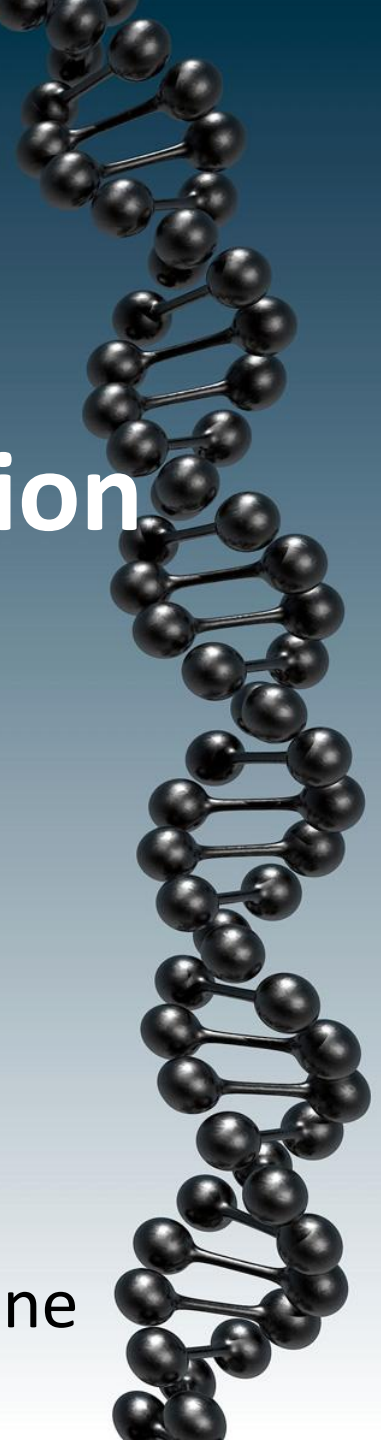
Genetics of gene expression



Stephen Montgomery
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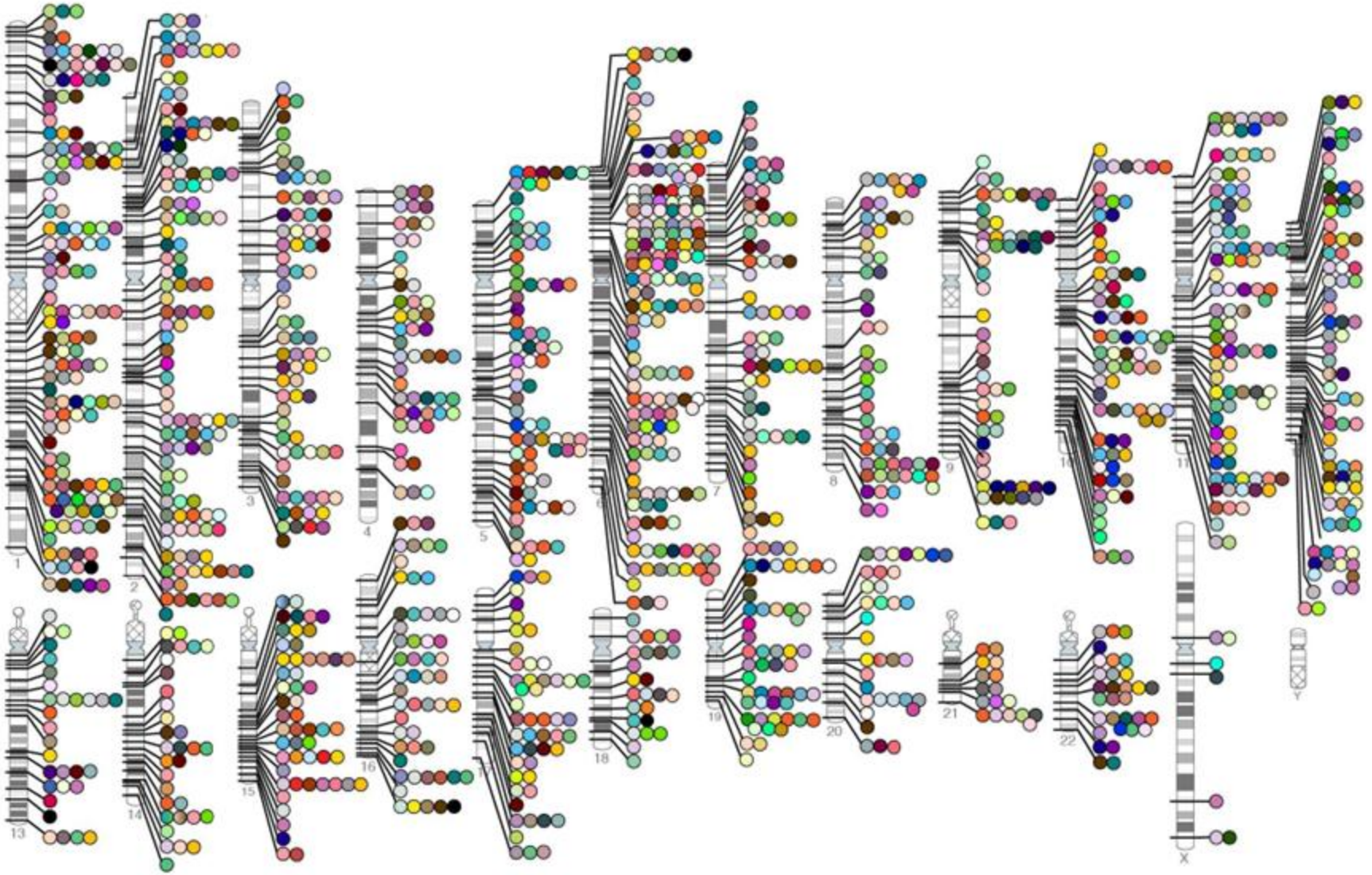
Stanford University School of Medicine

Chromosome map of disease-associated regions



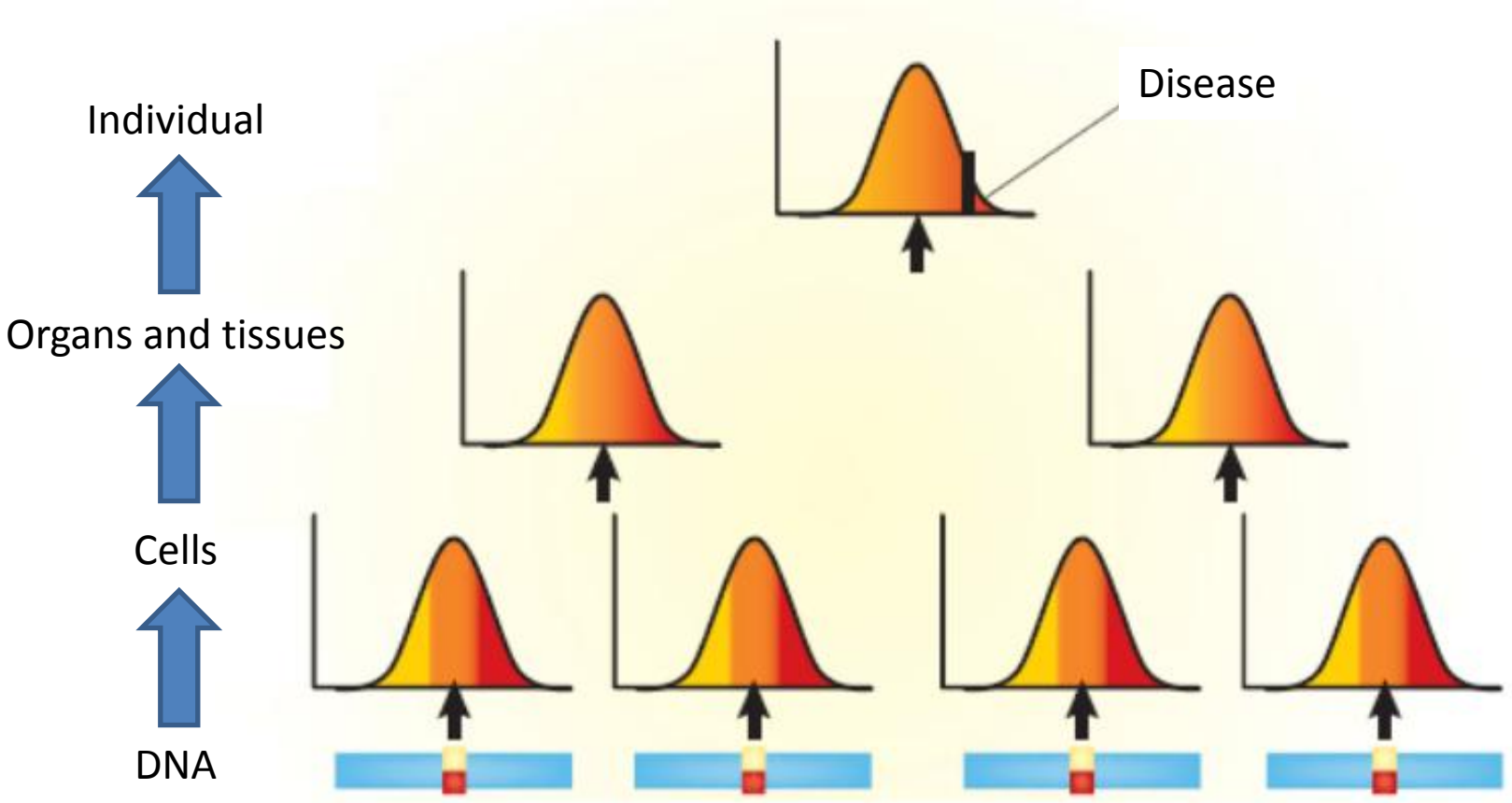
"GWAS have so far identified only a small fraction of the heritability of common diseases, so the ability to make meaningful predictions is still quite limited"

Francis Collins, Director of the NIH, *Nature*, April 2010

Trait	Heritability	Individuals studied	Heritability explained
Coronary artery disease	40%	86995	10%
Type 2 Diabetes	40%	47117	10%
BMI	50%	249796	3%
Blood pressure	50%	34433	1%
Circulating lipids	50%	100000	25%
Height	80%	183727	12.5%

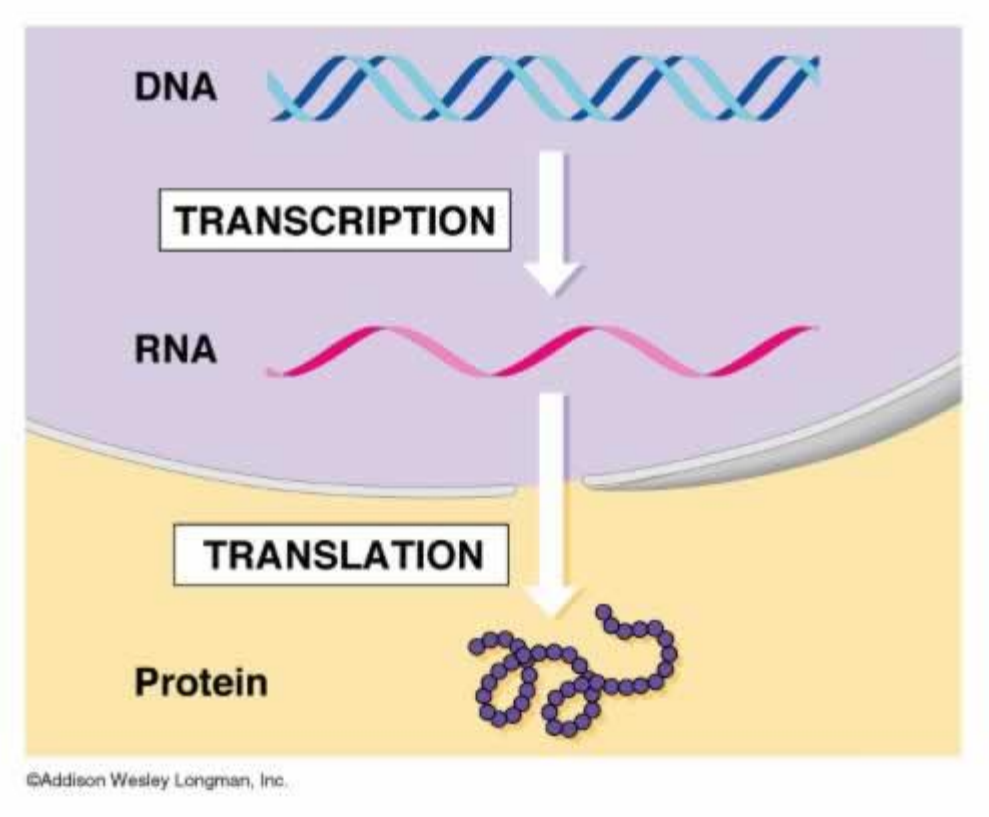
Where is the missing heritability?

Disease starts at a cellular level

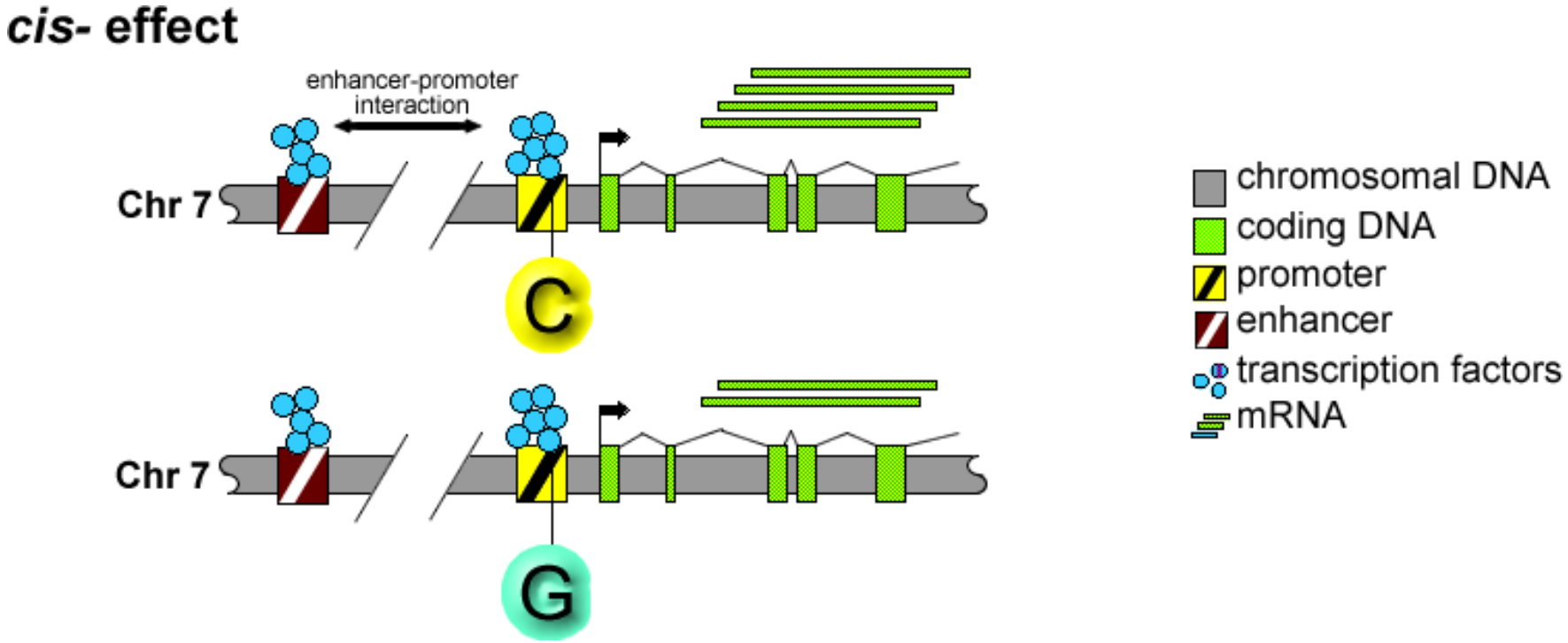


Understanding the influence of genetics on cells will improve our ability to predict disease risk

Genetics of gene expression

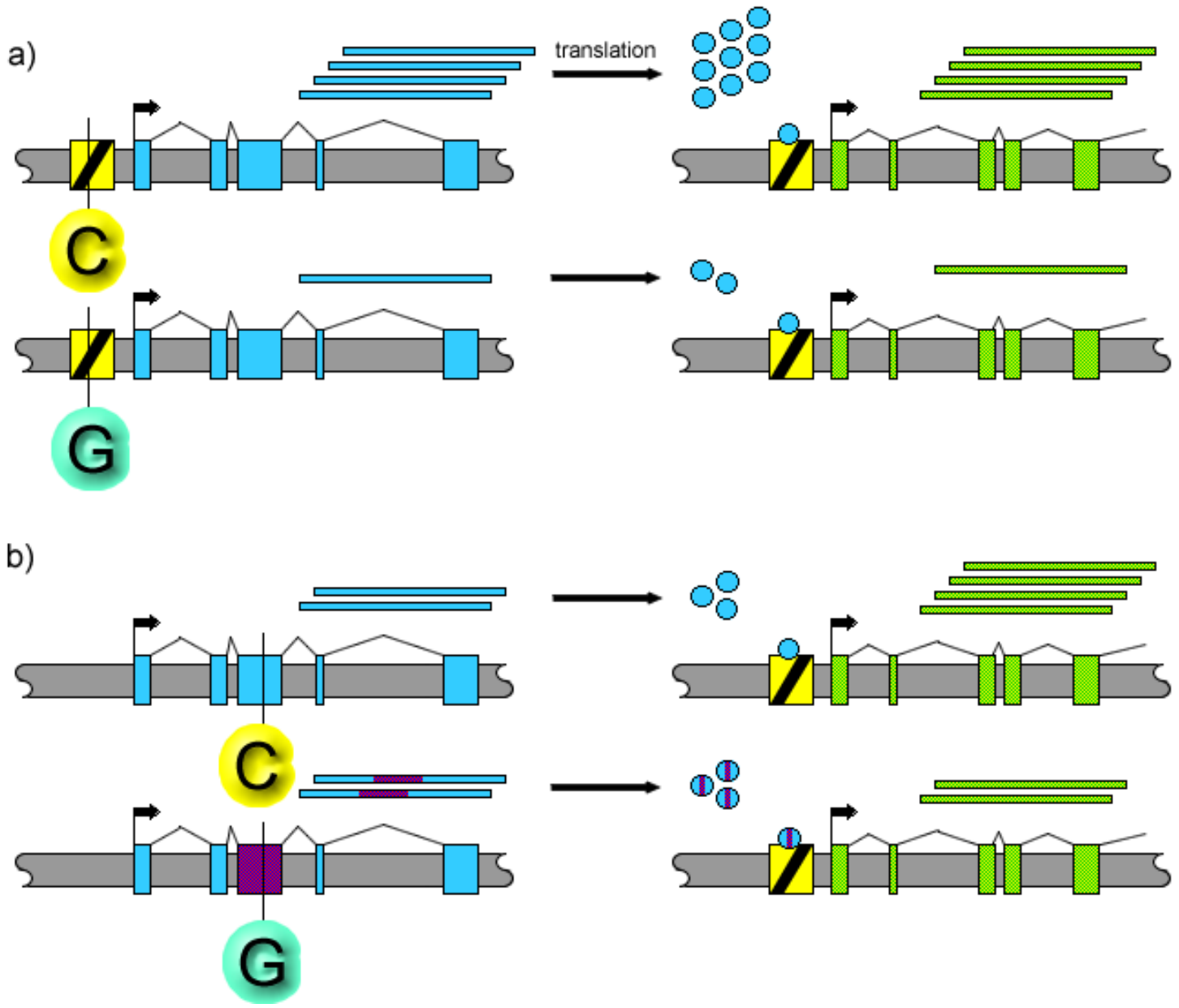


Insight into how genetic variants influence **transcription** in different tissues, individuals and populations

