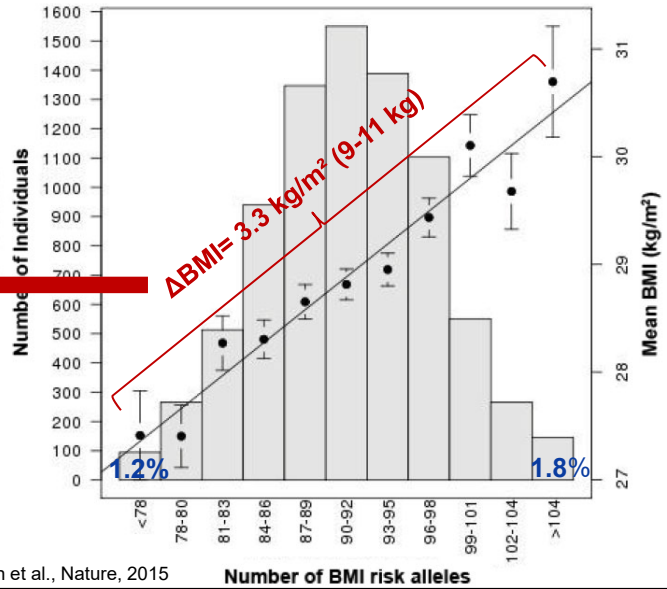
- ▶ 536 genetic loci detected
- ▶ Many of them play a role in the brain by
  - ⇒ **Regulation of appetite**
  - ⇒ Neuronal component of overweight
- ▶ Explain roughly 5% of BMI

## Contribution of single genes to overweight

- **Very few with strong effects:** risk increase by 10 to 30% per allele
- **More with moderate effects:** risk increase by 3 to 10% per allele
- **Many more with tiny effects:** risk increase by 0.1 to 3% per allele

## Development of SNP-Risk-Scores

## Risk score of 97 SNPs for BMI: 0-194 risk variants



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Shungin et al., Nature, 2015

Number of BMI risk alleles

## Risk score for 65 SNPs for diabetes



Many risk variants  
(top quintile)

2.7-fold increased  
risk to develop a  
diabetes in the  
future

Few risk variants  
(bottom quintile)

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Talmud et al., Diabetes 64:1830-40, 2015

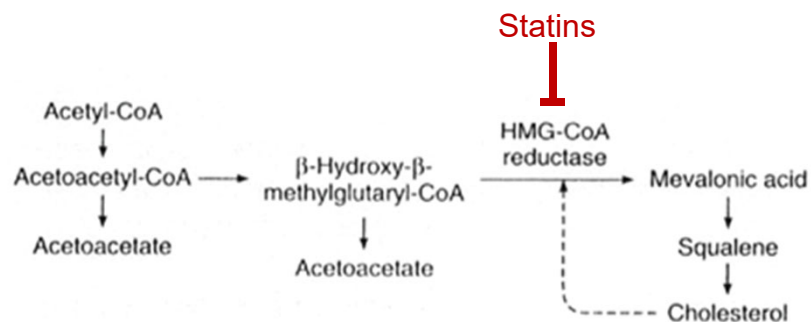
## Where is the reward?

**Can a single gene explaining less than 1% of the traits' variance still be useful for anything?**

## Statins: HMG-CoA-Reductase-Inhibitors

### ■ Mechanism of action

- ▶ Inhibition of HMG-CoA-Reductase: this enzyme catalyzes the conversion of HMG-CoA to mevalonic acid: an early and rate-limiting step in cholesterol biosynthesis.
- ▶ Results in higher expression of LDL receptor which decreases LDL cholesterol



## Polymorphisms in HMG-CoA-R gene region

### ■ GWAS results for HMG-CoA-reductase

- ▶ Very small effects
- ▶ Were not detected in the first GWAS
- ▶ This gene was only detected after investigation of at least 10.000 subjects
- ▶ **Single polymorphisms explain far less than 1% of the cholesterol concentrations within a population**
- ▶ Nevertheless, the most successful drug target for lipid metabolism

### ■ Other drug targets within the 157 lipid genes?

- ▶ CETP, ABCA1, PCSK9
- ▶ Others?

## Conclusions on GWAS

- An **hypothesis-free approach**
- Never before such a gain in gene-phenotypic information
- New genes for CAD, diabetes, cancer, kidney function...
- Odds ratios between **1.02** and 1.40
- To have the equipment is only the smallest step
- Very large studies of well phenotyped cohorts are necessary
- Works only within a very well constructed network between genetics, epidemiology, statistics, informatics, genomics
- Data sharing (a lot is already on the web)
- **Non-coding SNPs and "gene deserts" can no longer be neglected**
- A lot to learn about regulatory regions
- Functional characterization of "new" genes will need decades

## Why are we searching these many genes?

### Improvement of risk prediction (gene risk scores)

### Identification of new drug targets

- ▶ PCSK9 increases LDL cholesterol: discovered by genetic studies
- ▶ PCSK9 inhibitors lower LDL cholesterol by 60%

### Exclusion of drug targets

- ▶ CETP increases the "good" cholesterol
- ▶ Development of CETP inhibitors to increase HDL cholesterol
- ▶ Billions of investment without lowering of heart attacks
- ▶ Genetic studies would have predicted the failure of these drugs

## Gene hunting: an interdisciplinary approach

