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Analyze of the genome – GWAS and GRS

Structure

- Terminology
- The human genome project
- HapMap
- GWAS
- GWAS and oral cavity disease
- GRS

Terminology

- Allele
- Locus
- Single nucleotide polymorphism (SNP)
- Haplotype
- Linkage disequilibrium (LD)
- Imputation
- Genome wide association studies (GWAS)
- Genetic risk score (GRS)

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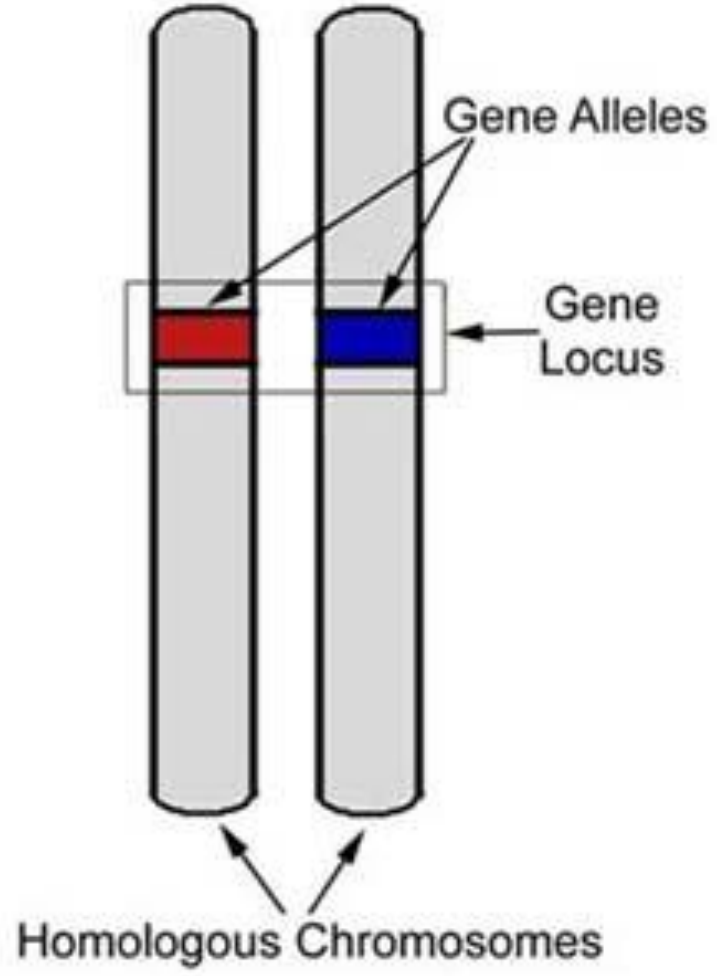
Allele a locus

- Allele is specific variant of the gene
- Locus determine specific position on the chromosome



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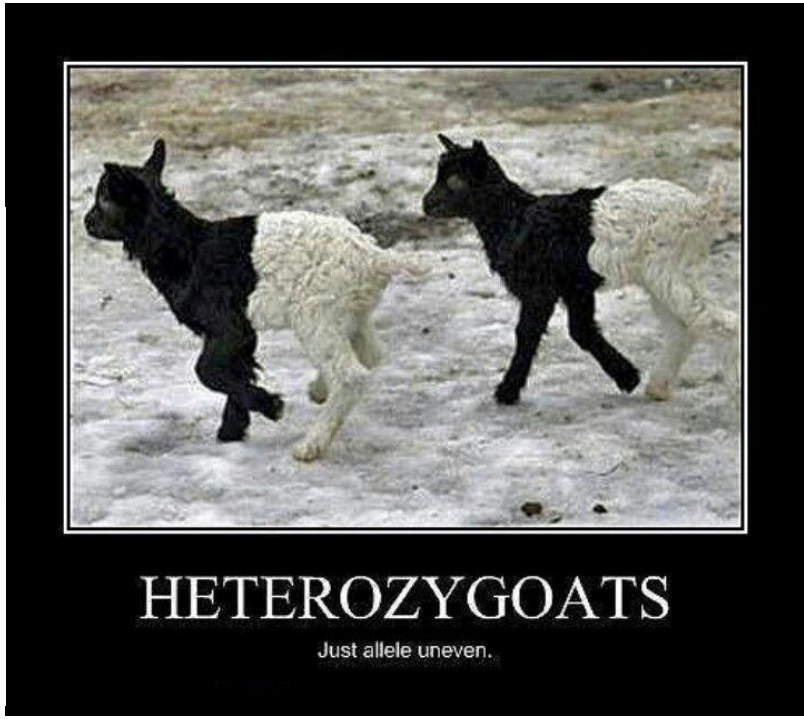
Single nucleotide polymorphism (SNP)



One nucleotide change with frequency higher than 1% in given population. This change does not have to impact function of the gene or protein.

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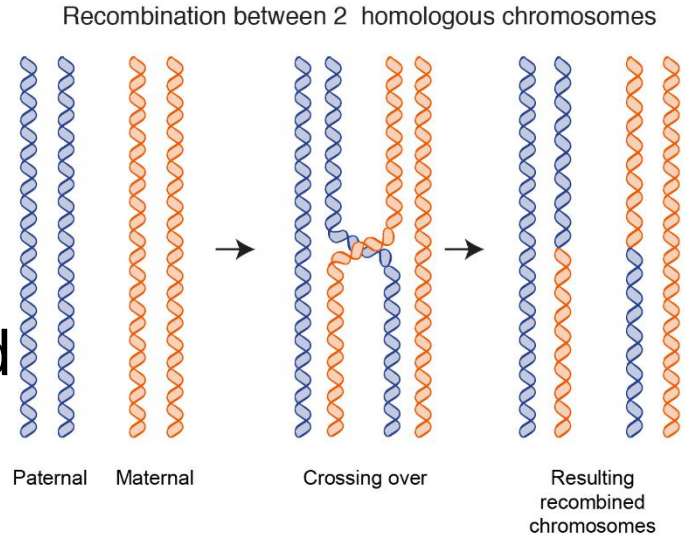
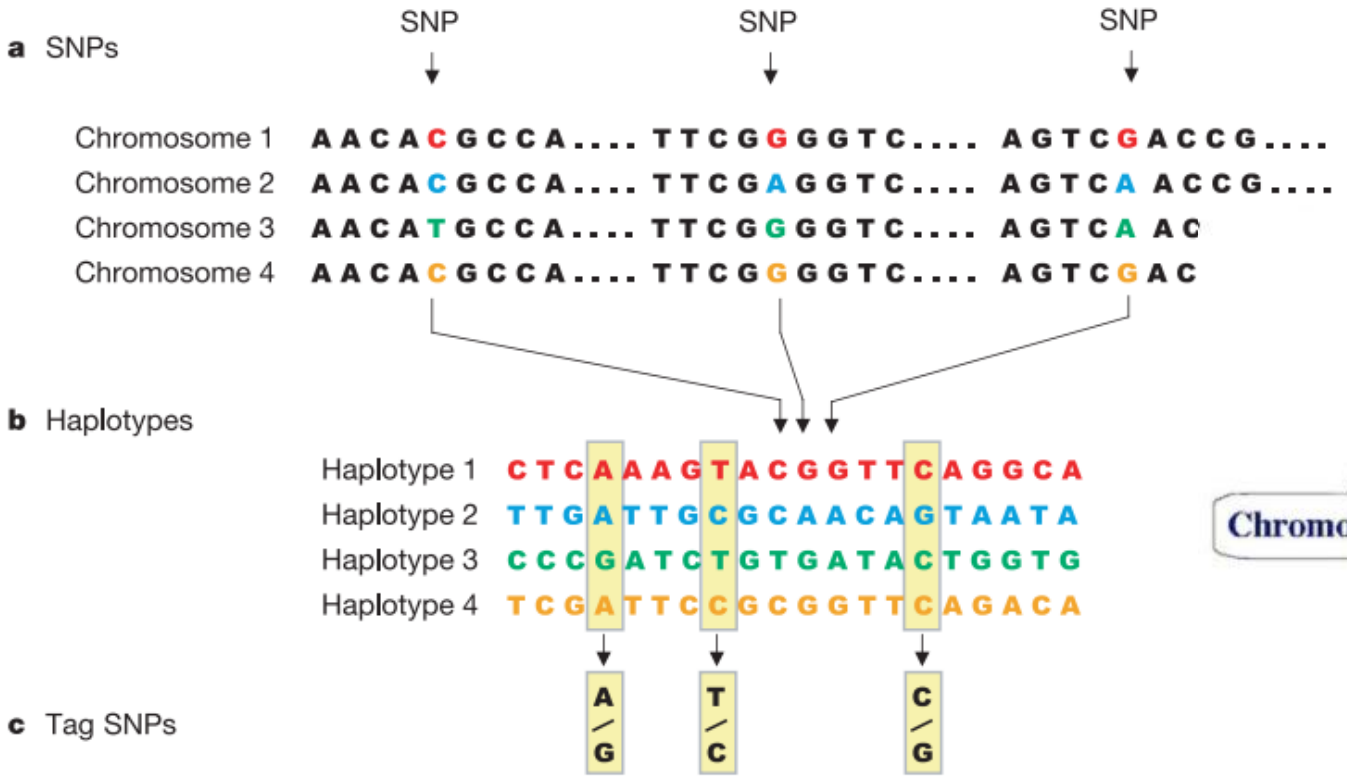
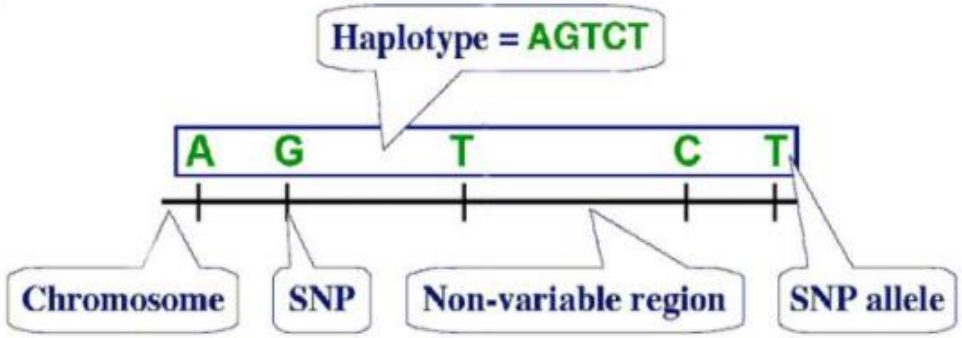
Haplotype