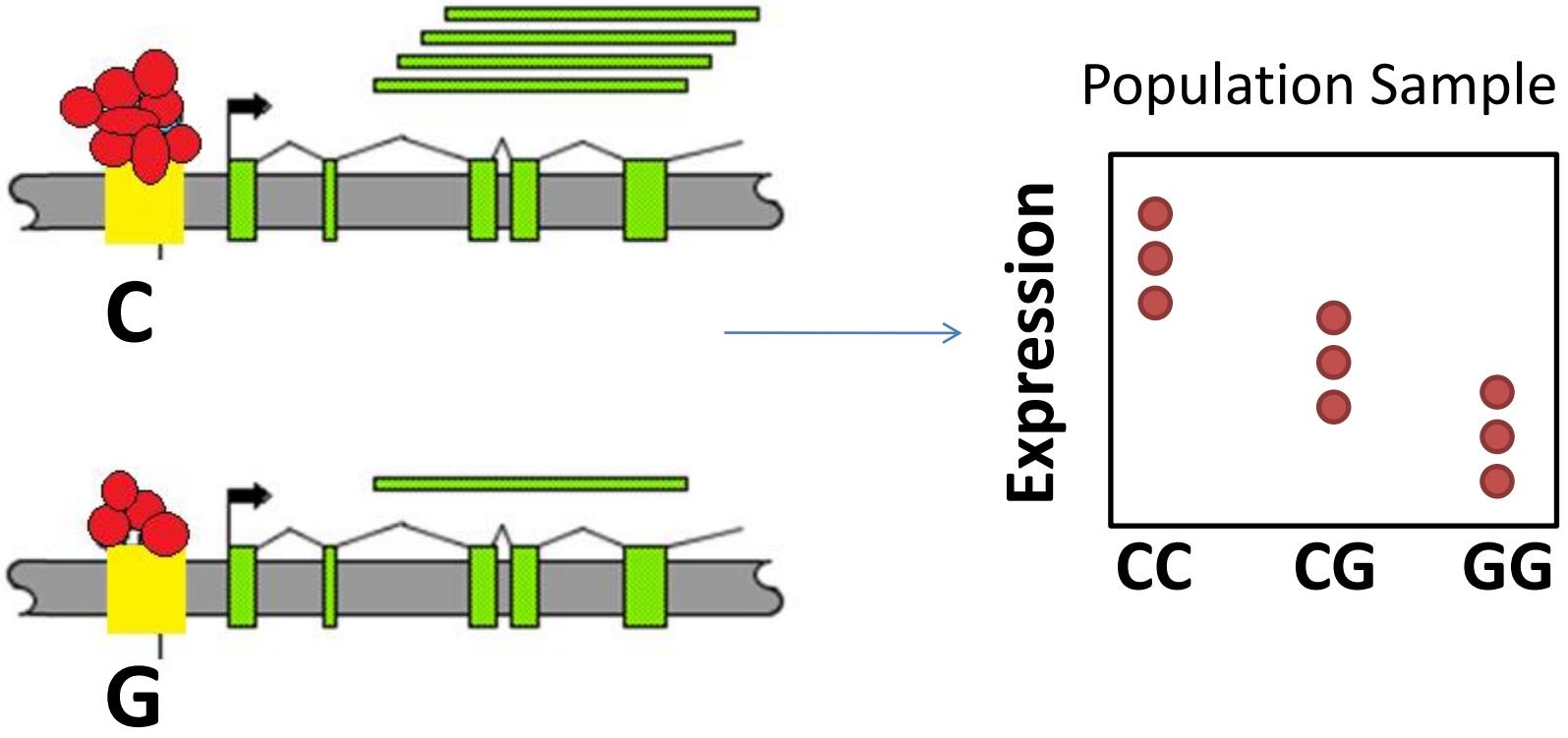


Canonical model

trans- effect

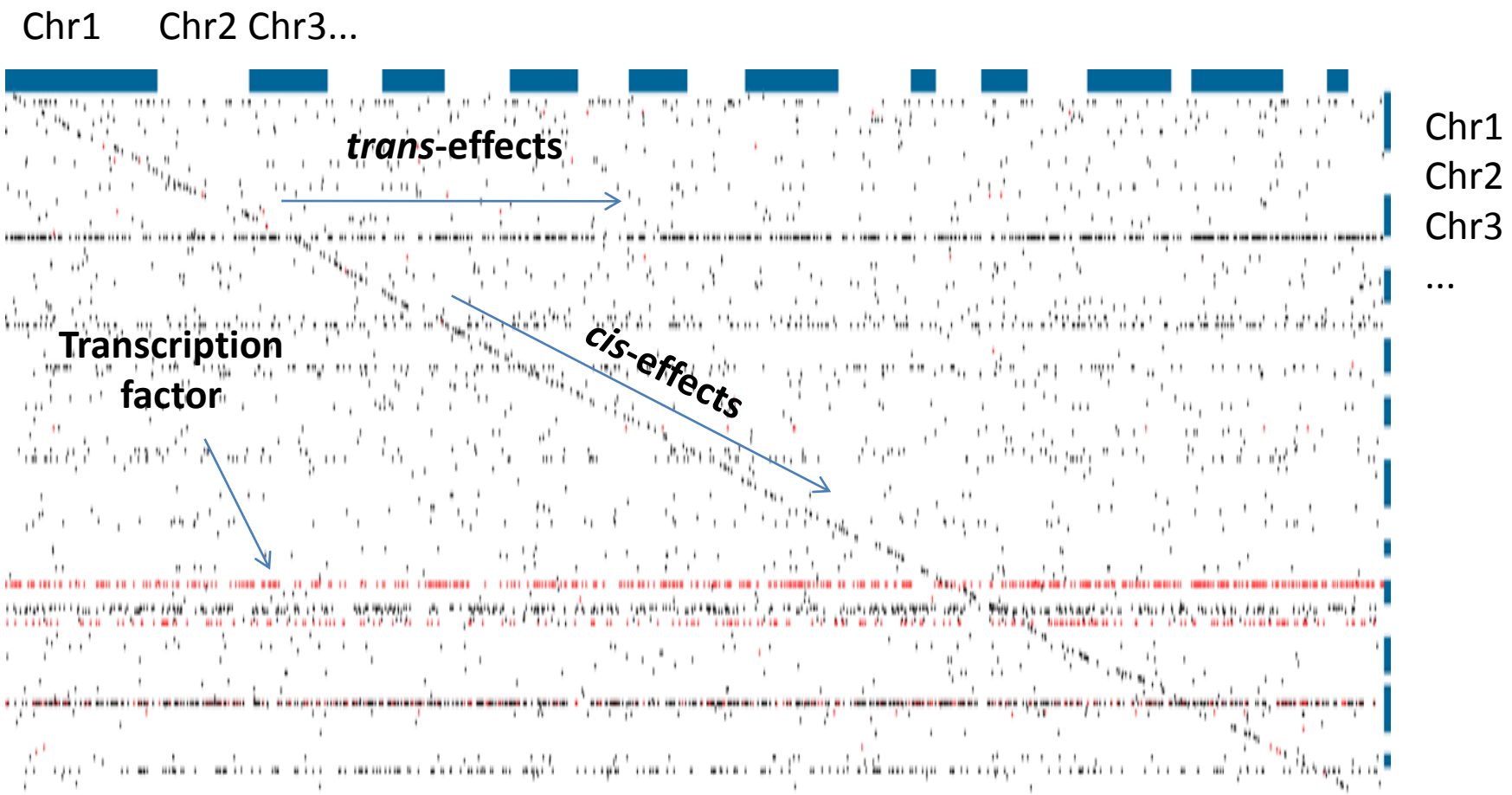


Genetic association can pinpoint regulatory haplotypes



We can identify genetic variants impacting gene expression (eQTLs)

THE LANDSCAPE OF REGULATORY VARIATION



Location of genetic variants by the gene's whose expression they impact

ADVANTAGES TO STUDYING GENETICS OF GENE EXPRESSION

Can rapidly evaluate 1000s of quantitative traits

Power to generalize patterns underlying classes of effects

Can easily transform or perturb the system

Direct connection to cellular mechanism!

Genetic differences in gene expression can identify candidate genes for gwas variants

Disease / trait study	Implicated eQTL genes
Asthma[24]	*ORMDL3*
Blood lipid levels[59,65]	*SORT1*, *PPP1R3B* and *TTC39B*
Body mass index[3]	*NEGR1*, *ZC3H4*, *TMEM160*, *MTCH2*, *NDUFS3*, *GTF3A*, *ADCY3*, *APOB48R*, *SH2B1*, *TUFM*, *GPRC5B*, *IQCK*, *SLC39A8*, *SULT1A1* and *SULT1A2*
Breast Cancer[66]	*RRP1B*
Celiac disease[2]	*MMEL1*, *NSF*, *PARK7*, *PLEK*, *TAGAP*, *RRP1*, *UBE2L3* and *ZMIZ1*
Crohn's disease[67] (add Franke reference, NG 2010)	*PTGER4*, *CARD9*, *ERAP2* and *TNFSF11*
Fat distribution[55]	*GRB14*
Height[58,68]	Multiple genes implicated
Kidney-aging[69]	*MMP20*
Migraine[4]	*MTDH*
Multiple diseases[70]	*CDKNA2A*, *CDKNA2B* and *ANRIL*
Osteoporosis-related[71,72]	*GPR177*, *MEF2C*, *FOXC2*, *IBSP*, *TBC1D8*, *OSBPL1A*, *RAP1A* and *TNFRSF11B*
Parkinson's[56,73]	*MAPT*, *LRRC37A*, *HLA-DRA*, *HLA-DQA2* and *HLA-DRB5*
Psoriasis[54]	*SDC4*, *SYS1*, *DBNDD2*, *PIGT* and *RPS26**
QRS duration and cardiac ventricular conduction[60]	*TKT*, *CDKN1A* and *C6orf204*
Type 2 diabetes[57,74]	*FADS1*, *FADS2*, *KLF14*, *CCNE2*, *IRS1*, *JAZF1* and *CAMK1D*

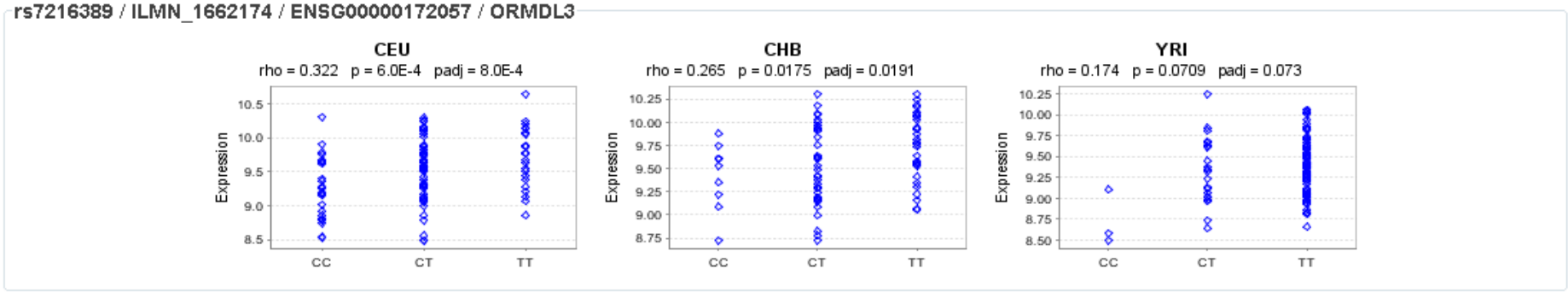
eQTL correlation helps pinpoint implicated genes and mode of effect

Montgomery, Nat Rev Genetics, 2011

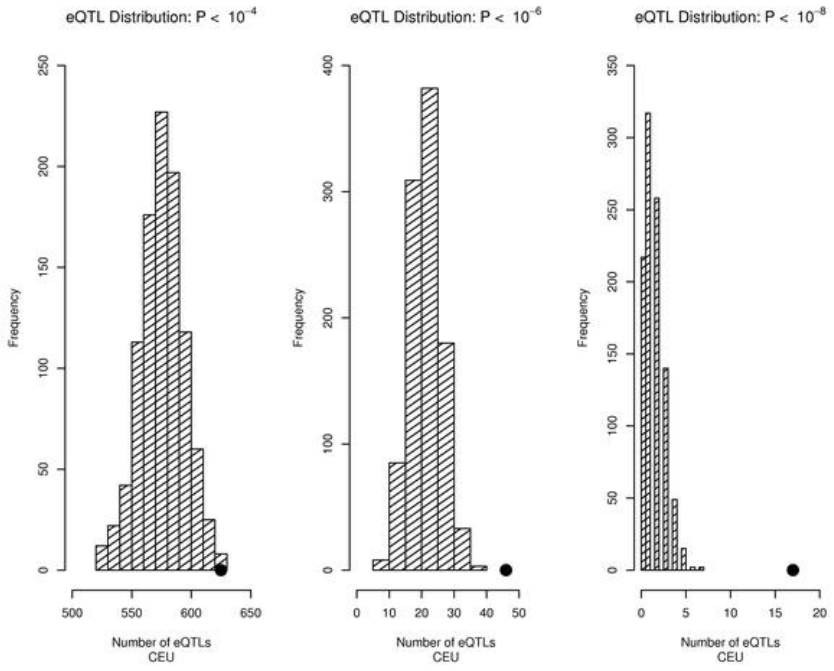
What are my asthma variants doing?

In the subset of individuals for whom expression data are available, the T nucleotide allele at *rs7216389* (the marker most strongly associated with disease in the combined GWA analysis) has a frequency of 62% amongst asthmatics compared to 52% in non-asthmatics (*P* = 0.005 in this sample).

Moffatt, Nature, 2007

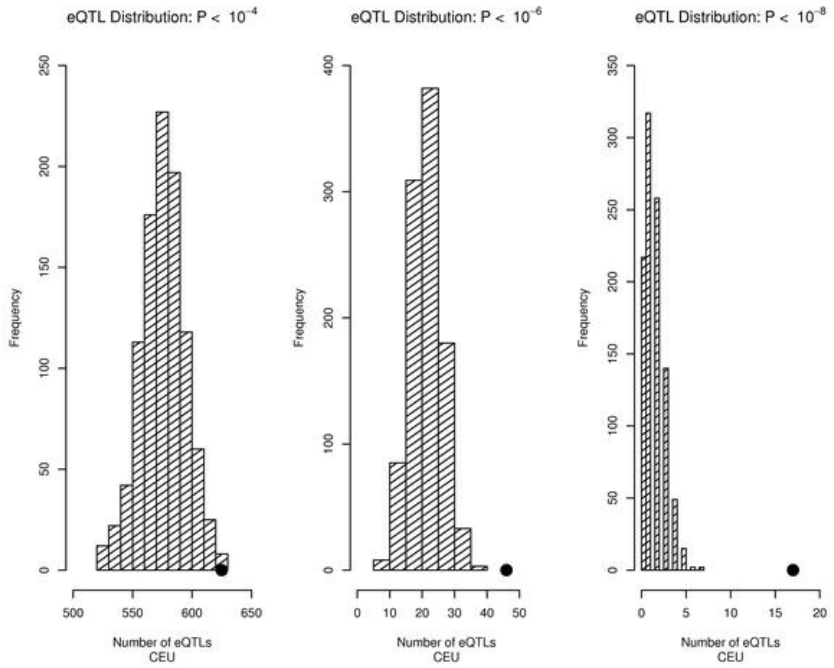


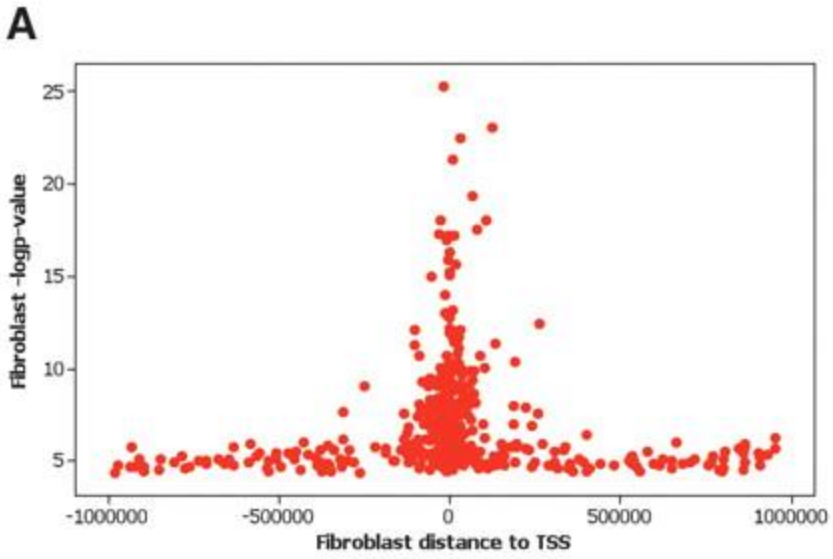
eQTLs are more likely to be trait SNPs



The null was a set of SNPs frequency matched to the GWAS SNPs.
Any problem with this?

GWA SNPs more likely to be near genes





The null was a set of SNPs frequency matched to the GWAS SNPs.
Any problem with this?

How are eQTLs detected?

Reported as the number of genes with significant heritability, linkage or association compared to an FDR

Example 1:
"Of the total set of genes, 2,340 were found to be expressed, of which 31% had significant heritability when a false-discovery rate of 0.05 was used."

- Monks, AJHG, 75(6): 1094–1105. 2004

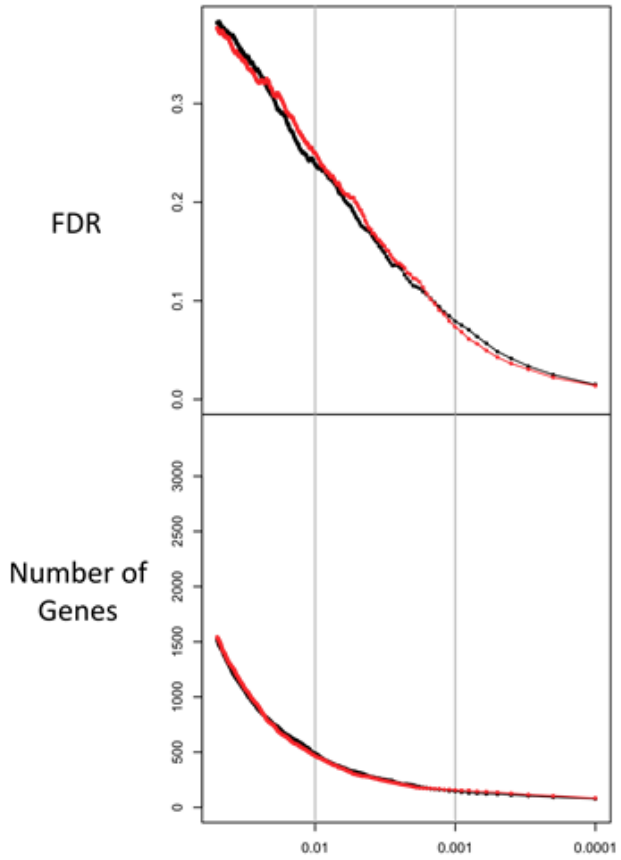
Example 2:
"Applying this genome-wide threshold to 3,554 scans we would expect only 3.5 genome scans to show any linkage evidence with a *P*-value this extreme by chance. Instead we found 142 expression phenotypes with evidence for linkage beyond the *P*-value threshold, and in some cases far beyond, so we conclude that false-positive linkage findings are at most a small fraction of the significant results."

- Morley, Nature, 430(7001): 743–747. 2004

Example 3:
"We detected 293, 274, 326 and 363 cis associations for CEU, CHB, JPT and YRI, respectively, corresponding to 783 distinct genes and an FDR of 4–5%."

- Stranger, Nat Genetics, 39, 1217–1224. 2007

eQTL definition depends on false discovery definition

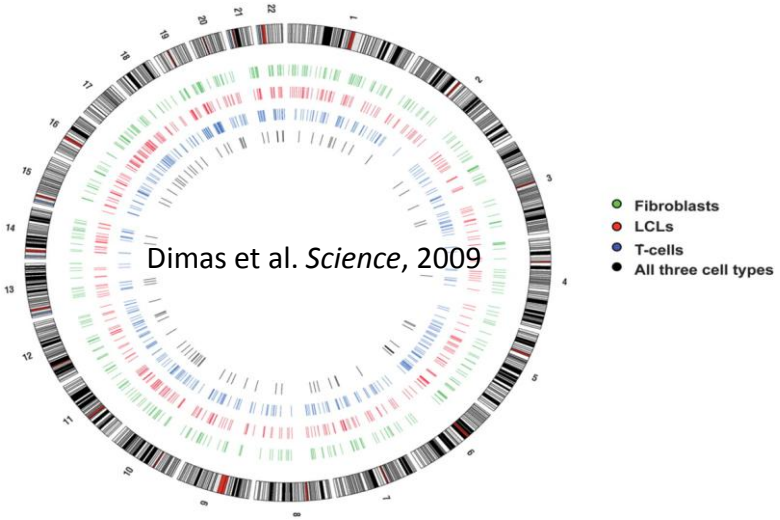


IMPORTANT: Understand the relationship Between false positive rate and eQTLs reported!

Discovery of eQTLs depends on:

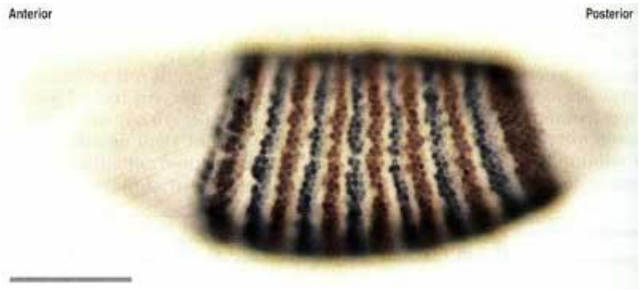
(A)Biological factors
(B) Technological factors

BIOLOGICAL FACTORS

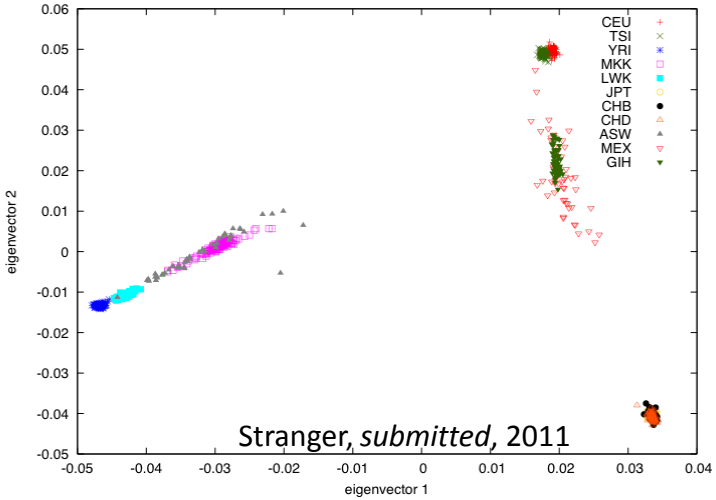


Dimas et al. *Science*, 2009

Trait biology

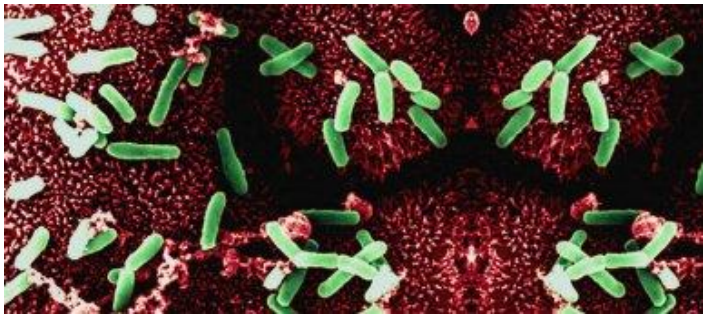


Ancestry



Stranger, *submitted*, 2011

Environment

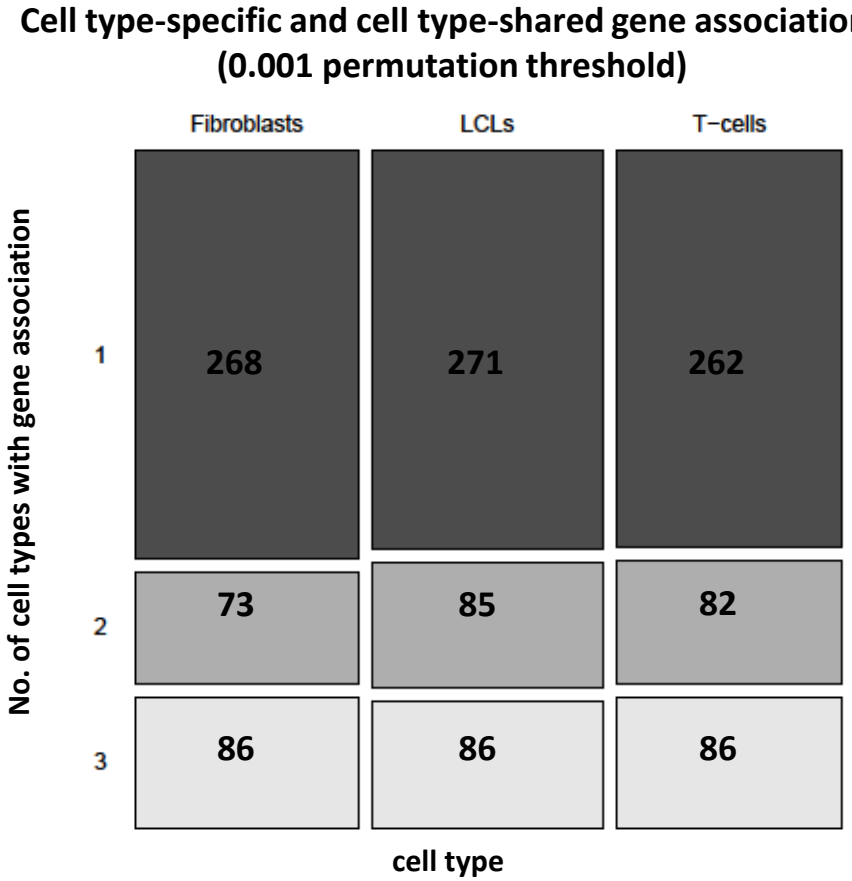


Multiple tissue studies

Determining how ubiquitous eQTL signals (and potential disease mechanism) are in different tissues.

i.e. if I find an eQTL in fat will it be informative of mechanism underlying disease risk for a disease based in muscle.

Answer: probably not



69-80% of cis associations are cell type-specific

Dimas *et al Science* 2009

50% specific (adipose and blood)

Emilsson *et al Nature* 2008

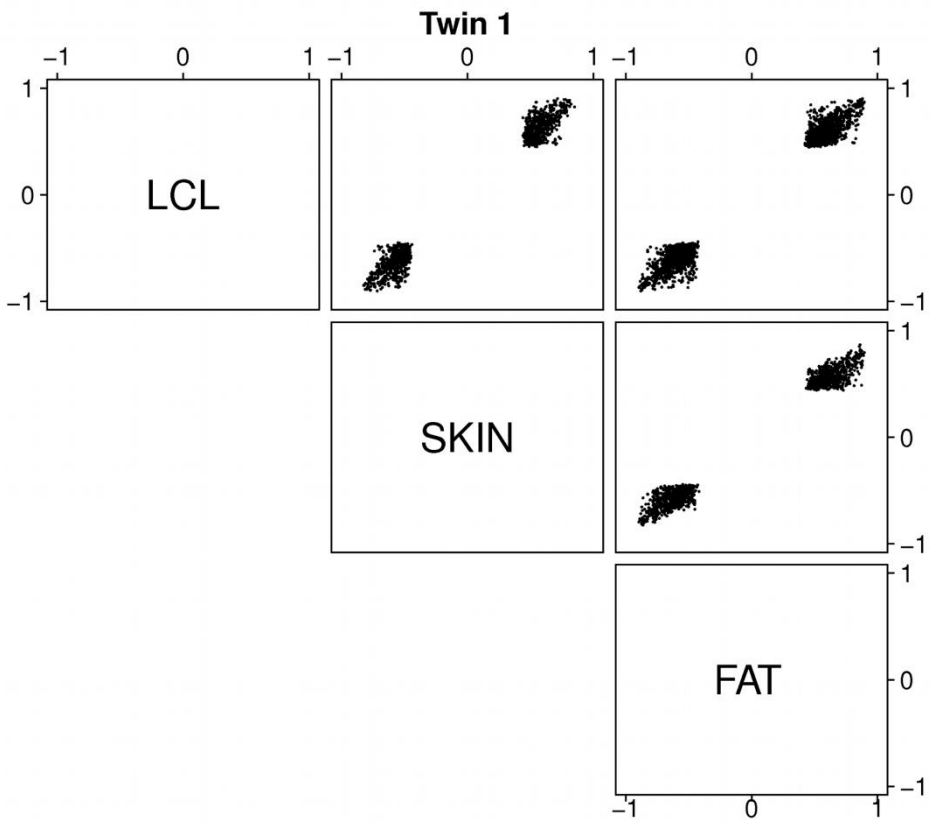
>50% specific (cortical tissue and peripheral blood)

Heinzen *et al PloS Biology* 2008

All estimates depend on eQTL definition and method for assessing sharing

SHARED EFFECTS BUT WEAKER?

10e-3 vs 10e-2 permutation threshold



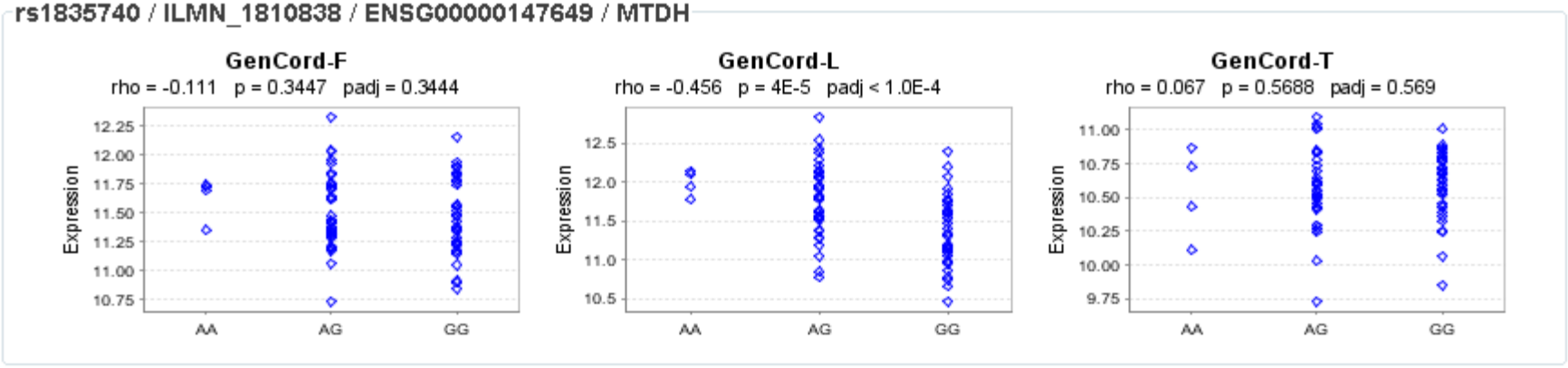
Nica et al., PloS Genetics, 2011

Issues of power may still dominate robust estimates of eQTL sharing.

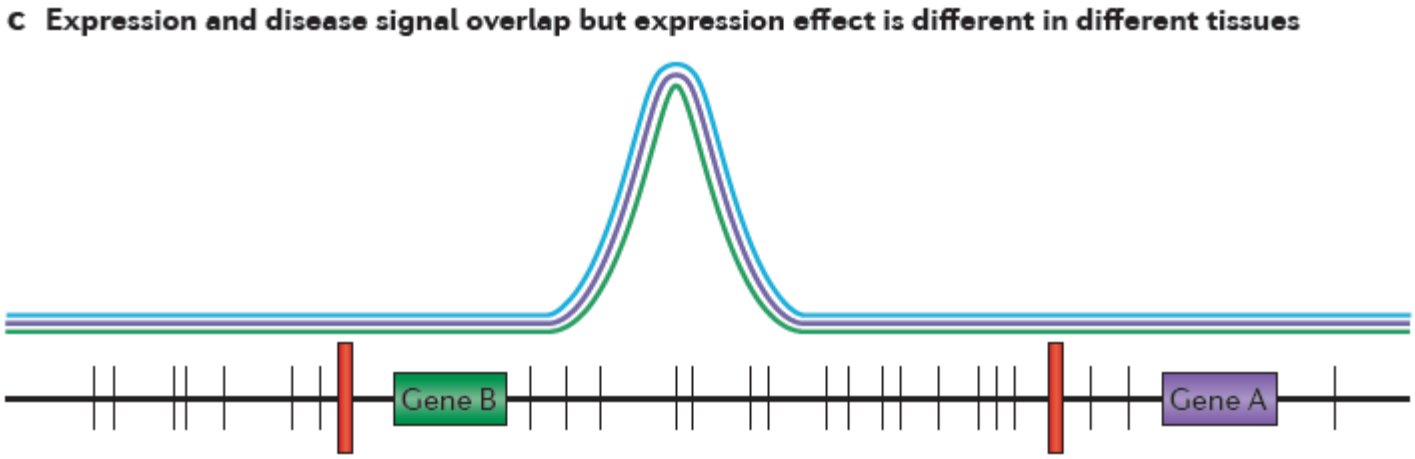
What are my migraine variants doing in different tissues?

We identified the minor allele of rs1835740 on chromosome 8q22.1 to be associated with migraine ($P = 5.38 \times 10^{-9}$, odds ratio = 1.23, 95% CI 1.150–1.324) in a genome-wide association study of 2,731 migraine cases ascertained from three European headache clinics and 10,747 population-matched controls. In an expression quantitative trait study in lymphoblastoid cell lines, transcript levels of the *MTDH* were found to have a significant correlation to rs1835740 ($P = 3.96 \times 10^{-5}$, permuted threshold for genome-wide significance 7.7×10^{-5}).