– It is combination of alleles on different parts of the DNA (usually one chromosome or its part) which are inherited together







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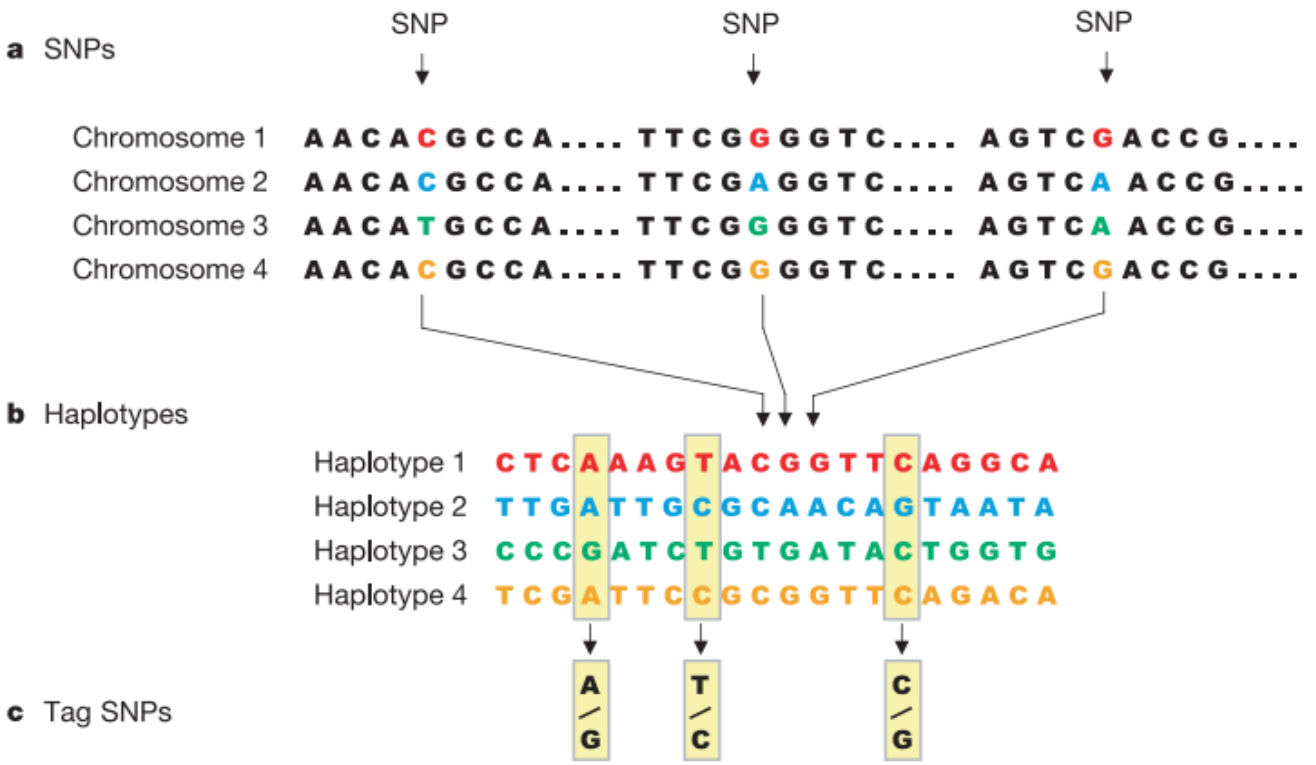
Linkage disequilibrium (LD) and imputation