Anttila, Nature Genetics, 2011



Predictive value of gene expression dependent on proximity to pathological tissue

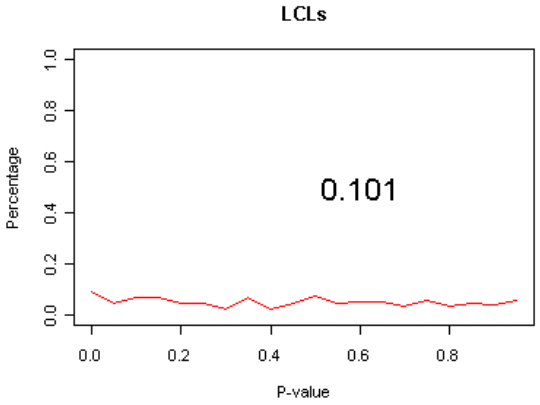


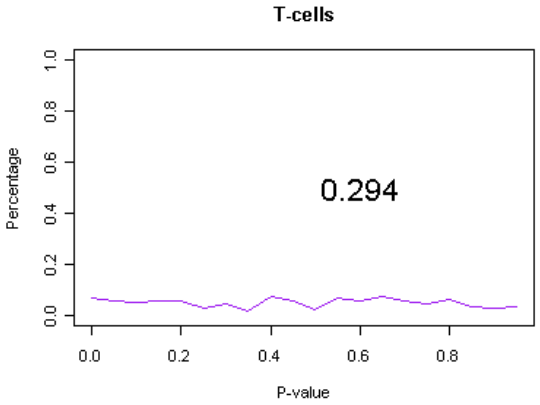
We have limited understanding of the Type I and II error rate

However, a lack of sharing may allow us to discover the pathological tissue

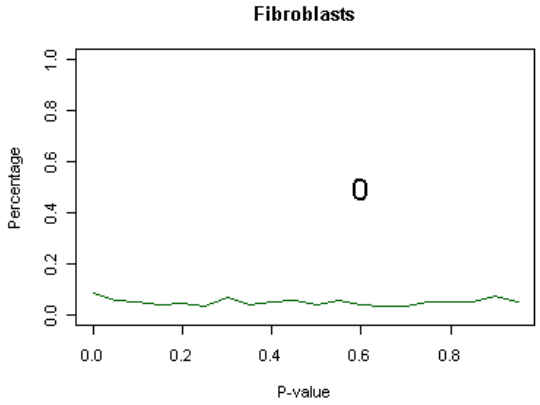
TISSUE SPECIFIC GWAS EQTLS

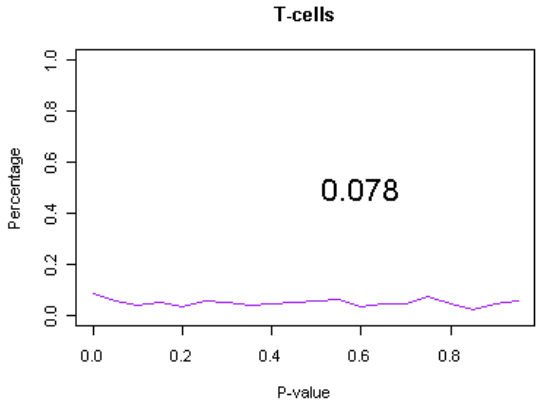
F: 306 eQTL genes



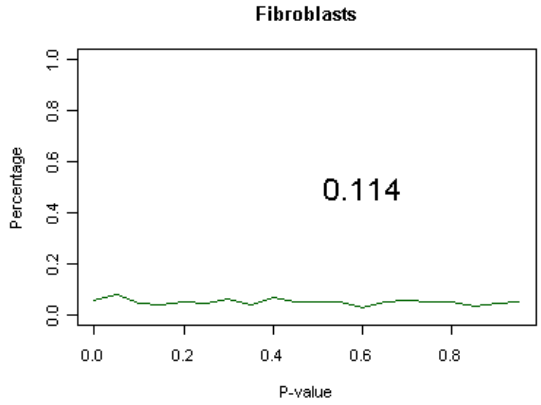


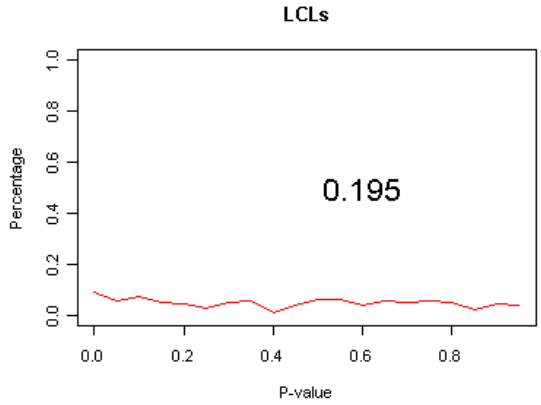
L: 377 eQTL genes





T: 299 eQTL genes



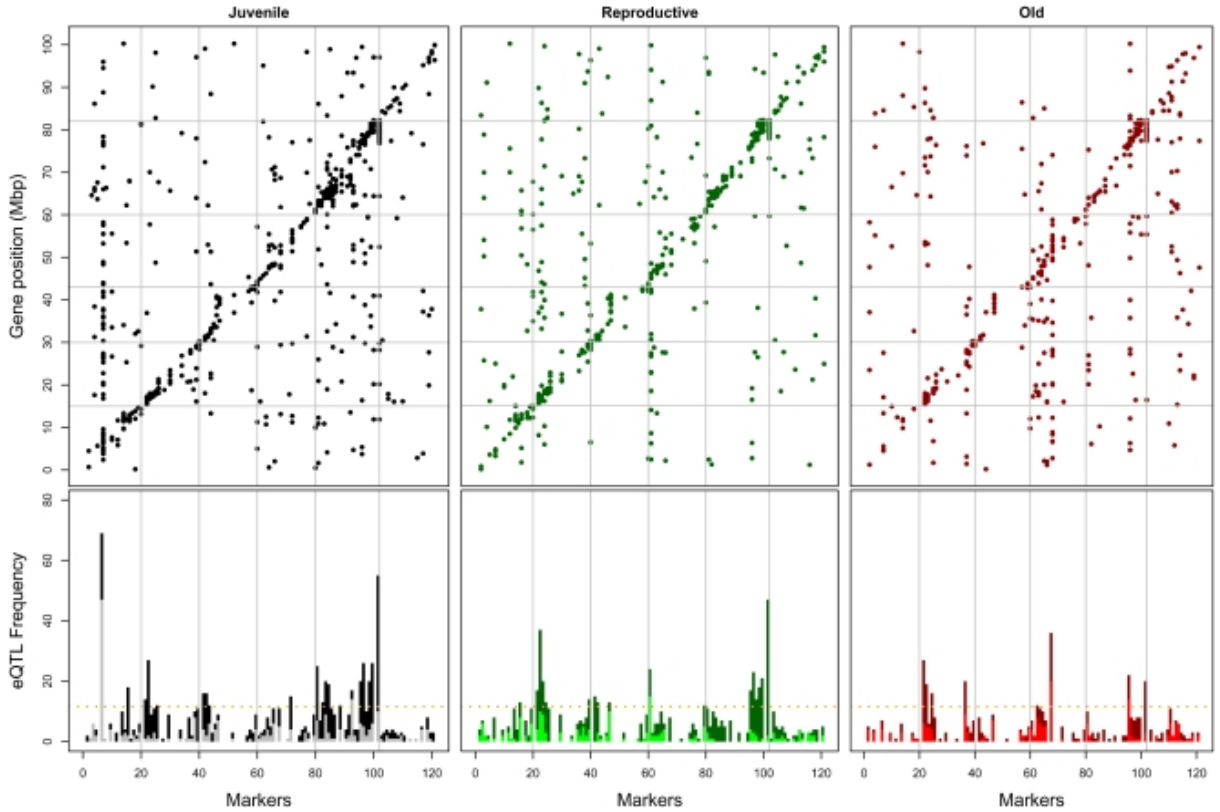


Development-specific studies

Determining how eQTLs behave over time

i.e. if I find an eQTL in pluripotent cell state will it be informative of mechanism underlying an differentiated state.

Less eQTLs in older individuals



Recombinant inbred *C.elegans*

More interruption by somatic or environmental effects?

Viñuela A, Genome Research 20(7):929-37. 2010

Multiple Population studies

Determining how ubiquitous eQTL signals (and potential disease mechanism) are in different populations.

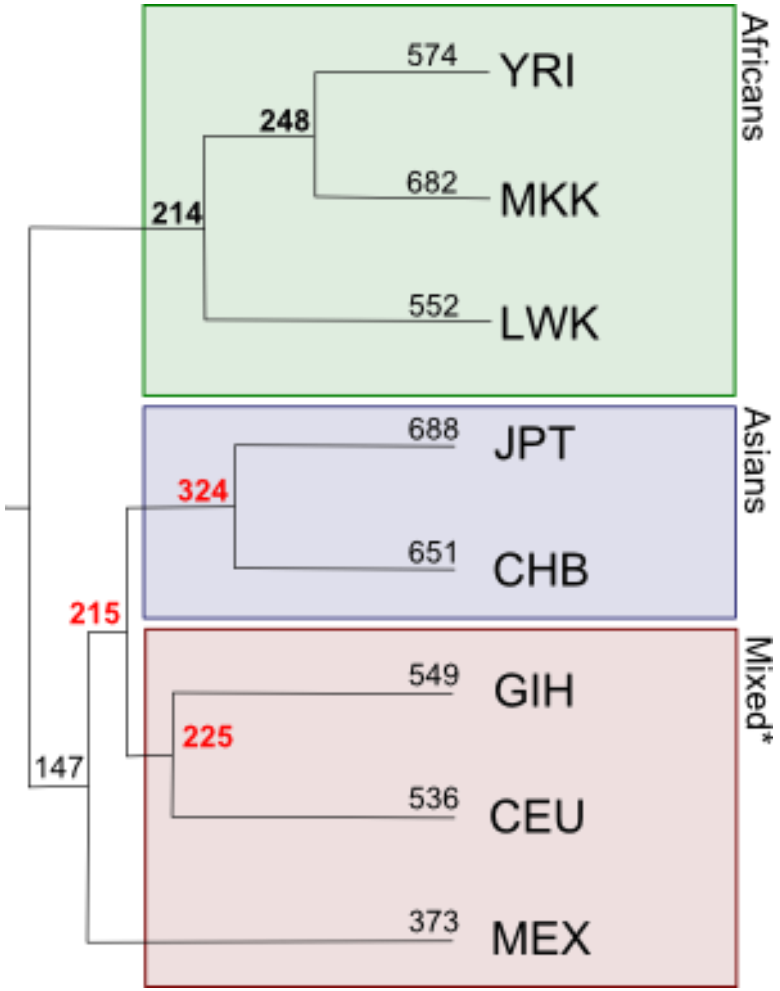
i.e. if I find an eQTL in Europeans will it be informative of mechanism underlying disease risk for a disease found in Chinese.

NOT ALL EQTLS SHARED ACROSS POPULATIONS

"We have reported that many genes showing cis associations at the 0.001 permutation threshold are shared (about 37%) in at least two populations … In 95–97% of the shared associations, the direction of the allelic effect was the same across populations, and the discordant 3–5% was of the same order as the FDR."

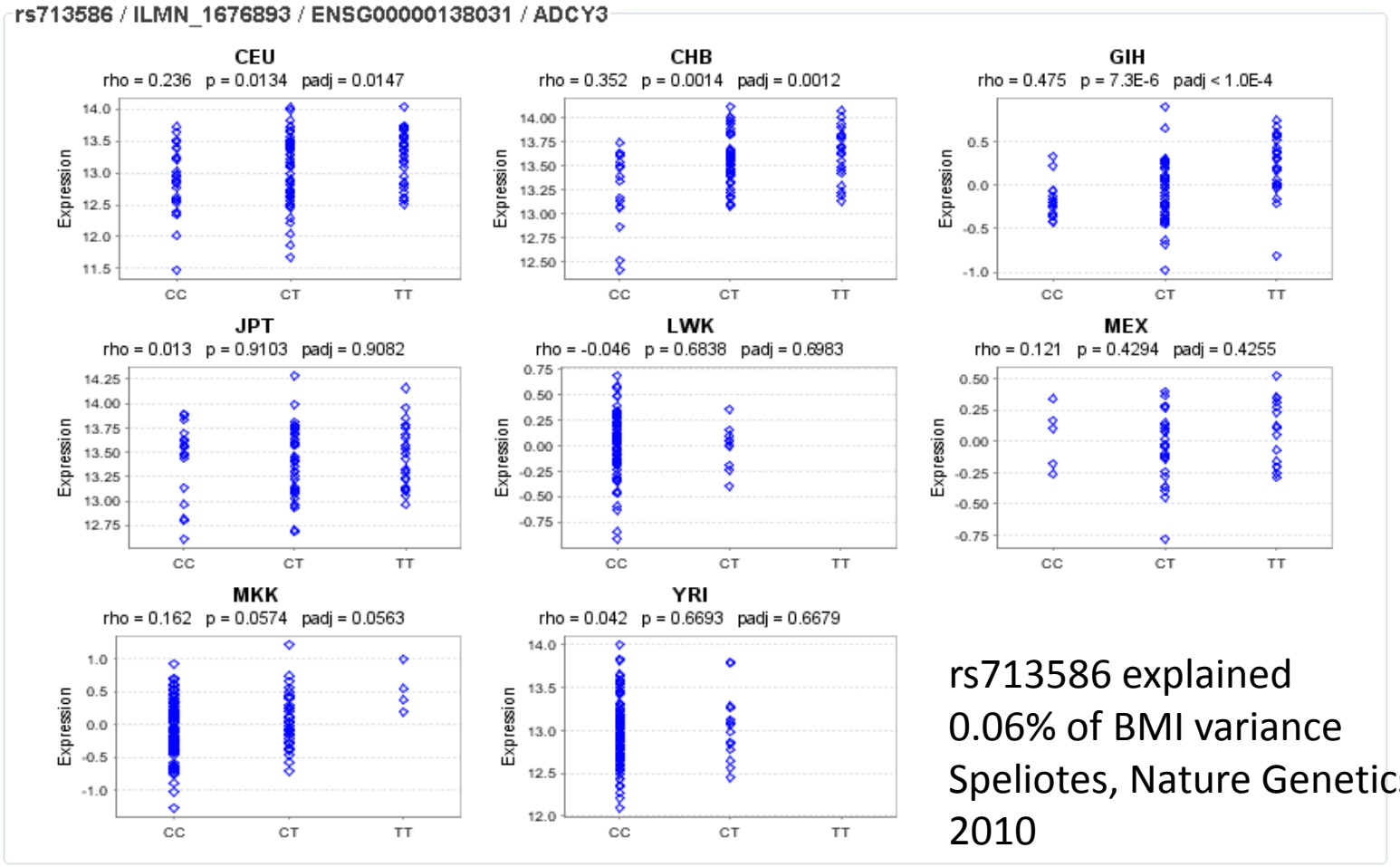
Stranger, Nat Genetics, 2007

If we know the etiology of a disease can we predict its population frequency from cellular models of that disease?



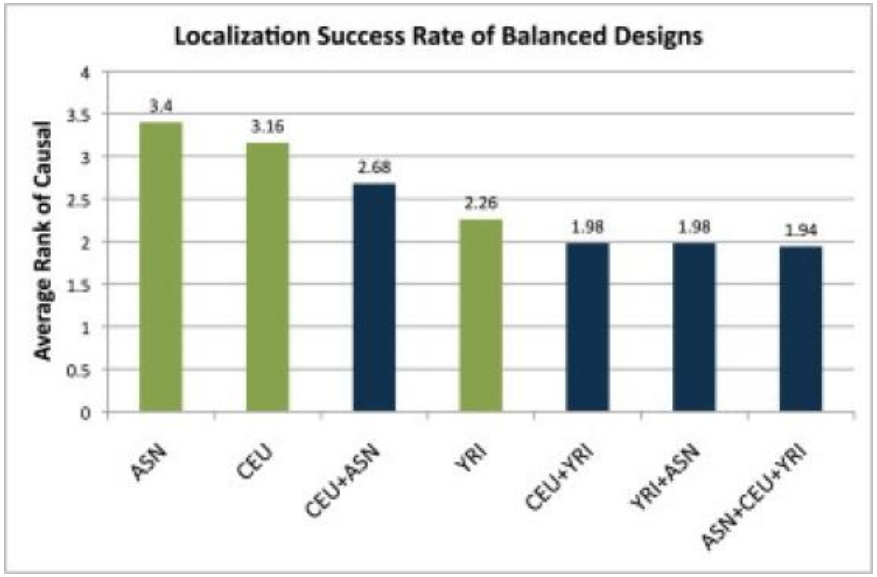
Stranger, Montgomery, Dimas, PLoS Genetics, 2012

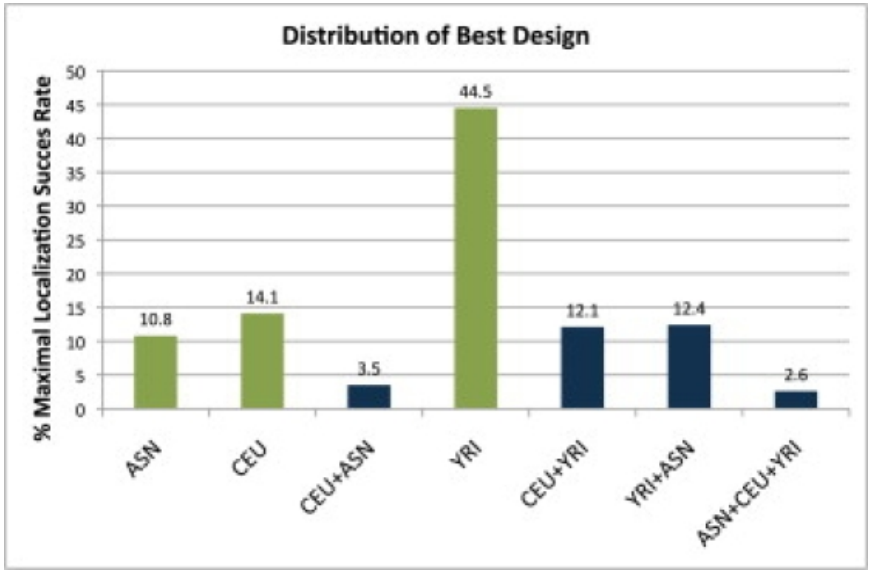
What are my BMI variants doing in different populations?



rs713586 explained 0.06% of BMI variance
Speliotes, Nature Genetics, 2010

Multiple population study designs: Recombination mapping can get at causal variants

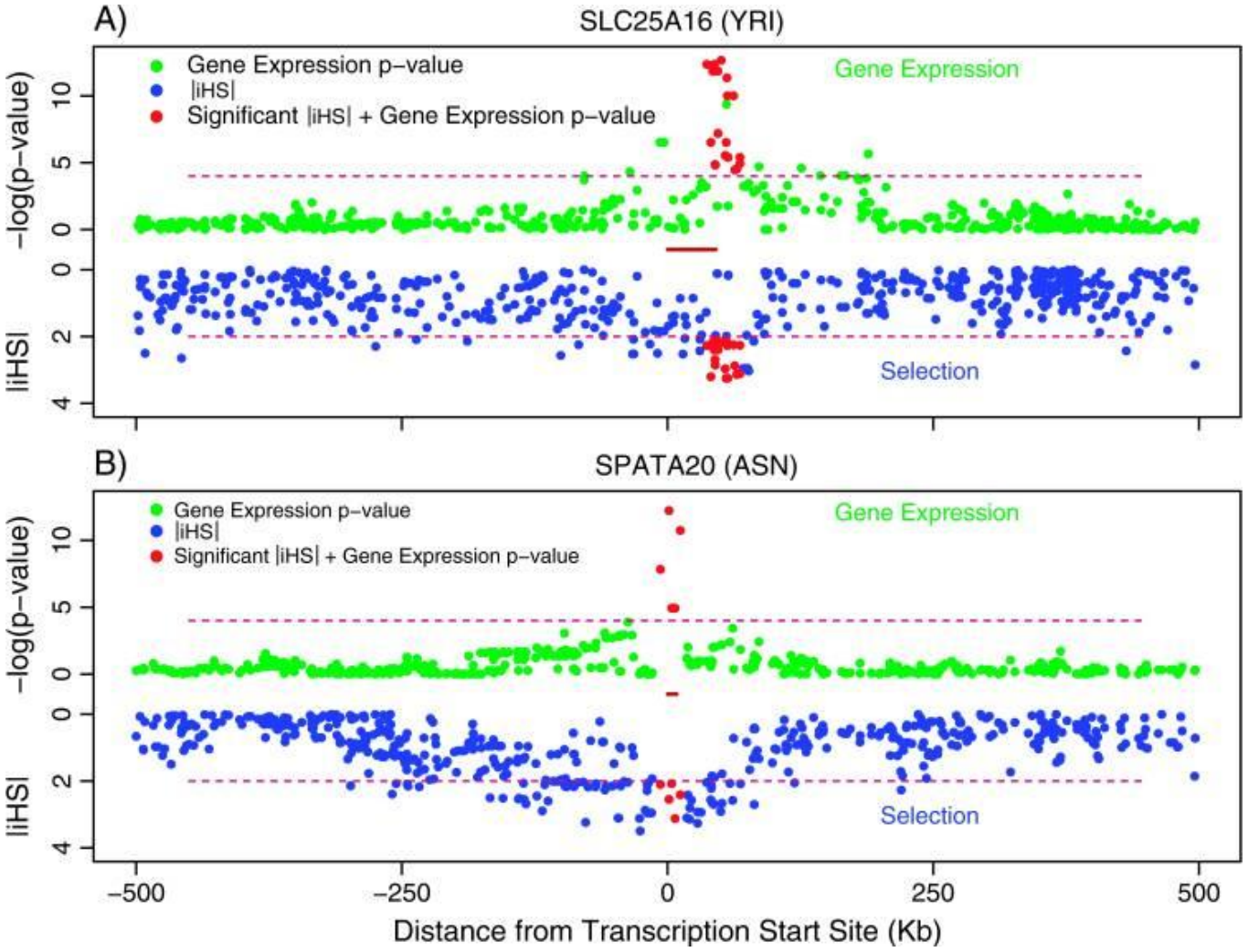




Zaitlen, AJHG, ; 86(1): 23–33. 2010

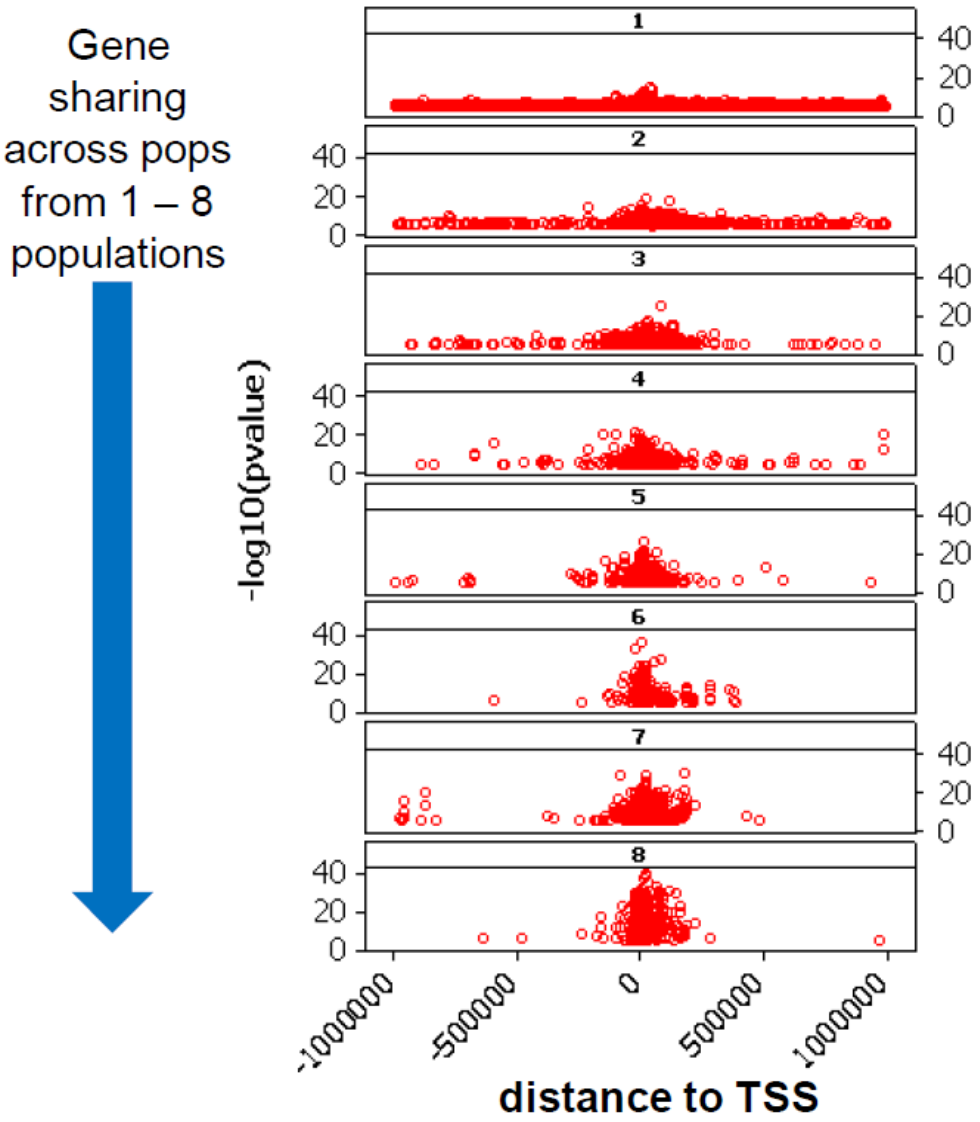
Multiple populations do well at mapping causal variants; however their design results in a reduction of power

eQTLs under selection



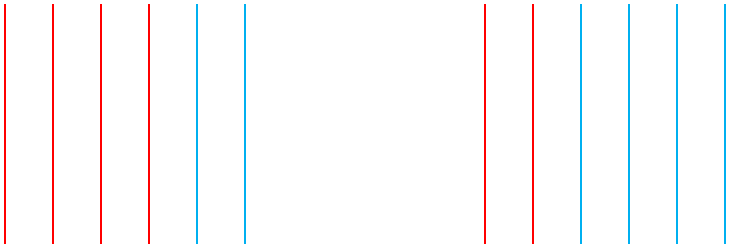
Kudaravalli, MBE, 2009

Finding causal variants



Admixed populations

- Challenges: Loss of power if local ancestry not known or inflation in significance if frequency differences are large and effect is trans-acting.



Eur (red): mean 3.0 Afr (blue): mean 4.0

If mean expression invariant to genotype then
allele frequency differences will create false association

Solution: Add local ancestry as a covariate

Environment studies

Determining how eQTLs behave under stimulus

i.e. if I find an eQTL in resting state will it be informative of mechanism underlying an responsive state.

Answer: not much difference to date

"We carried out large-scale induction experiments using primary human bone cells derived from unrelated donors of Swedish origin treated with 18 different stimuli (7 treatments and 2 controls, each assessed at 2 time points). … We found that 93% of cis-eQTLs at 1% FDR were observed in at least one additional treatment, and in fact, on average, only 1.4% of the cis-eQTLs were considered as treatment-specific at high confidence. "

- Grundberg PloS Genetics 7(1). 2011

Discovery of eQTL depends on technology

Gene expression technology

PCR-based, array-based, **sequencing-based**

Genotyping technology

array-based, sequencing-based

Sample size

More individuals and/or families yields more power to detect association with particular effect sizes. (Lowers FDR). Early studies used 18-30 families or 45-60 unrelated individuals.

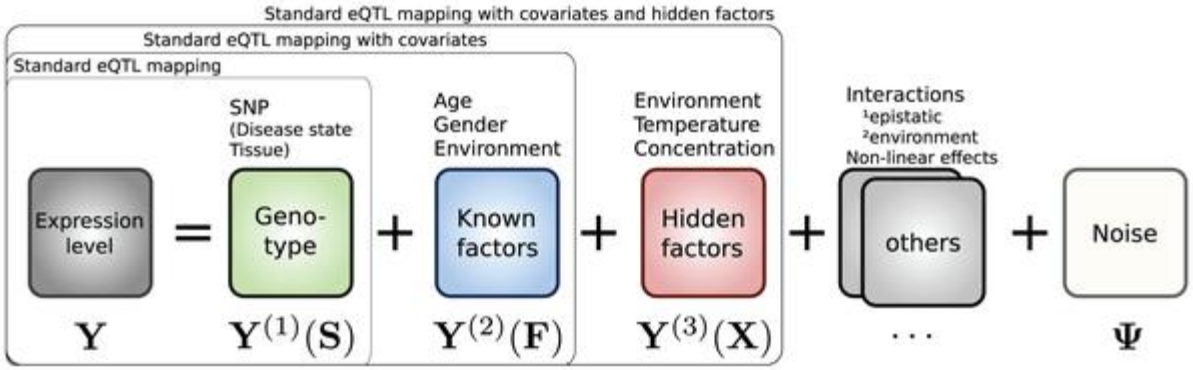
THE biases we don't know about:
Hidden factors can cause false associations

- Hidden technical and biological variables. i.e. population, sex, date of processing
- However, correcting these factors can remove true signals (i.e. master regulators)

Methods to correct hidden factors

- Factor analysis on *40* global factors has tripled eQTL discovery.

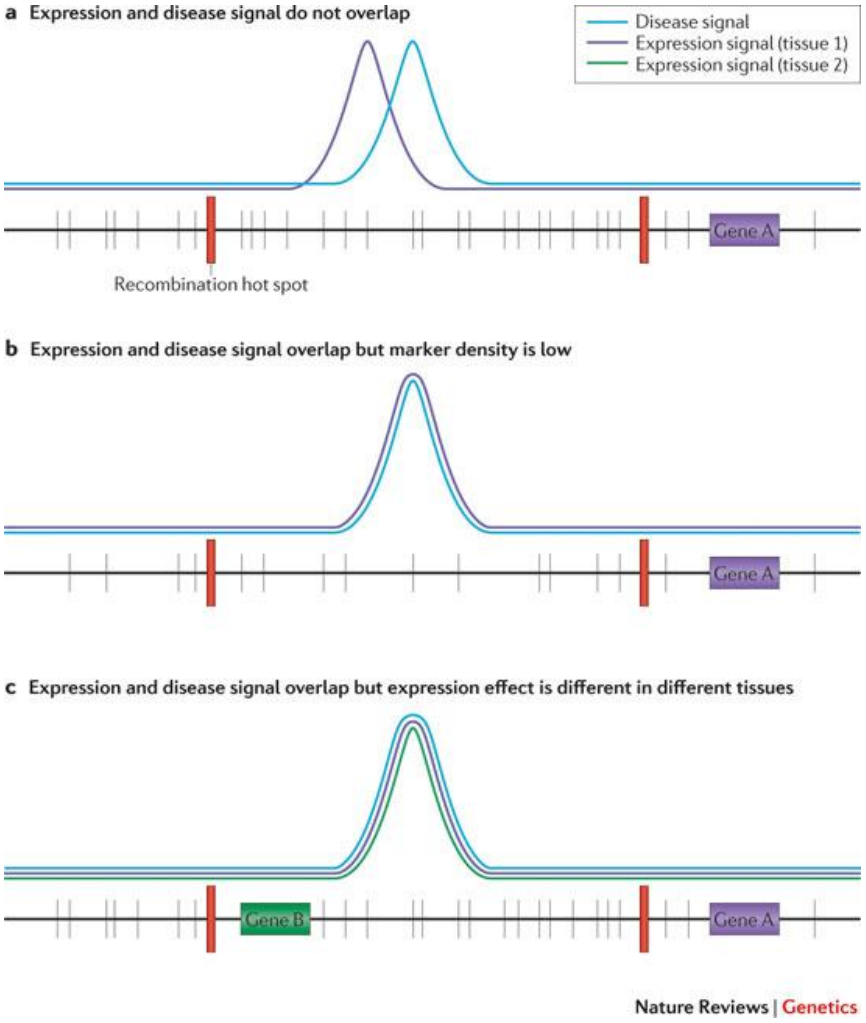
  - Stegle, PLoS Computational Biology, 2010



- Surrogate variable analysis, has increased by 20% eQTL discovery

  - Leek, PLoS Genetics, 2007

Why are biological and technological contexts important for understanding eQTL role in disease?



eQTL data can open up new biology

- Without traits and disease we can find variants influencing expression level.
- We can speculate and investigate what these effects might do.

What are my TCF3 variants doing

Cell. 2006 Oct 6;127(1):171-83.

Tcf3 governs stem cell features and represses cell fate determination in skin.

Nguyen H, Rendl M, Fuchs E.

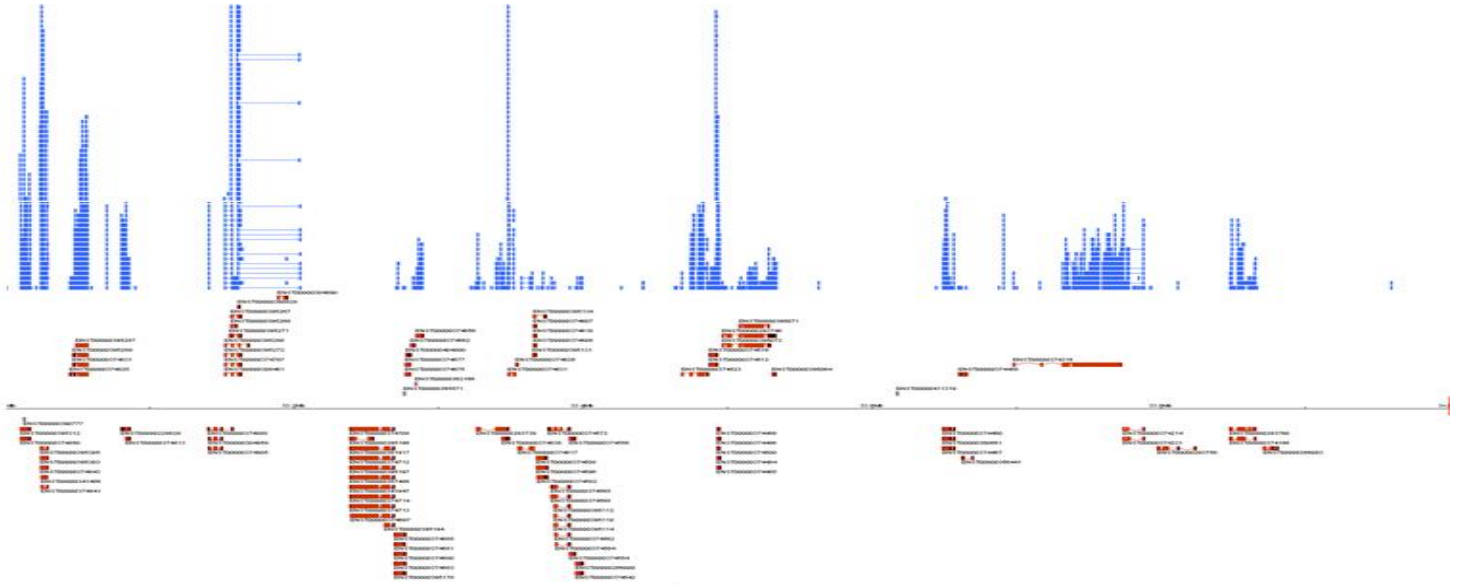
Howard Hughes Medical Institute, Department of Mammalian Cell Biology and Development, The Rockefeller University, 1230 York Avenue, Box 300, New York, NY 10021, USA.

Abstract

Many stem cells (SCs) respond to Wnt signaling, but whether beta-catenin's DNA binding partners, the Tcfs, play a role in SCs in the absence of Wnts, is unknown. In adult skin, quiescent multipotent progenitors express Tcf3 and commit to a hair cell fate in response to Wnt signaling. We find that embryonic skin progenitors also express Tcf3. Using an inducible system in mice, we show that upon Tcf3 reactivation, committed epidermal cells induce genes associated with an undifferentiated, Wnt-inhibited state and Tcf3 promotes a transcriptional program shared by embryonic and postnatal SCs. Further, Tcf3-repressed genes include transcriptional regulators of the epidermal, sebaceous gland and hair follicle differentiation programs, and correspondingly, all three terminal differentiation pathways are suppressed when Tcf3 is induced postnatally. These data suggest that in the absence of Wnt signals, Tcf3 may function in skin SCs to maintain an undifferentiated state and, through Wnt signaling, directs these cells along the hair lineage.

dbSNP	Genotype	Reference	Alternate	Gene	Rho	P-value
350146	CT	C	T	TCF3	0.545	0.00000687

Next generation sequencing has increased our ability to survey the transcriptome.