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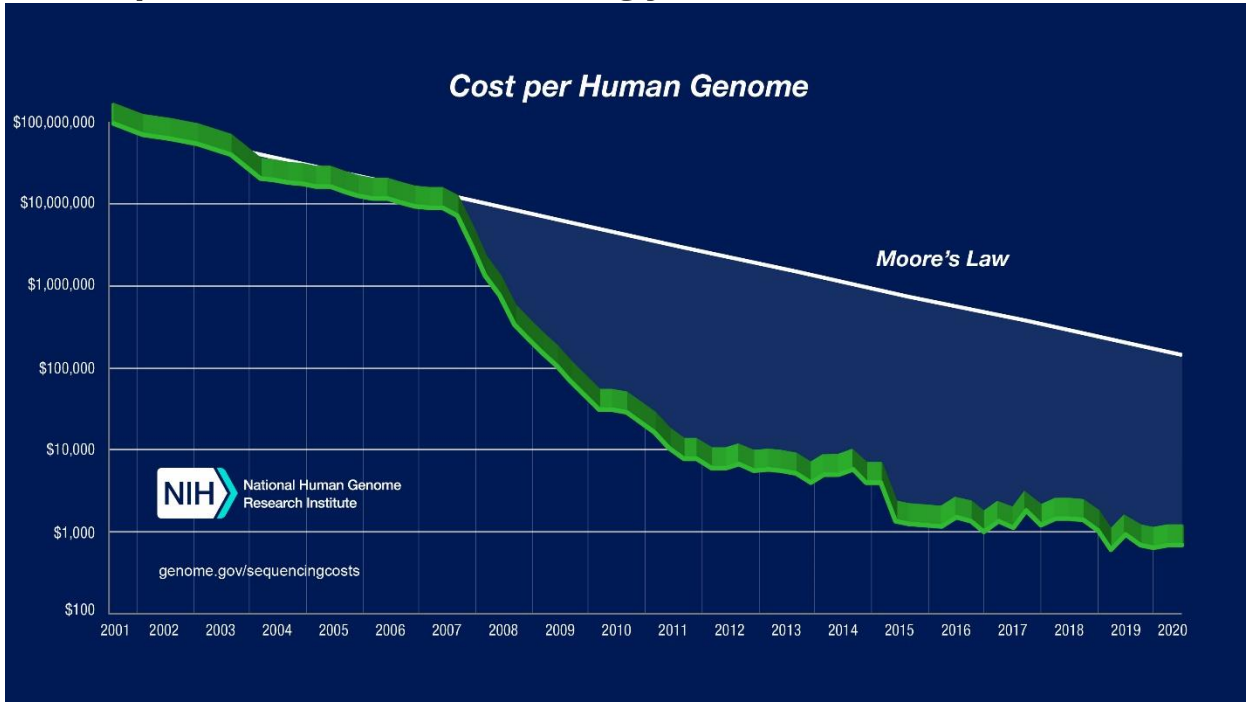
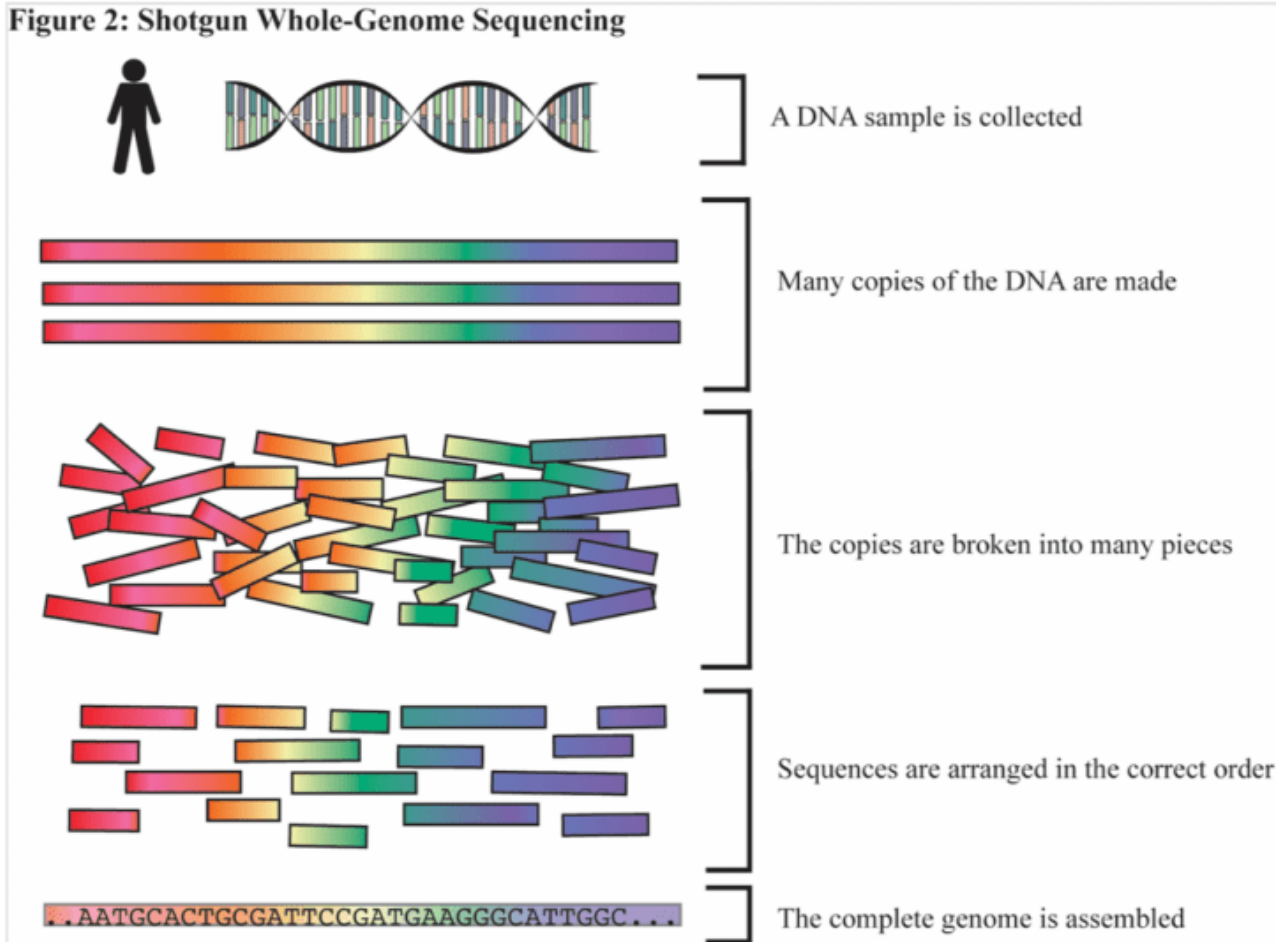
Human genome project

- Started in 1980´s, results published in 2001
- Estimated cost approx. $3 billions and 50 thousand „man-years“
  - Approx. 1/3 of cost for moon landing
- At the beginning under the jurisdiction of Department of Energy of the USA (labs and scientist all over the world were enrolled), later private company started to compete (Celera Genomics)
- Race in the sequencing has begun



Human genome project

- Celera wanted to keep its result private and sell them for profit – in the contrast to the government project
- Results were published at 15.2.2001 in the Nature (gov.) and 16.2.2001 in the Science (Celera project)
- Map of human genome was established, but without variability between individuals



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The HapMap Project

- DNA between each other is different in only about 0.1% of nucleotides - most commonly SNPs, which is known about 10 millions. These SNPs represents about 90% of total genome variability (rest are mutation, deletion and insertion)
- Based on math and statistic approx. 45 unrelated samples should be able to find 99% of all haplotypes with frequency higher than 5%

The HapMap Project

- Started in 2002 – two phases – at first production of „blank map“ and then fill up the blank spaces
- In the first phase was found about 1 mil of SNPs – results in 2005
- Second phase found another 2 mil of SNPs – results in 2007
- **Discovery of approx. 1 mil of LD blocks**
- Scientists from all over the world were enrolled
- Samples from USA, China, Japan, Kenya, UK, Canada

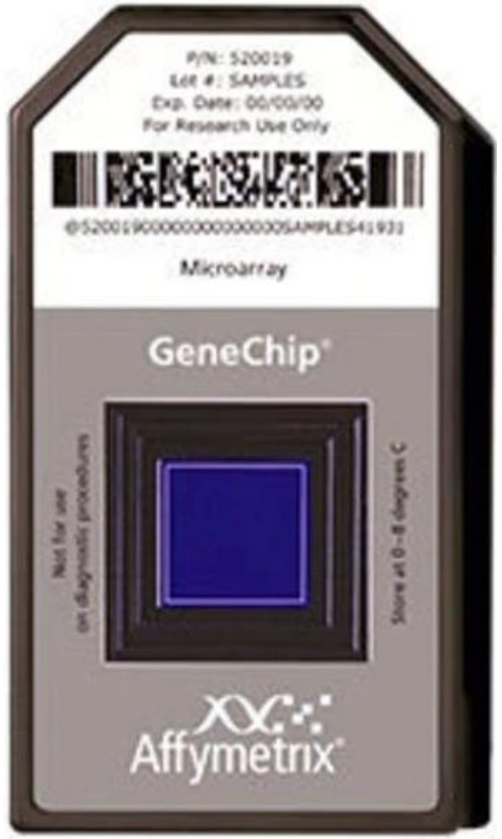
Genome-wide association studies (GWAS)

Combination of epidemiologic studies and new possibilities of genotyping

Ten thousands up to hundred of thousands of SNPs are determined (+ imputation and LD)

Need for huge set of patients, thousands more likely tens of thousands (control group + group with studied phenotype)

Necessary to proper describe phenotype of both, patients and control group



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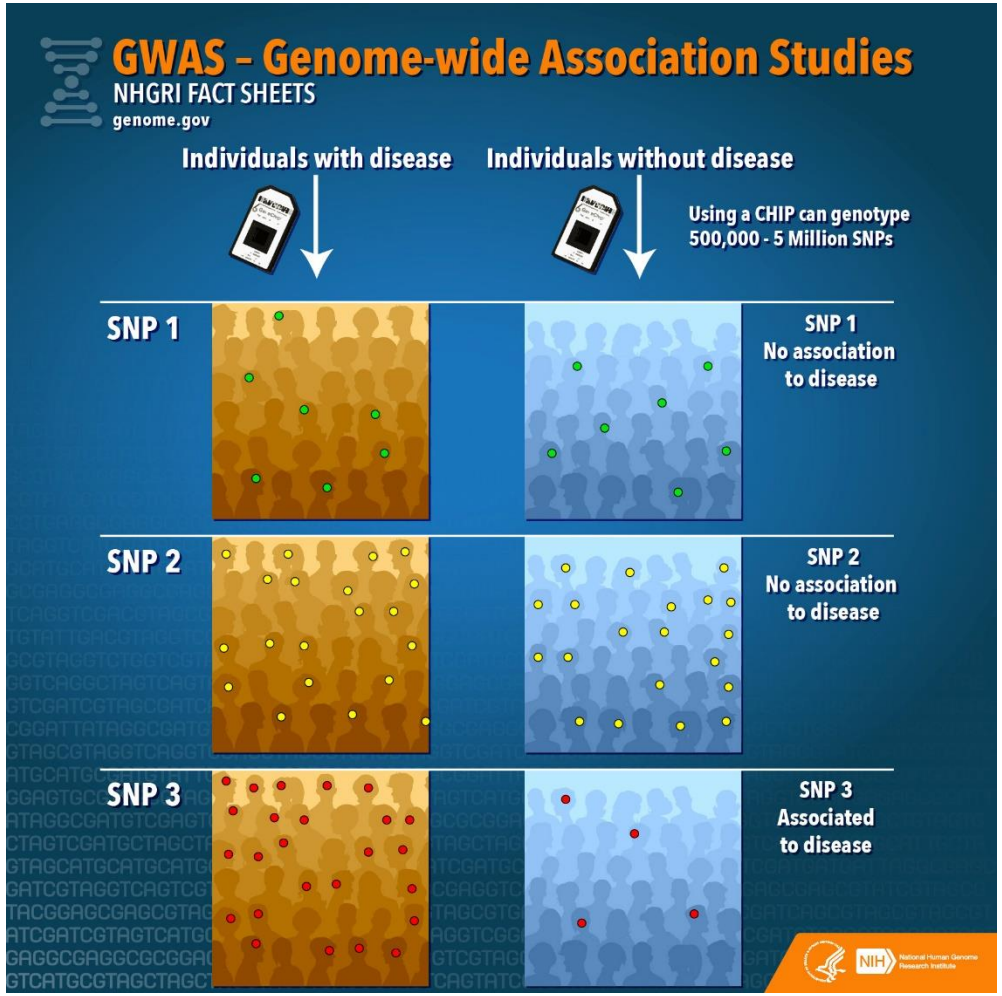
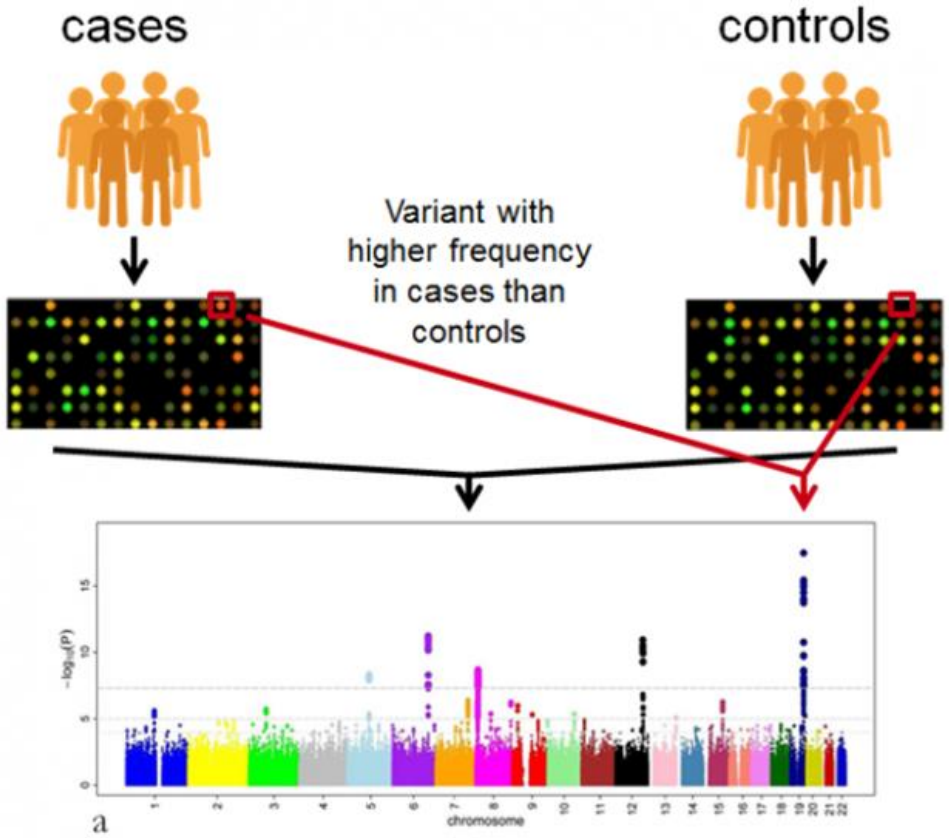
GWAS





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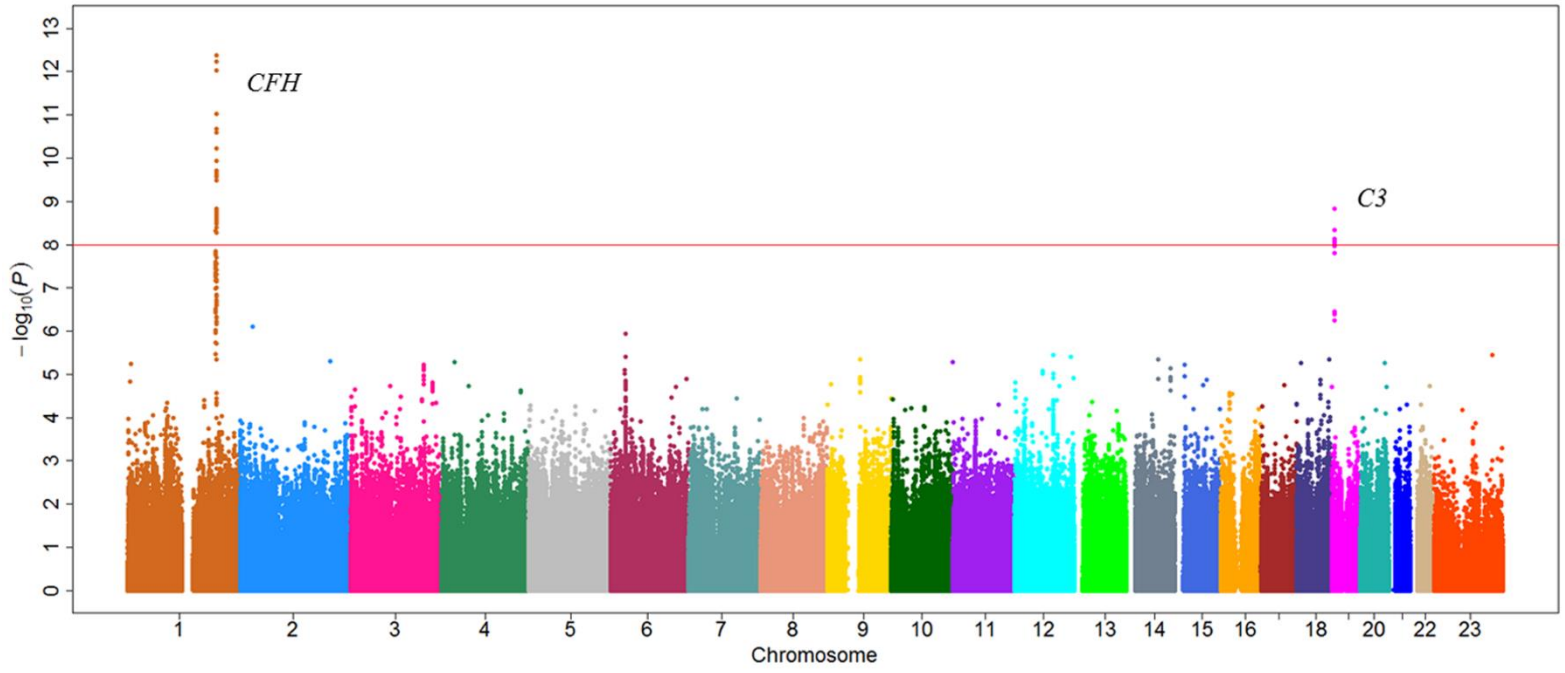
GWAS

- Great computing power is necessary for evaluation (approx few GBs for one patient and approx. 15 TB for 10 000 patients)
- As statistically significant is considered $P < 5*10^{-8}$
- P values between $1*10^{-6}$ to $5*10^{-8}$ are further replicated for possible association



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GWAS – pros and cons

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- Successful method for findings of new variants associated with given phenotype
  - Approx. 40 000 SNPs associated with different traits (cancers, T2DM, anorexia, depression, schizophrenia, BMI, insomnia,…)
- Could lead to discovery of new biological mechanism
  - Study of associated SNPs and their function
- Wide clinical application
  - Identification of risk groups of patients
  - Genetic risk score
- GWAS are able to explain differences between various ethnics in the complex trait
  - E.g. T2DM

-

- Each variant, by itself, have very limited indicative power
- Huge amount of patient is needed
  - Due to high demand for statistical power
- SNPs associated in GWAs represent only portion of inheritability of complex diseases
  - It is estimated that 1/3 to 2/3 of total heritability of complex diseases
- GWAS are able to find only locus associated with trait, not specific SNP
  - Another steps for determination of specific SNP are needed
- Can not find all variants associated with defined trait
  - Hard to find common variant with low effect or very rare variants with big impact

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GWAS – pros and cons

+

- Can find genetic variants with low frequency in population
  - Bigger set of patients, rarer SNPs can be associated
- Data can be used in another use
  - Determination of ancestry, estimate place of birth, forensic analysis, paternity,…
- Data can be loaded and shared to public databases
- Data presented so far represent only tip of the iceberg
  - Bigger set of patients the better information we can get
- Reliable genotyping technology
- Cheap method (price/performance ratio)

-

- Population stratification
  - Differences in allele frequency between patient and controls can be caused by different ancestry rather than association for the gene with specific trait
- Limited clinical predictive ability
  - Rare to predict disease based on specific variant
  - GRS
- Need to know genetic background of investigated population
  - LD can differ between ethnics
  - Could be problem in native Americans, island nation in Pacific, Pygmy
- Does not count with gene-environment interaction
- Big team with various expert is needed for this kind of study

What does the studies say?