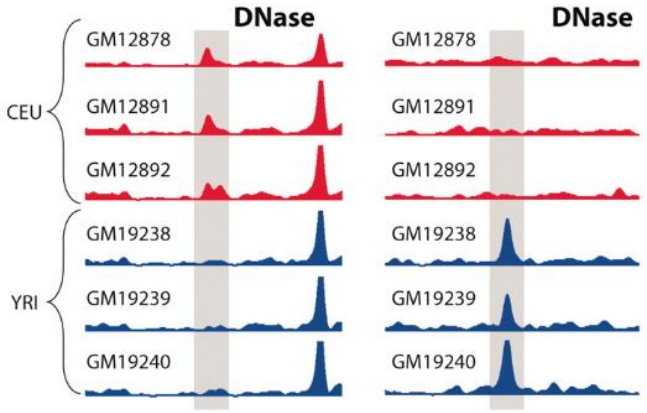


RNA-Seq

Montgomery, Nature 2010
Pickrell, Nature 2010

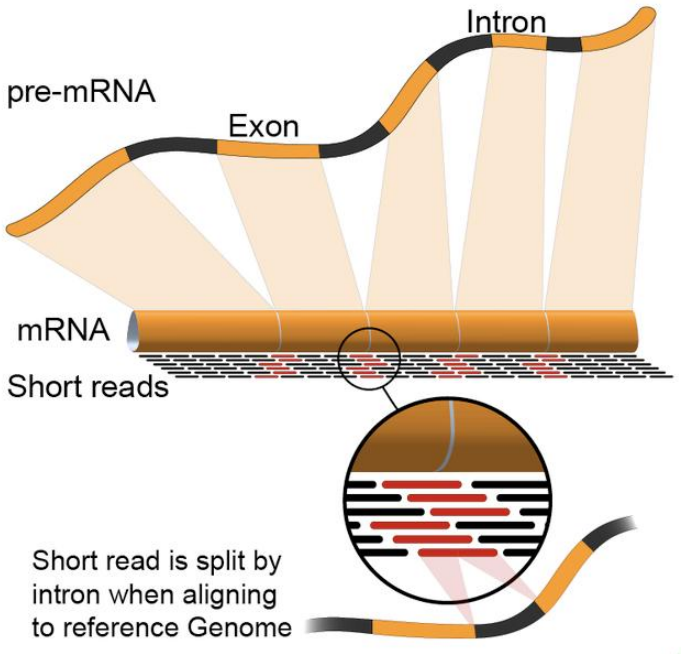
ChIP-Seq

McDaniell, Science 2010

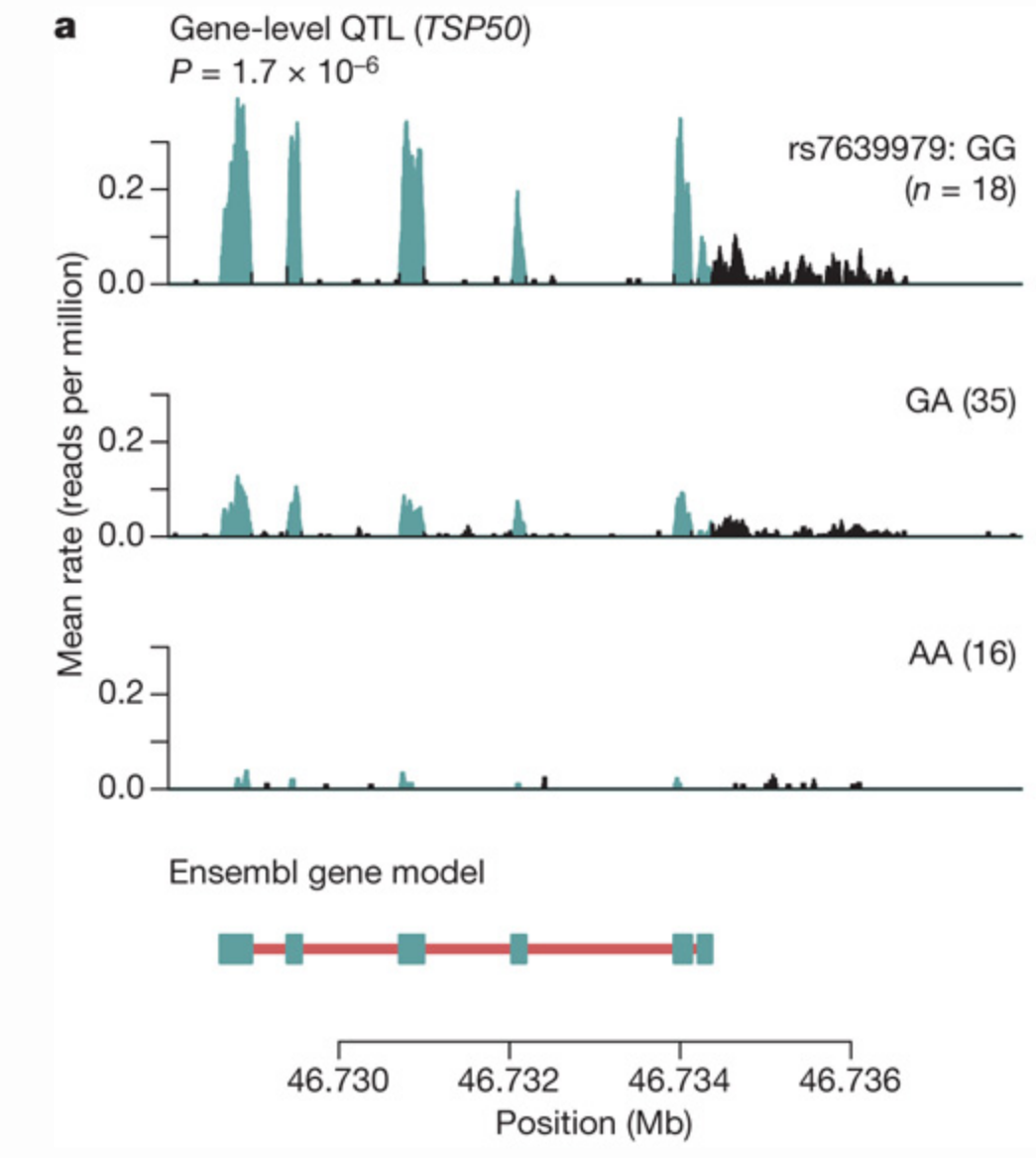


What is RNA-seq

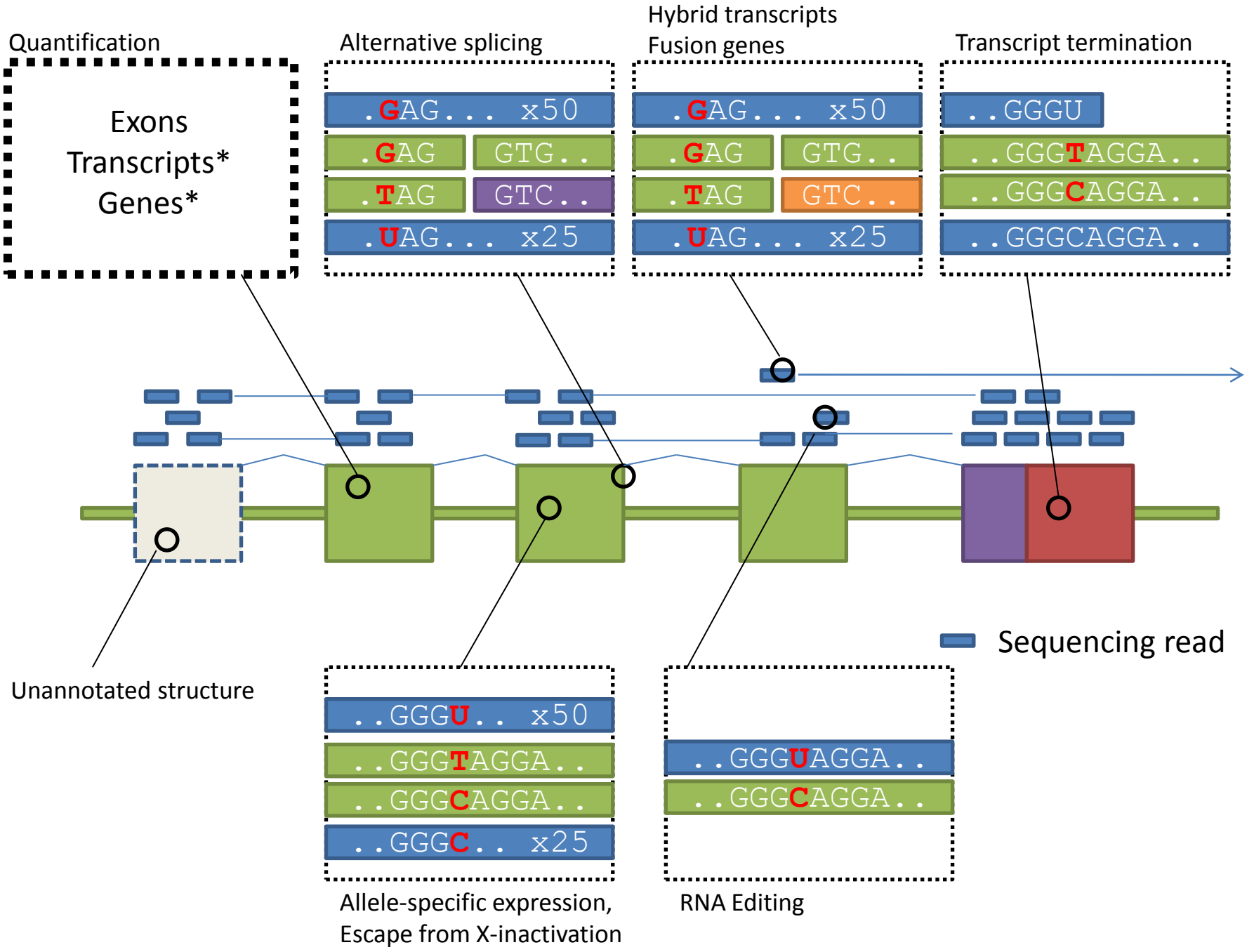
High-throughput sequencing of cDNA to
understand/quantify a sample's gene expression profile
Output: millions of short, single or paired-end sequences (reads)



Genetics of gene expression using RNA-Seq



Increased resolution of transcriptome through RNA- sequencing

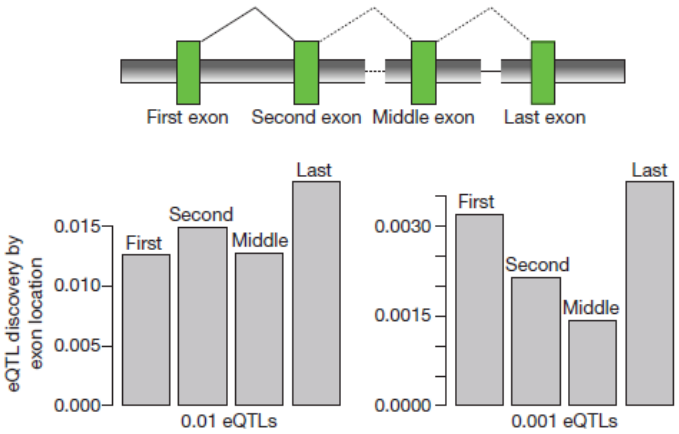


RNA-seq provides resolution of more QTLs

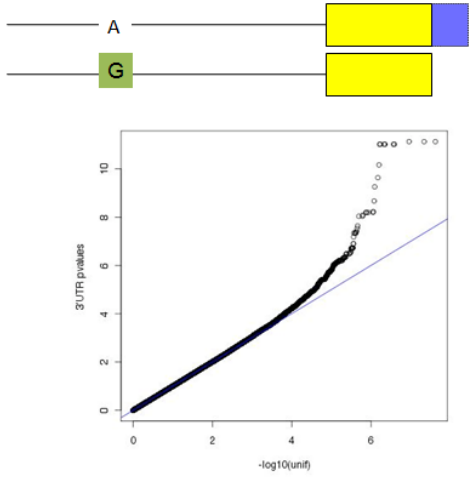
RNA-sequencing in 60 Europeans (HapMap genotypes; LCLs)

Found 2x more expression Quantitative Trait Loci (eQTLs) and...

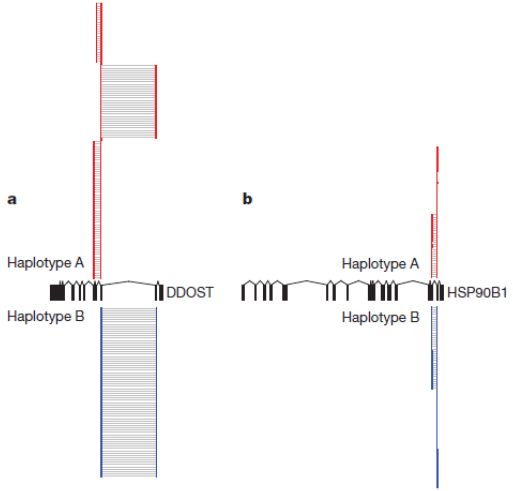
Exon-eQTLs



UTR Length-QTLs



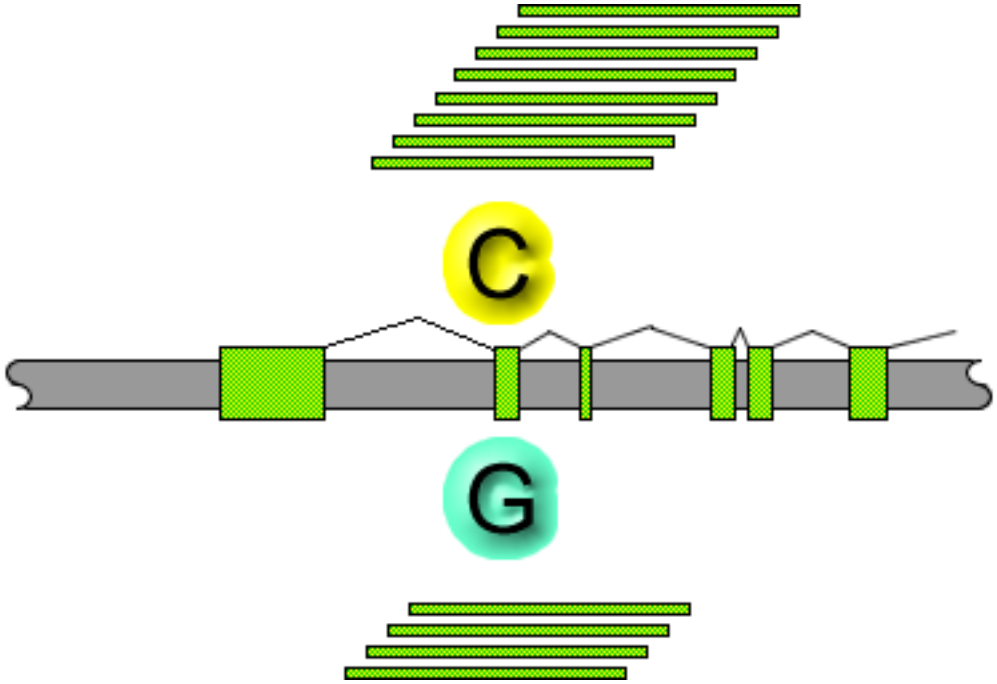
Splicing eQTLs



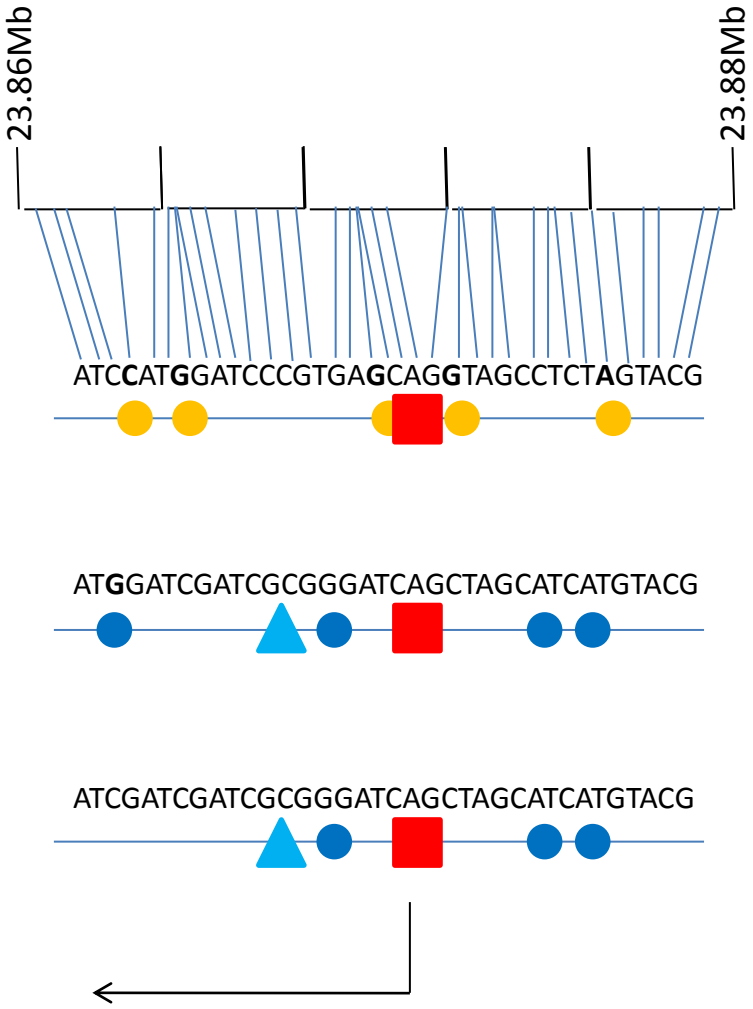
Rare eQTLs with allele specific expression-based approaches

Advantages of ASE

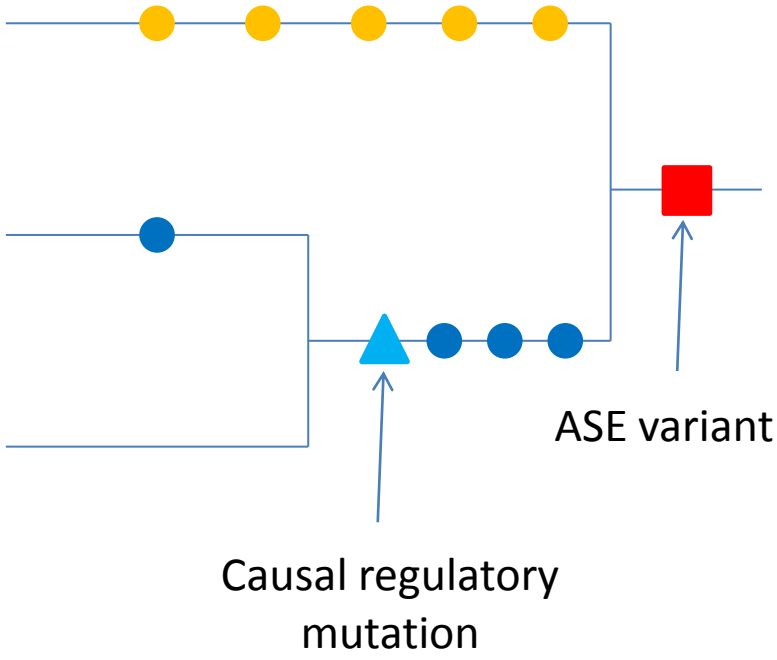
- Test within an individual allelic imbalance, given one has sufficient reads.