- First GWAS studying childhood caries
- 1305 children at age 3-12 years
- Genotyped 580 000 SNPs, with imputation 1,4 M SNPs
- No significant SNPs found

J Dent Res. 2011 Dec;90(12):1457-62. doi: 10.1177/0022034511422910. Epub 2011 Sep 21.

Genome-wide association scan for childhood caries implicates novel genes

J R Shaffer [1], X Wang, E Feingold, M Lee, F Begum, D E Weeks, K T Cuenco, M M Barmada, S K Wendell, D R Crosslin, C C Laurie, K F Doheny, E W Pugh, Q Zhang, B Feenstra, F Geller, H A Boyd, H Zhang, M Melbye, J C Murray, R J Weyant, R Crout, D W McNeil, S M Levy, R L Slayton, M C Willing, B Broffitt, A R Vieira, M L Marazita

Affiliations + expand

PMID: 21940522 PMCID: PMC3215757 DOI: 10.1177/0022034511422910

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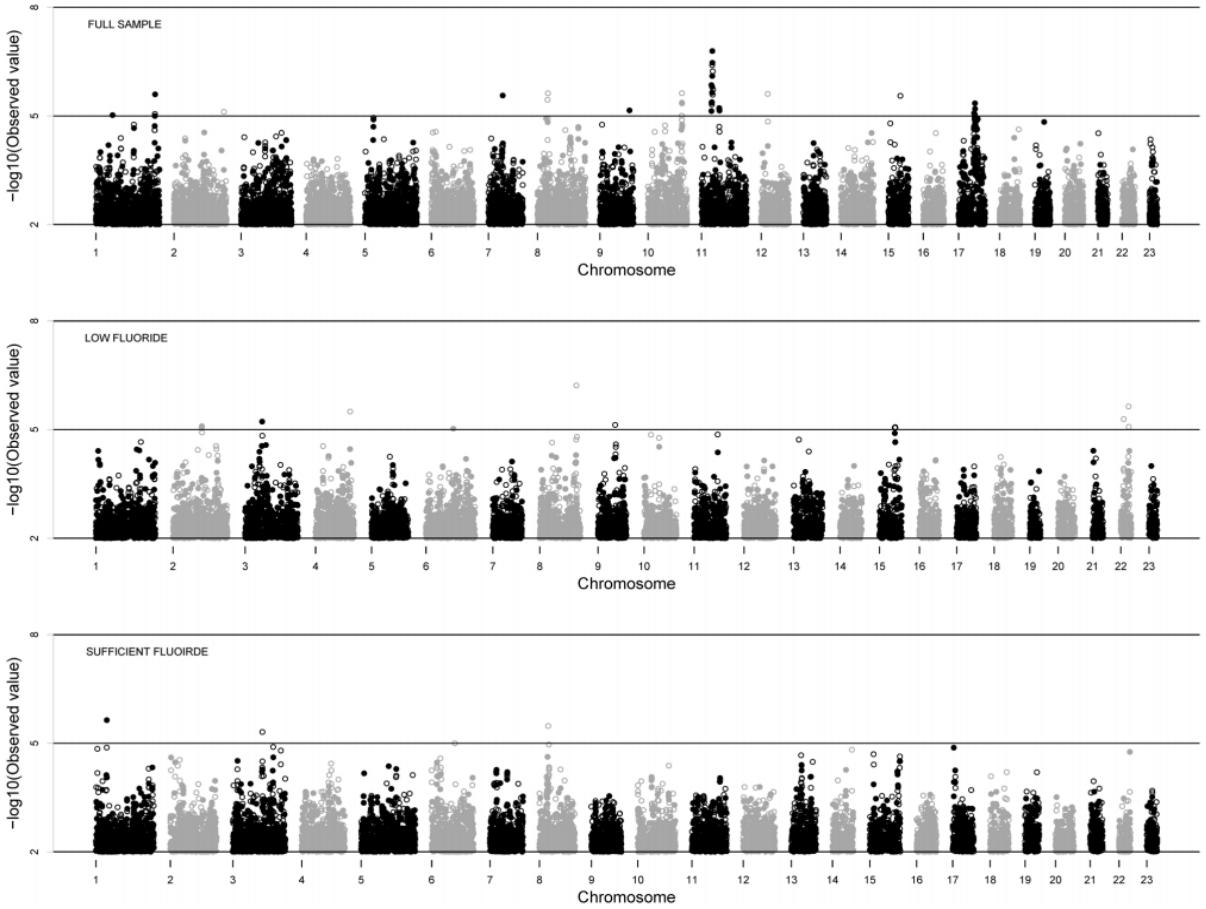
Shaffer et al.



- No significant SNPs were found

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Shaffer et al.

- 920 participants at age18-75 years
- 520 000 SNPs
- Patients were divided into groups based on DMFS (decay-missing-filled surface index)

J Dent Res. 2013 Jan;92(1):38-44. doi: 10.1177/0022034512463579. Epub 2012 Oct 11.

GWAS of dental caries patterns in the permanent dentition

J R Shaffer [1], E Feingold, X Wang, M Lee, K Tcuenco, D E Weeks, R J Weyant, R Crout, D W McNeil, M L Marazita

Affiliations + expand

PMID: 23064961 PMCID: PMC3521449 DOI: 10.1177/0022034512463579

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- Two significant locus were found
  - AJAP1 – involved in development of the tooth together with MMP
  - LYZL2 – lysozyme-like gene, bacteriolytic factor
- Another 31 „suspicious“ loci

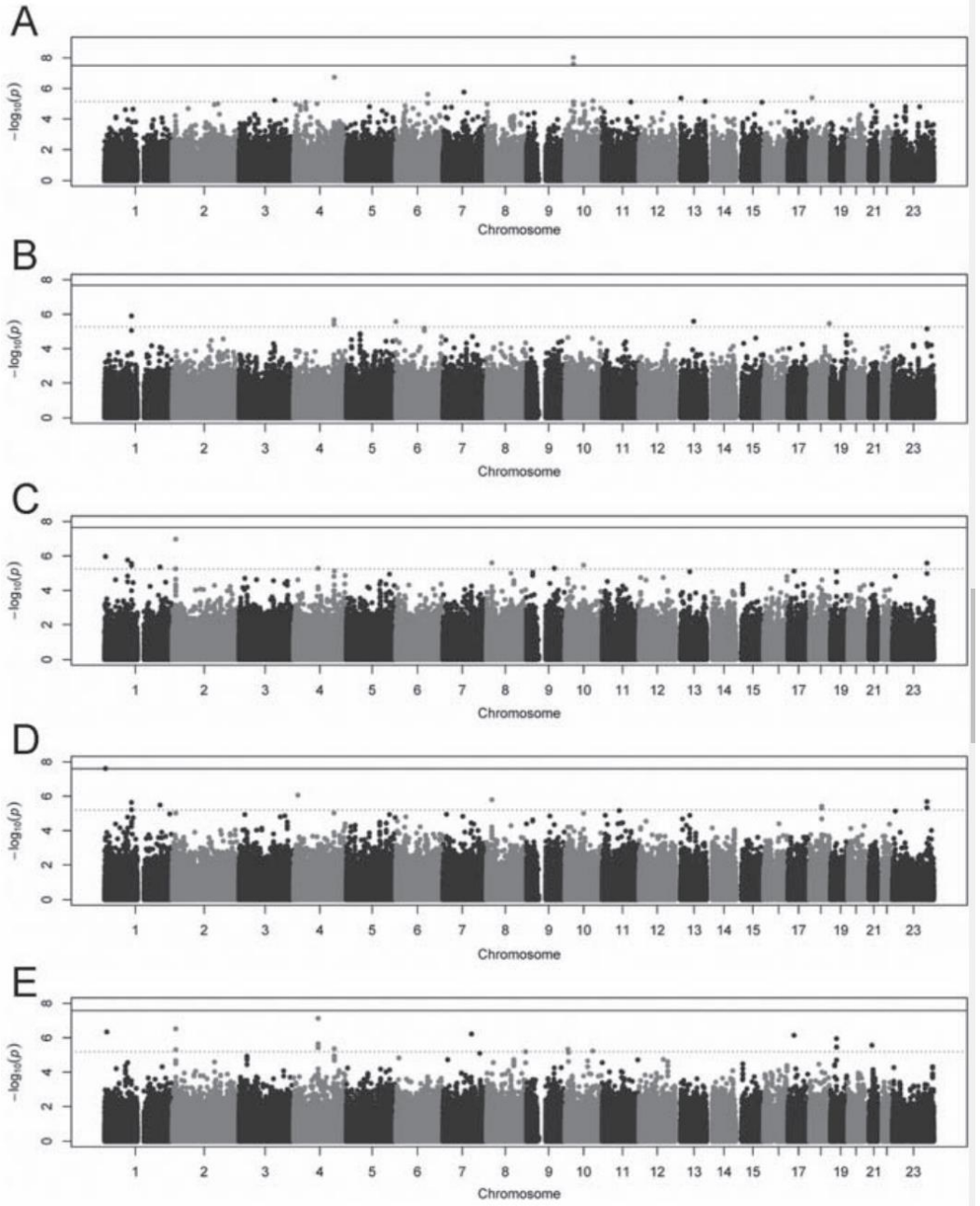

Figure. Manhattan plots showing GWAS results for **(A)** DMFS2, **(B)** DMFS3, **(C)** DMFS5, **(D)** $DMFS5_{max}$, and **(E)** $DMFS5_{mand}$. Solid lines represent thresholds for genome-wide significance (p value < $10^{-7.3}$). Dotted lines represent thresholds for suggestive significance (p value < 10^{-5})

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Zeng et al.

- Two sets of patients – 1006 children at age 3-12 (SM) and 979 children at age 4-14 (PF)
  - DMFS divided into two phenotypes – smooth teeth surface and teeth with fissure
- Genotyped 530 000 SNPs, with imputation 1 200 000 SNPs

› Caries Res. 2014;48(4):330-8. doi: 10.1159/000356299.

Genome-wide association study of primary dentition pit-and-fissure and smooth surface caries

Z Zeng, E Feingold, X Wang, D E Weeks, M Lee, D T Cuenco, B Broffitt, R J Weyant, R Crout, D W McNeil, S M Levy, M L Marazita, J R Shaffer

PMID: 24556642 PMCID: PMC4043868 DOI: 10.1159/000356299

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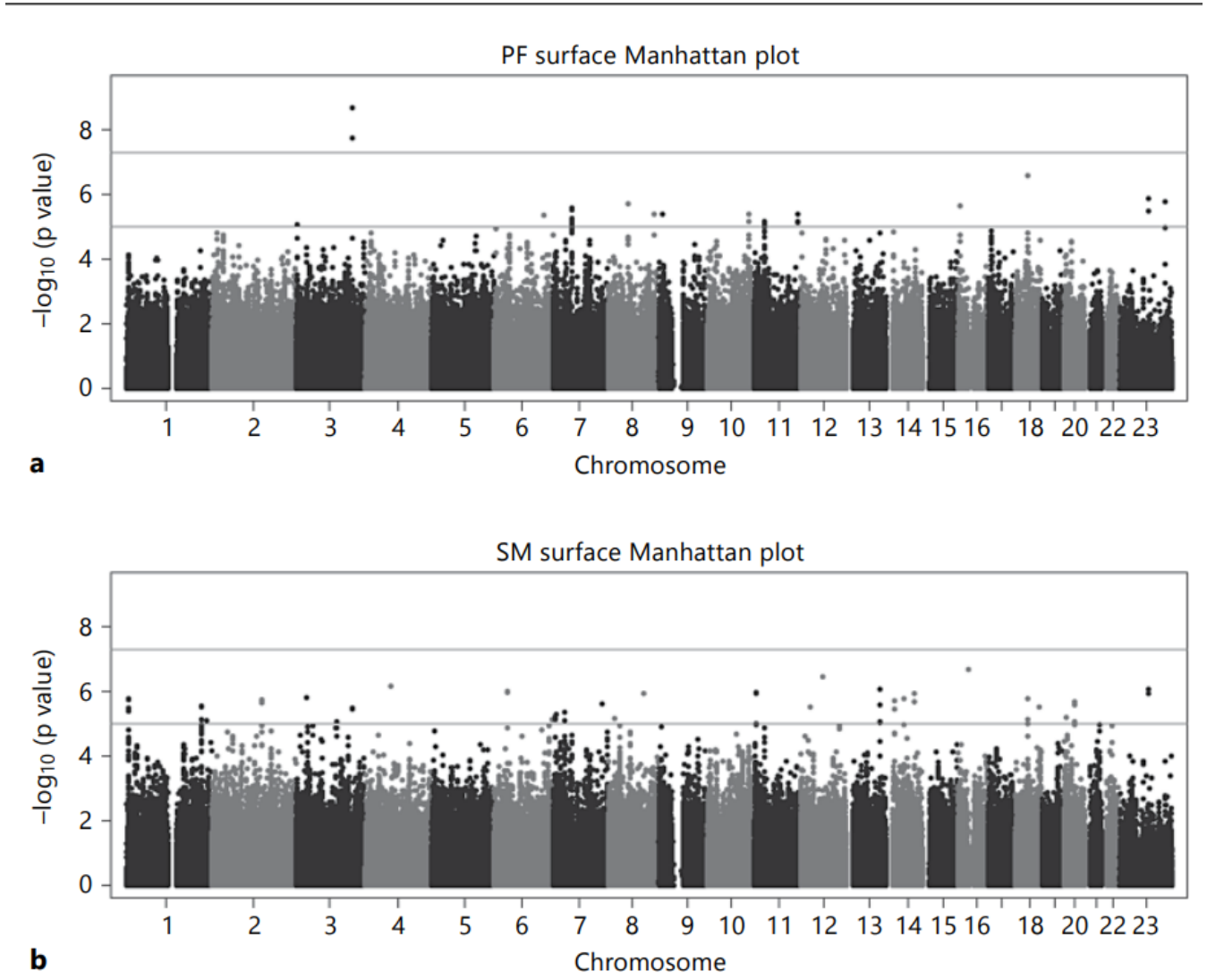
- In PF group KPNA4 gene was significantly associated
- No statistically significant association in SM group
- Another 5 suspicious loci



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Shungin et al.

- Two biobanks were used – UKB and GLIDE (Gene-lifestyle interactions in dental endpoints)
  - Over 500 000 patients
- Genotyped approx. 500 000 SNPs + imputation (together 8.9M SNPs)
- 47 new variants were associated with dental caries

Article | Open Access | Published: 24 June 2019

Genome-wide analysis of dental caries and periodontitis combining clinical and self-reported data