Looking for rare regulatory haplotypes

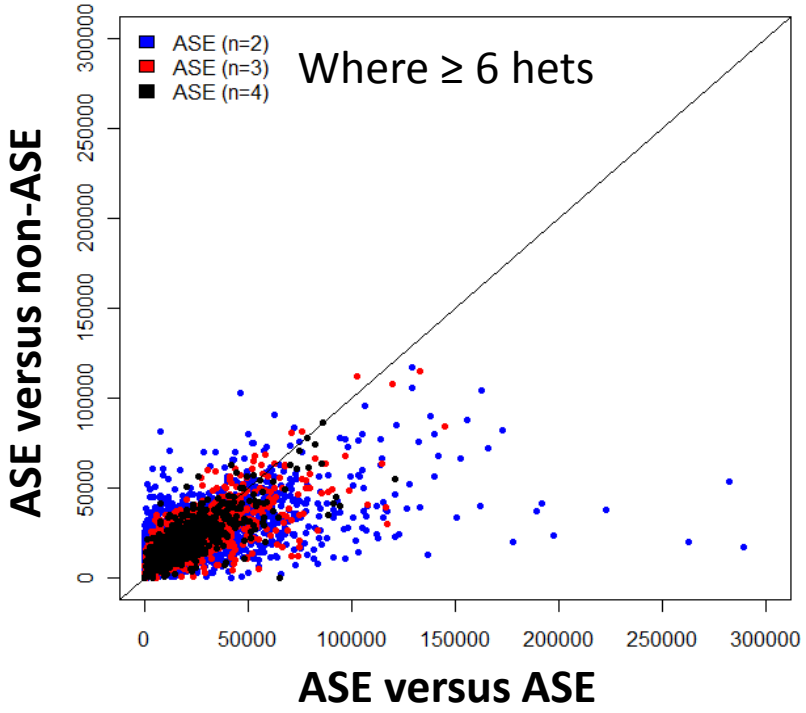


SNP Location



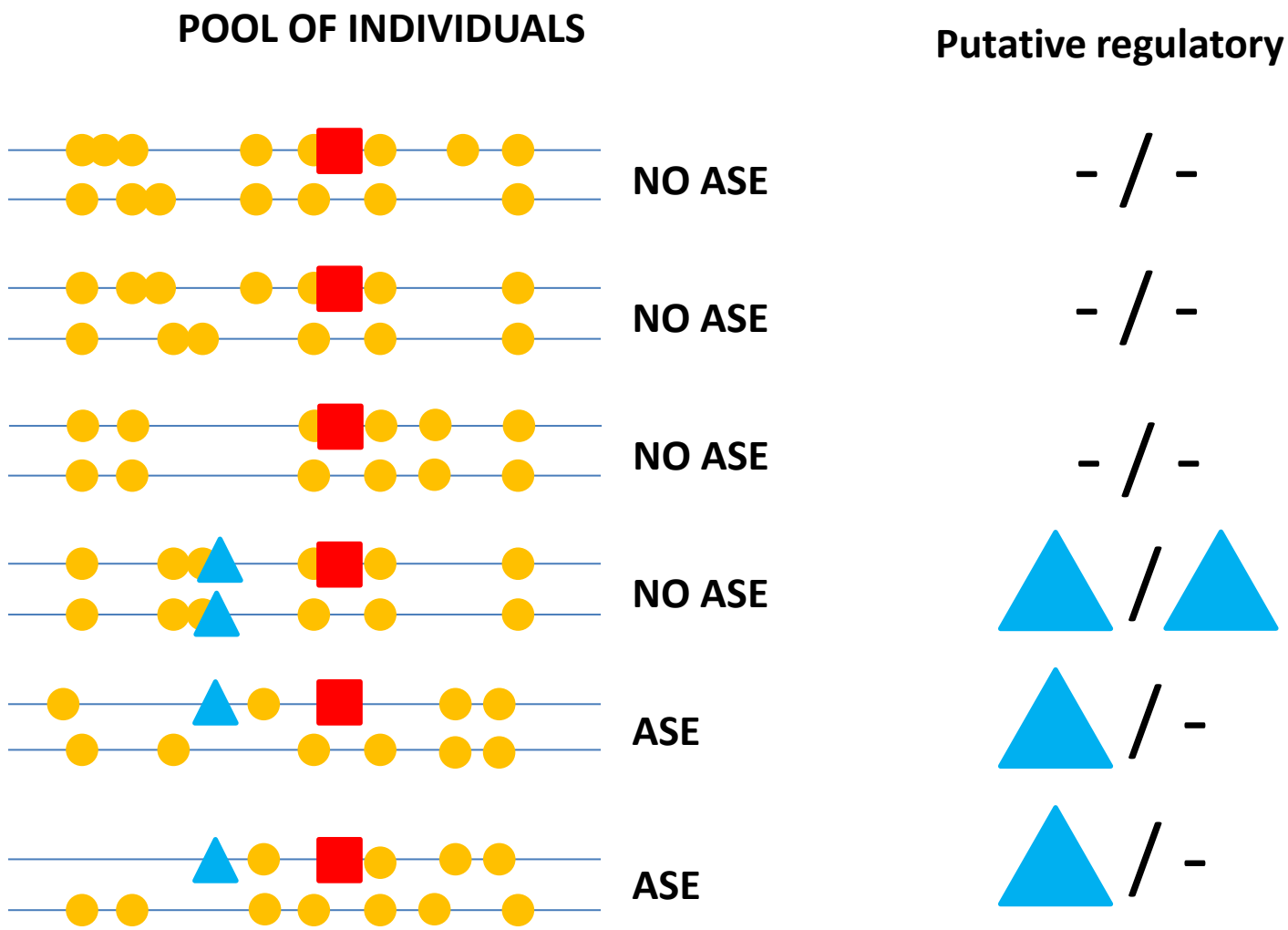
Can measure extent of haplotype homozygosity since shared ASE assumed to have common genealogy

Evidence of recent and rare eQTLs

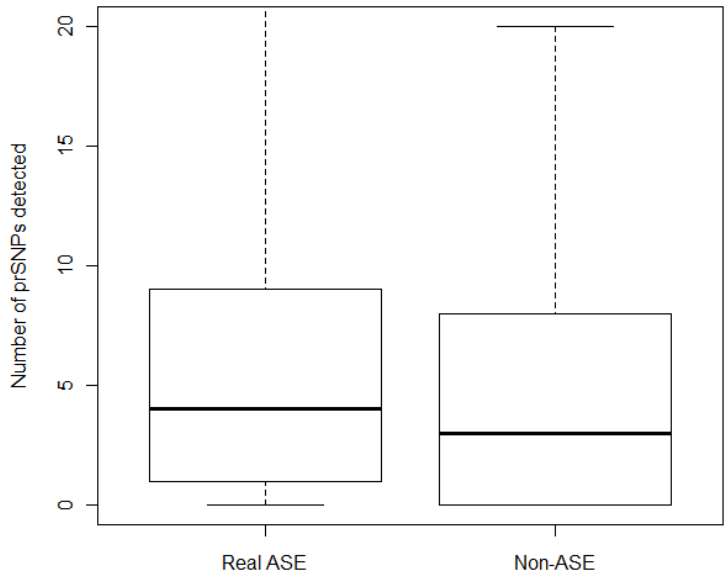


When ASE individuals compared, we observed longer tracts of haplotype homozygosity

Can we find the recent and rare causal regulatory variants?



More putative regulatory SNPs found for real ASE versus non-ASE

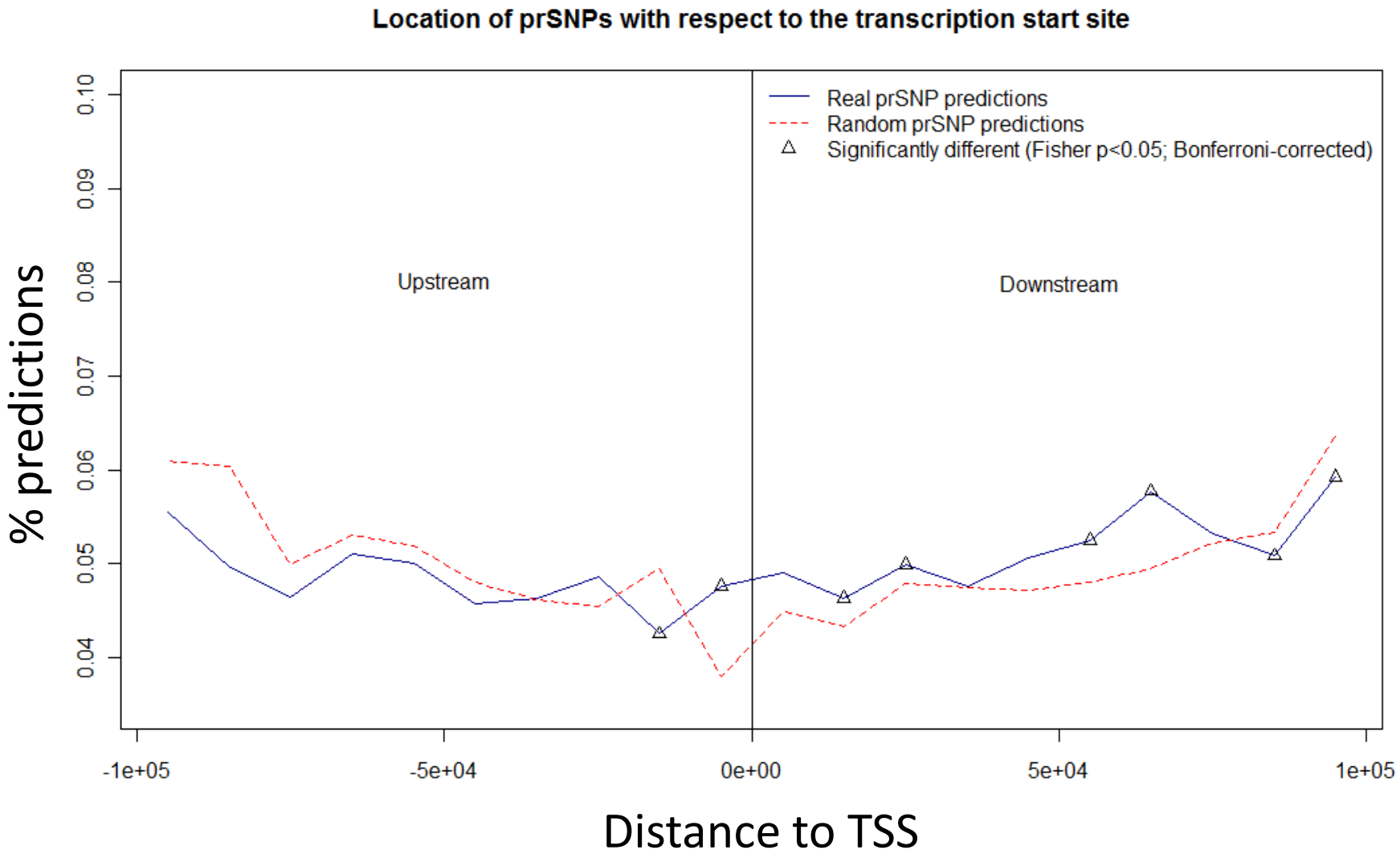


Mann Whitney
p<2e-16

We see 1 more prSNP on average in real ASE versus non-ASE

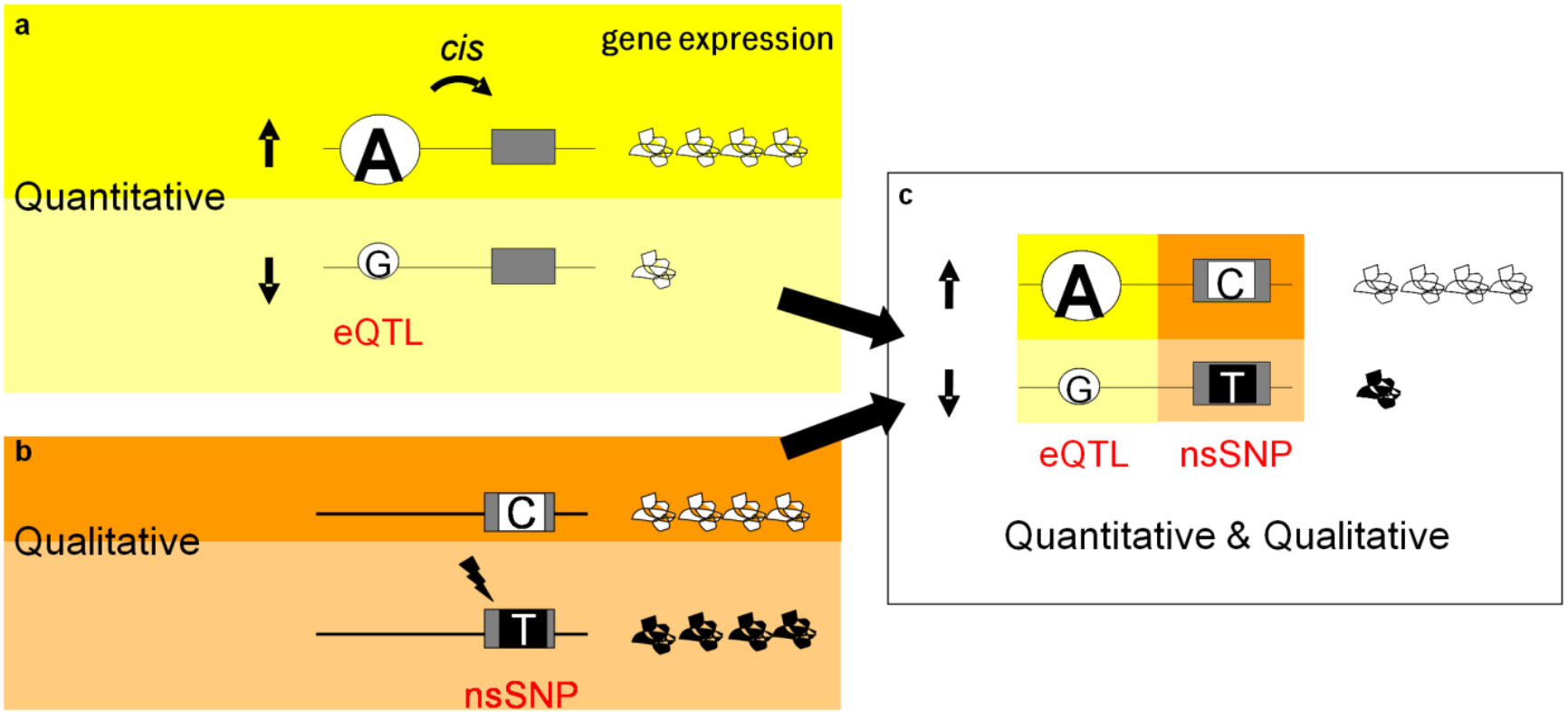
Montgomery, PLoS Genetics, 2011

Putative regulatory SNPs are enriched around TSS



EPISTATIC EFFECTS

- Evaluate outcome through joint assessment of genome and transcriptome



23.3% (9022 of 38645) nonsynonymous sites where ASE can be detected are significant; **46.2% of variants DE in 1 indiv.**

Compound inheritance of regulatory and coding polymorphism causes disease

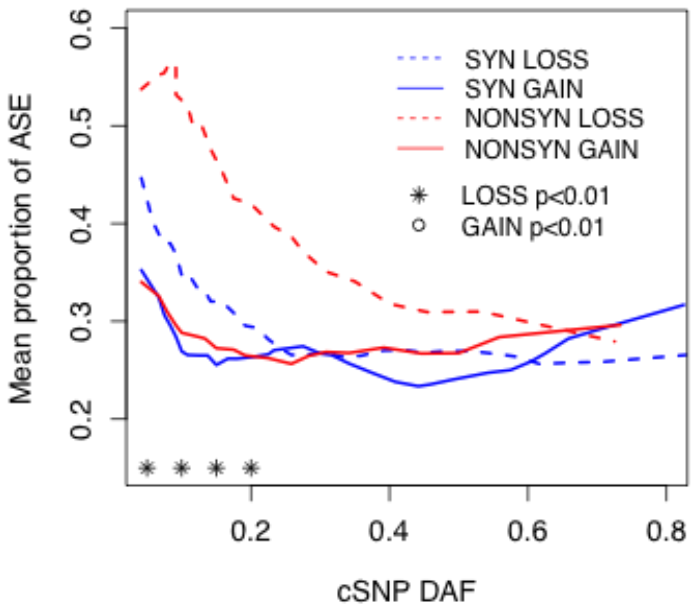
Compound inheritance of a low-frequency regulatory SNP and a rare null mutation in exon-junction complex subunit *RBM8A* causes TAR syndrome

The exon-junction complex (EJC) performs essential RNA processing tasks1–5. Here, we describe the first human disorder, thrombocytopenia with absent radii (TAR)6, caused by deficiency in one of the four EJC subunits.