Dmitry Shungin, Simon Haworth ✉, [...] Ingegerd Johansson

Nature Communications **10**, Article number: 2773 (2019) | Cite this article

7904 Accesses | **30** Citations | **129** Altmetric | Metrics

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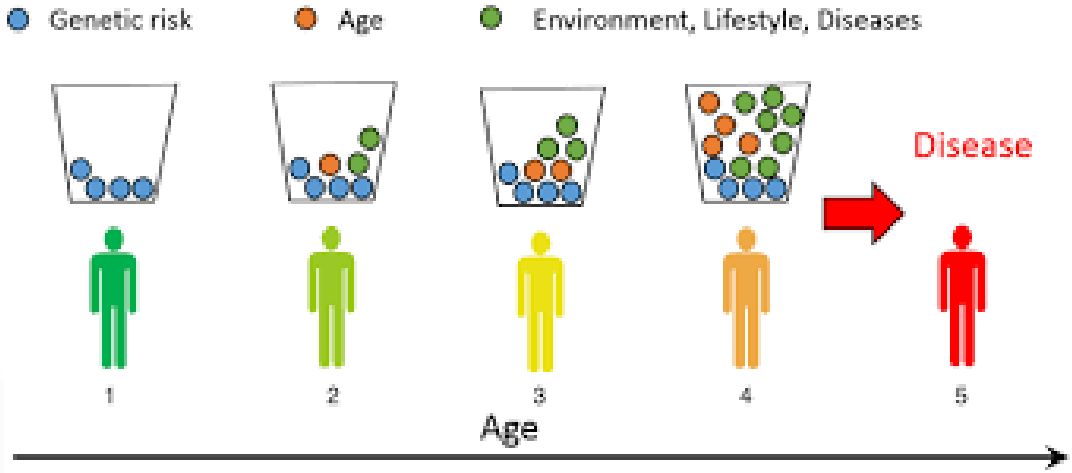
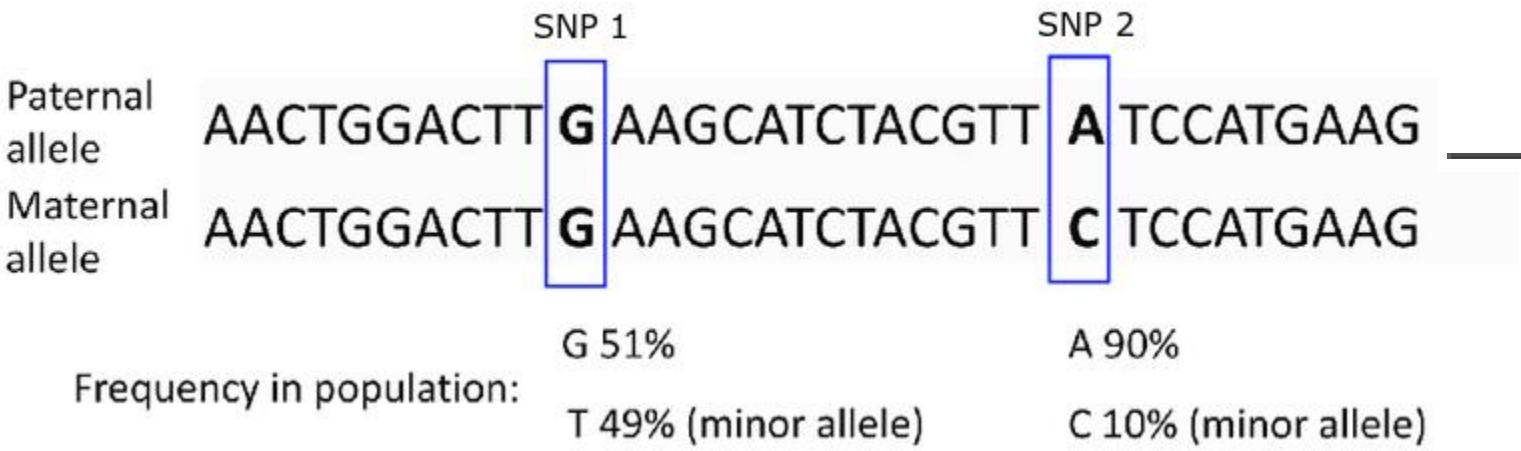
Genetic/polygenic risk score (GRS/PRS)

- Number, determining risk of development of observed phenotype
- Weighted and unweighted





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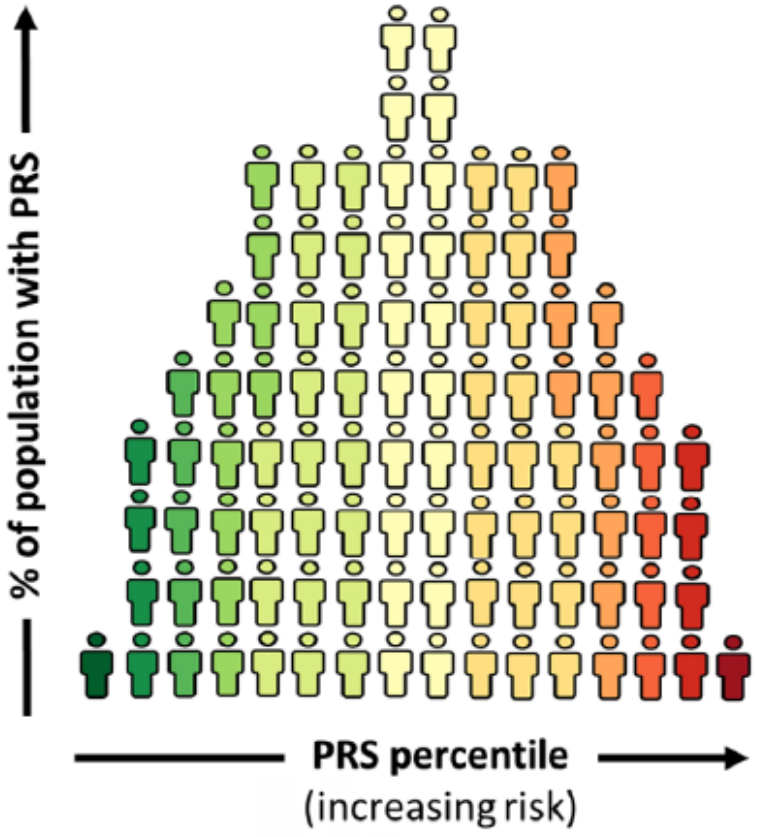
Genetic/polygenic risk score (GRS/PRS)

PRS percentile	Risk of disease vs. reference group
0-1	Lowest
1-5	
5-10	
10-20	
20-40	
40-60 (reference)	1
60-80	
80-90	
90-95	
95-99	
99-100	Highest



Source: RGA

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Morelli et al.

- 40 most strongly associated SNPs from the GWAS and they constructed unweighted GRS
  - Theoretical values 0-80, mean 37,1 ± 3,9; range of values 24 – 52
  - European-American population

Review > Periodontol 2000. 2020 Feb;82(1):143-156. doi: 10.1111/prd.12320.

Genomics of periodontal disease and tooth morbidity

Thiago Morelli [1], Cary S Agler [2], Kimon Divaris [3 4]

Affiliations + expand

PMID: 31850632 PMCID: PMC6972532 DOI: 10.1111/prd.12320

Free PMC article

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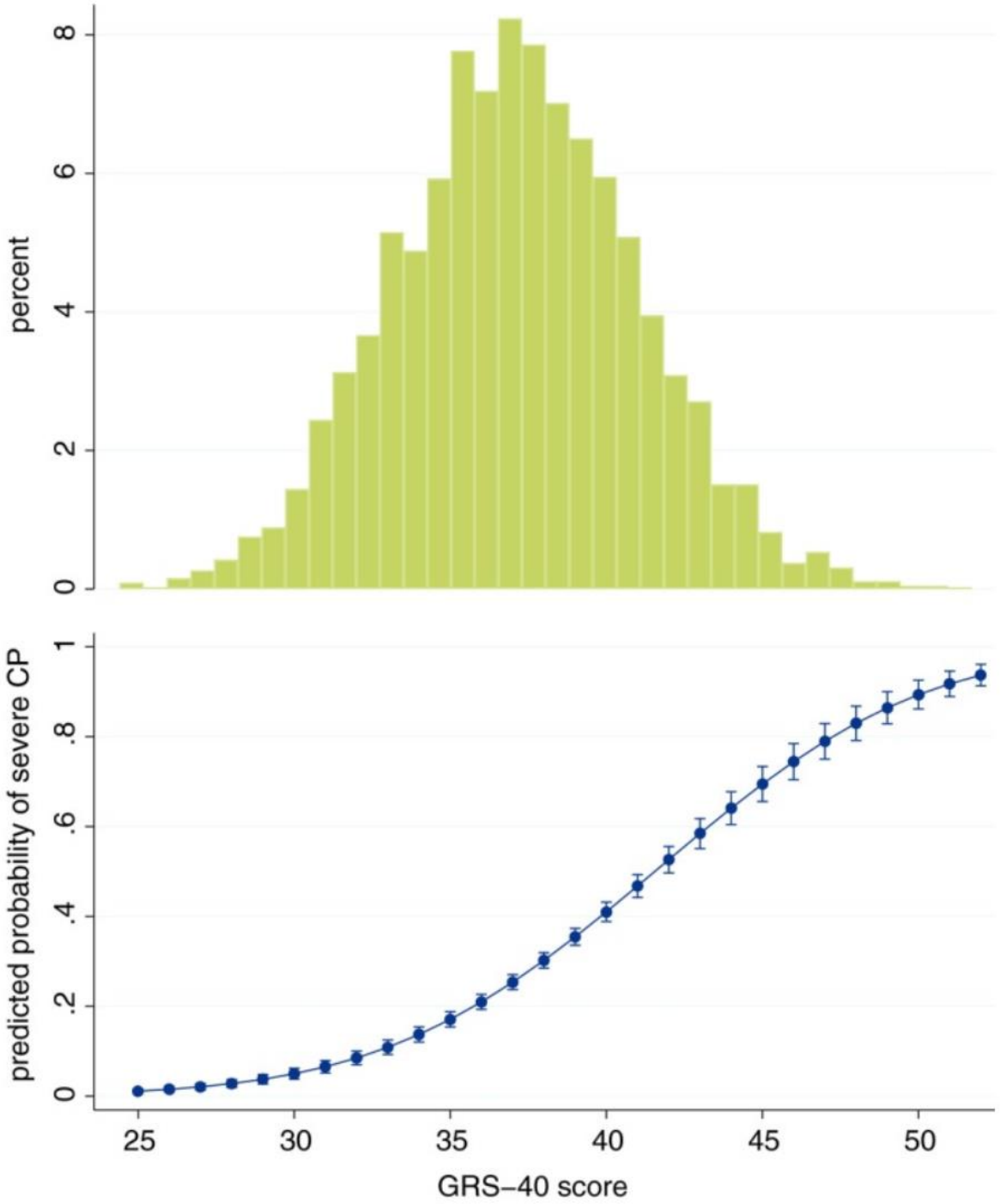
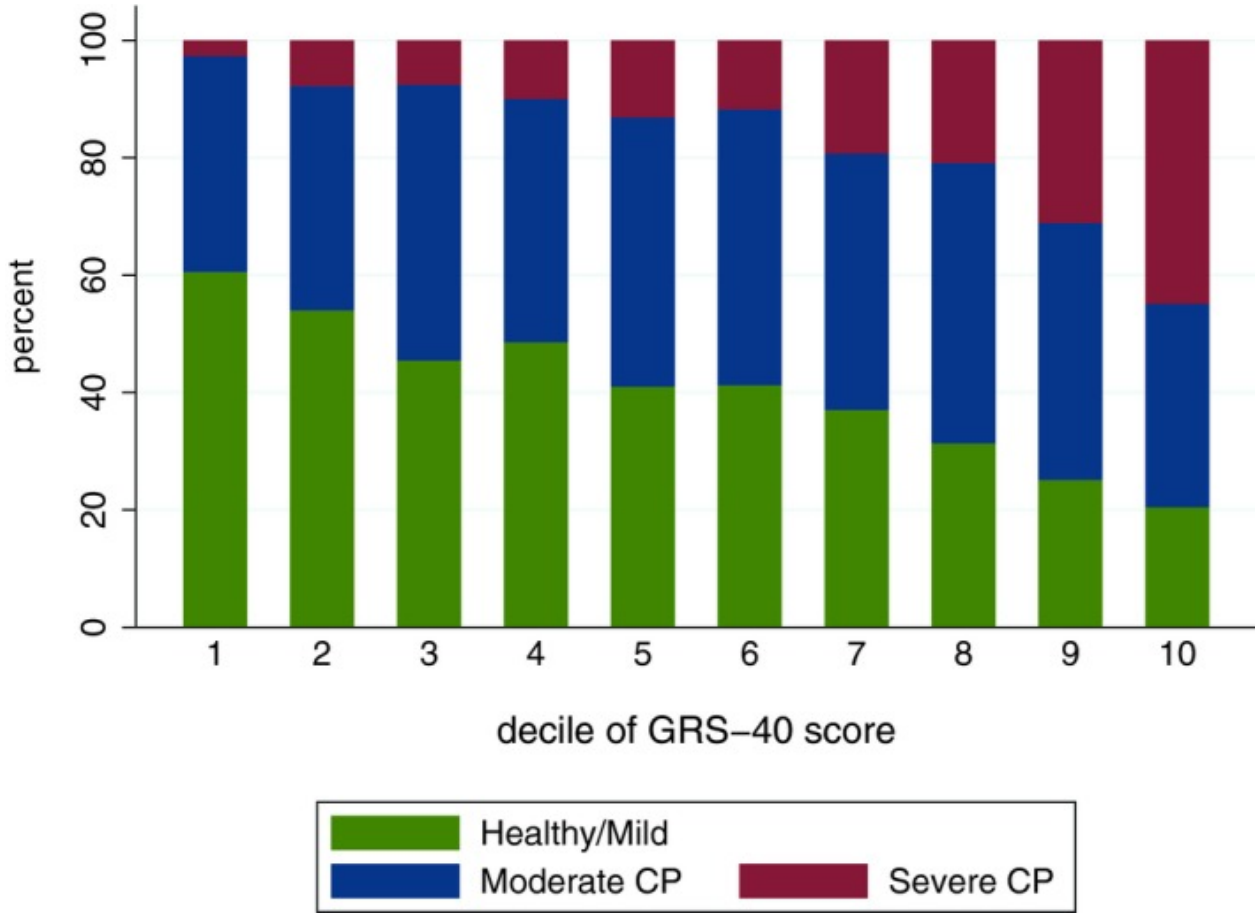

percent
0
2
4
6
8
predicted probability of severe CP
0
.2
.4
.6
.8
1
25
30
35
40
45
50
GRS–40 score


percent
0
20
40
60
80
100
1
2
3
4
5
6
7
8
9
10
decile of GRS–40 score
Healthy/Mild
Moderate CP
Severe CP

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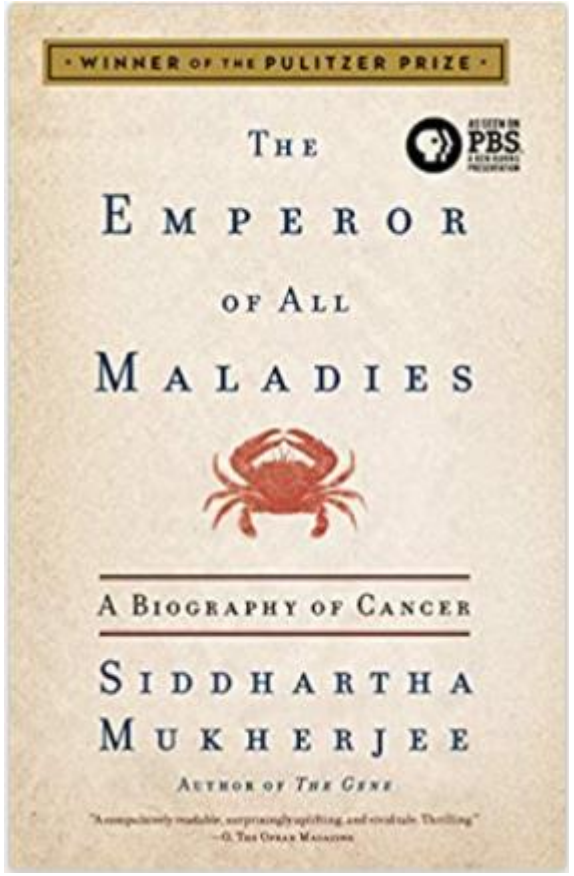
Morelli et al.

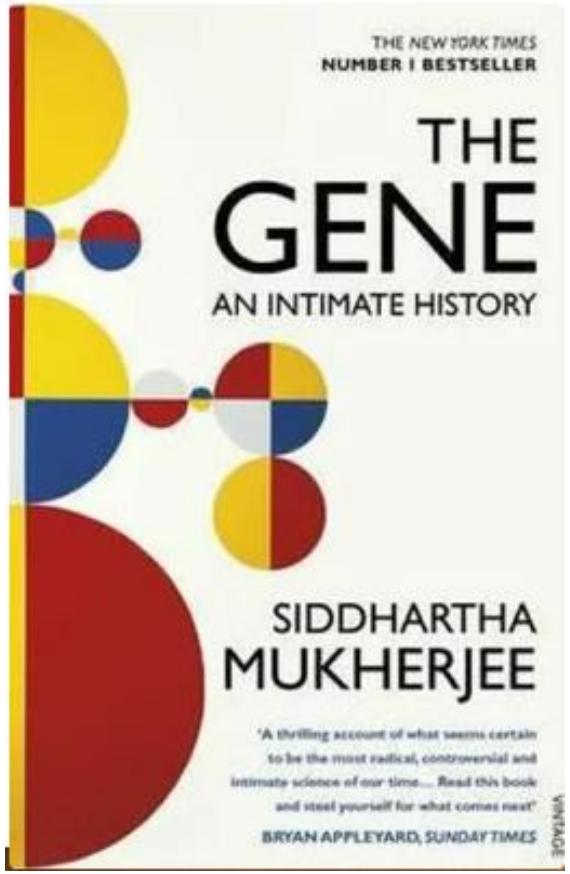
- Authors posted three reasons why these score need further adjustment
  - SNPs used in this study were associated only on one set of patients – does not have to be true for other ethic groups. At first validation ad replication of the results are needed
  - Participants were at middle age and only European-American ancestry
  - Other factors than genetic may play a role on progression of diseases in the oral cavity (habits, socio-economical status, dental care access)
- Tendency to create universal GRS for all people capable of determining individual risk for particular disease. These individuals could be under more frequent screening, they could alter their habits,…

Conclusion

- Era before GWAS
- What are the GWAS – pros and cons
- Summarizing of recent GWAS studies
- Construction of GRS

Recommended literature





Interview with Eric Lander: https://www.ceskatelevize.cz/porady/10441294653-hyde-park-civilizace/220411058090919/

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