The thrombocytopenia with absent radii (TAR) syndrome is characterized by a reduction in the number of platelets (the cells that make blood clot)

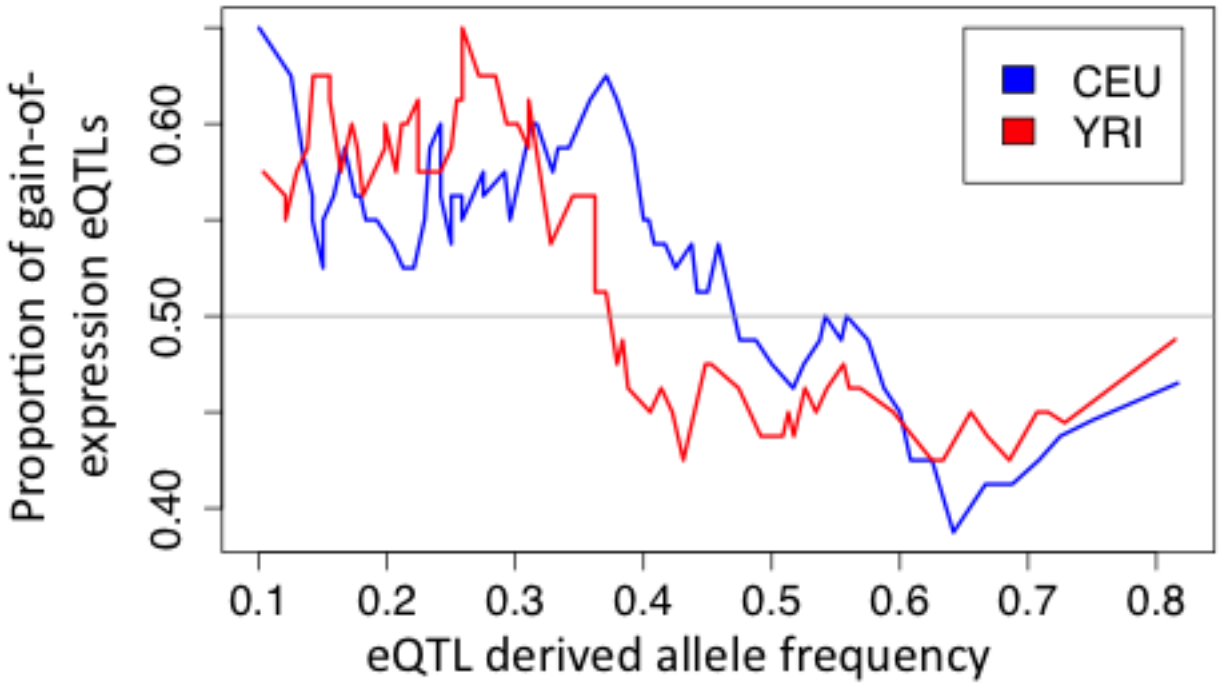
Albers, Nature Genetics, 2012

Non-synonymous variants more often linked to loss haplotype.

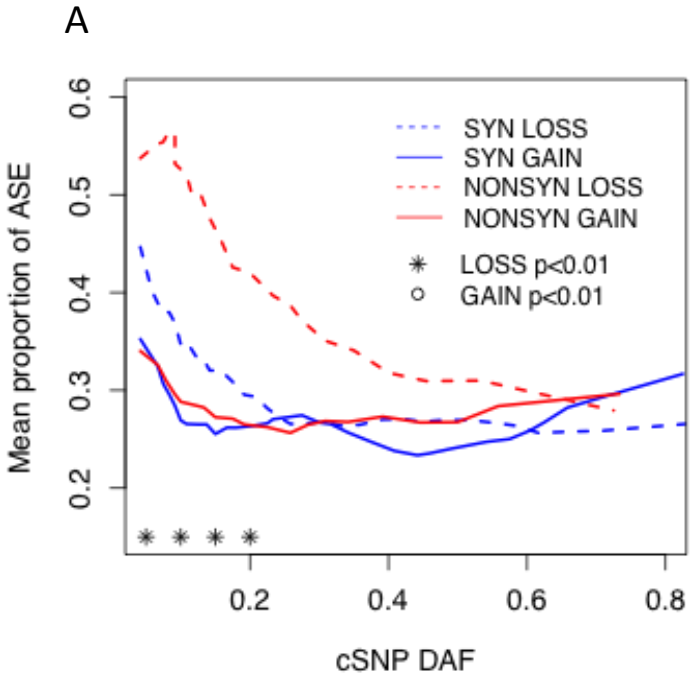


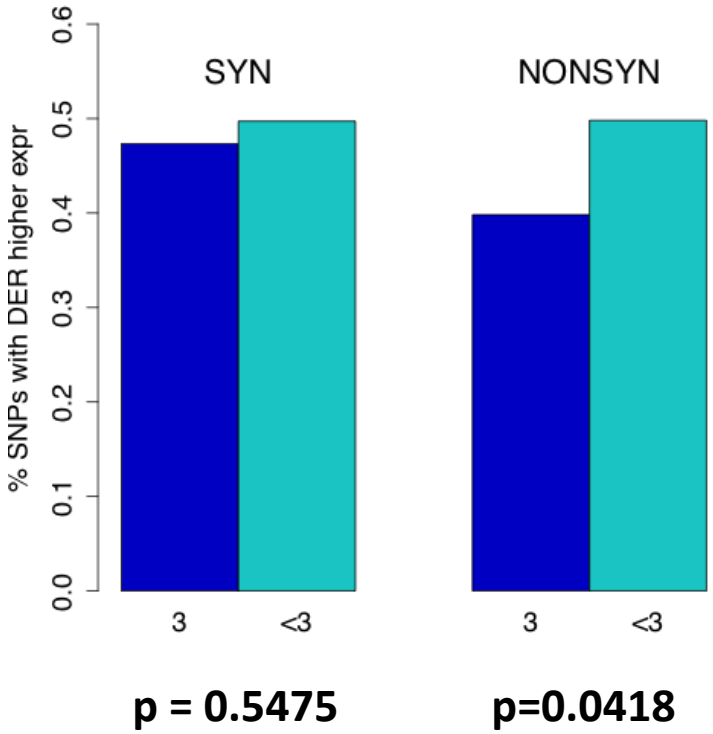
A variant that is deleterious may be compensated

Gain of expression eQTLs have lower derived allele frequencies



Stronger epistatic selection in genes with shared regulatory variation

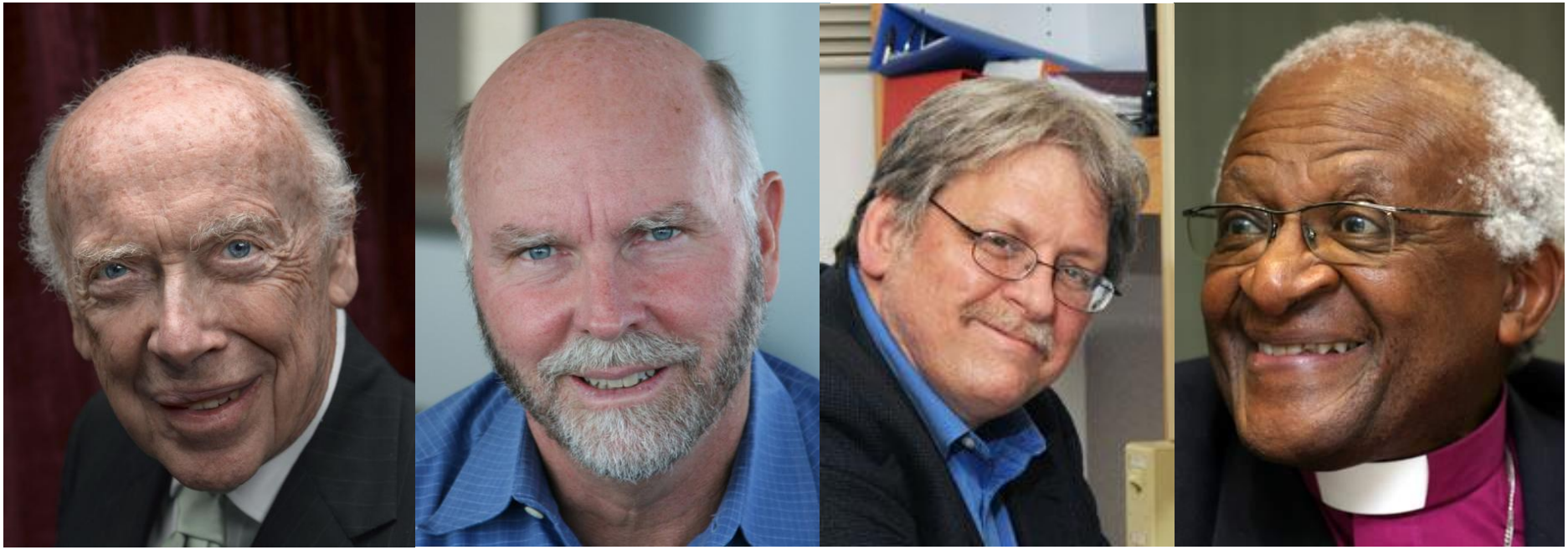




p = 0.5475 **p=0.0418**

Multi-tissue expression will inform likelihood of deleterious mutations being compensated

We are all dysfunctional

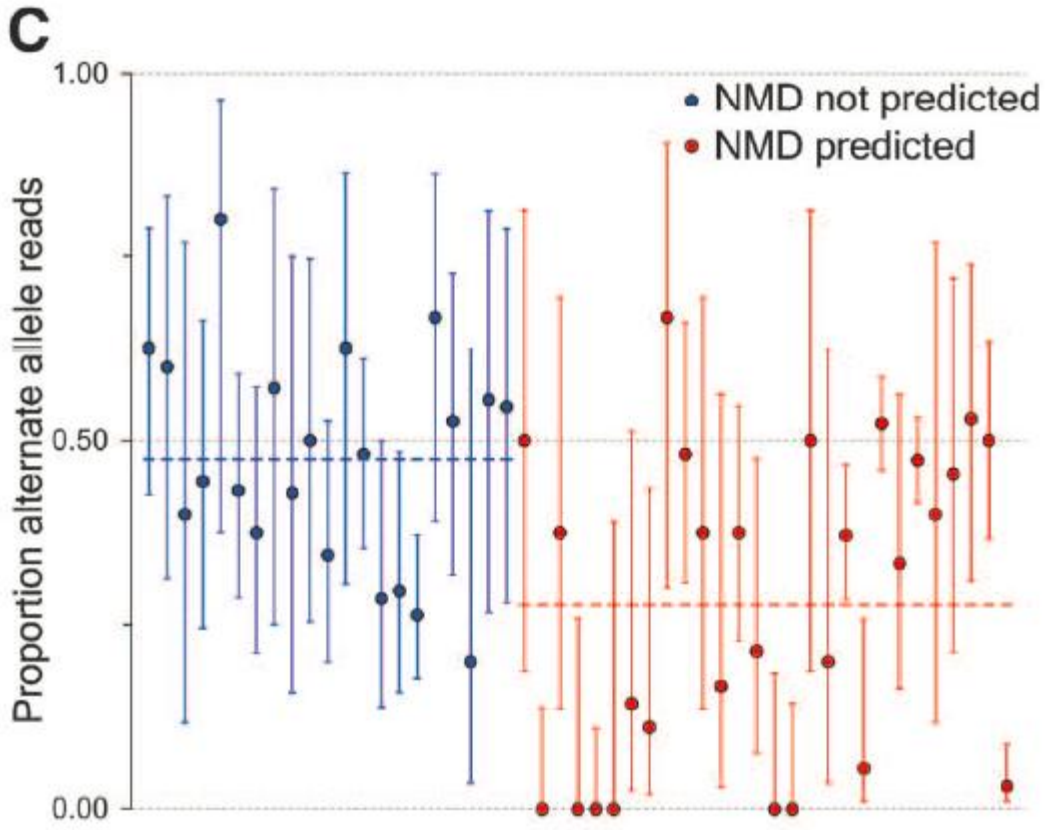


All sequenced genomes contain ~100 variants predicted to severely disrupt gene function.

Why do healthy people have disease variants?

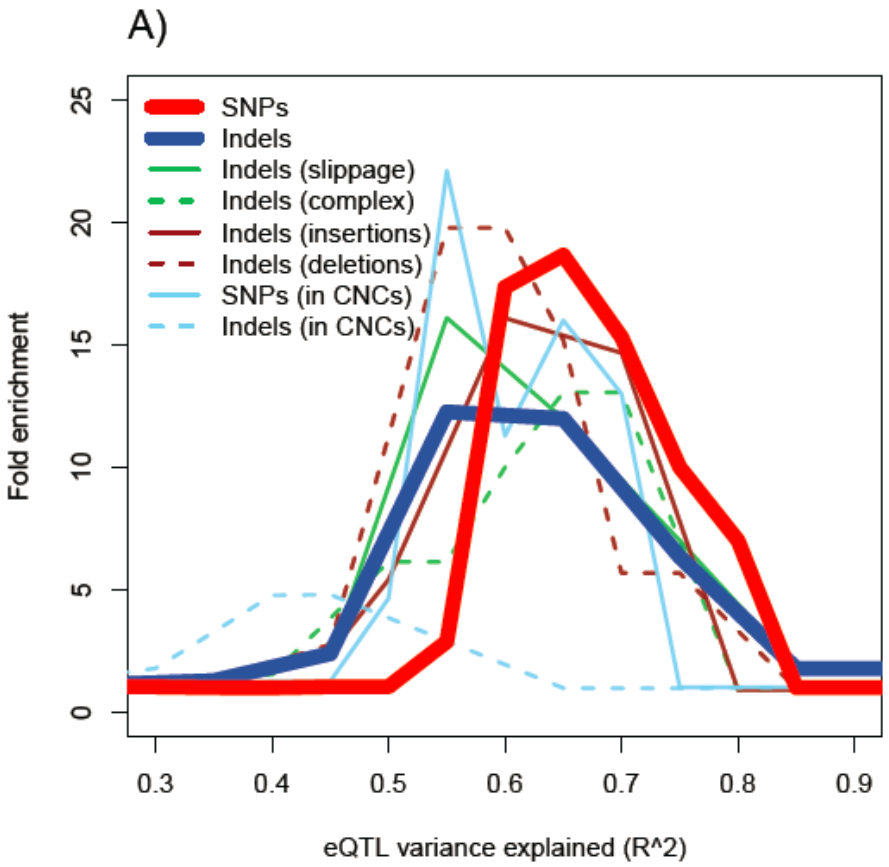
MacArthur et al, *Science*, 2012

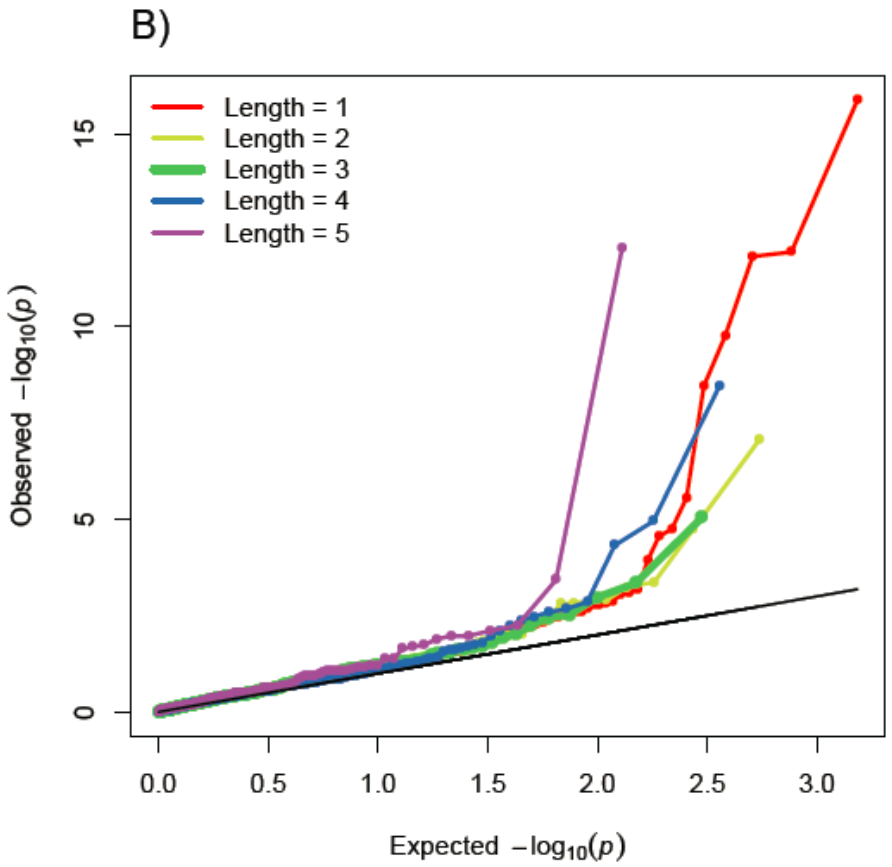
Interpreting completed genomes with gene expression



MacArthur et al., Science, 2012

The impact of short insertion deletion variation

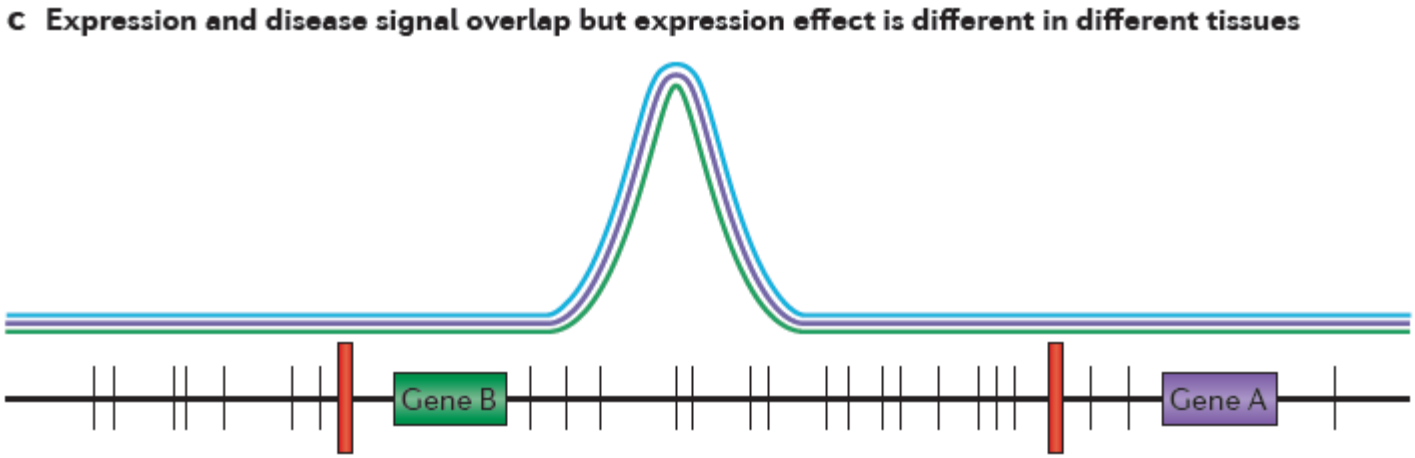




1000 Genomes Indel Analysis Group
Montgomery, Goode, Kvikstad et al, in submission

Understanding disease mechanism

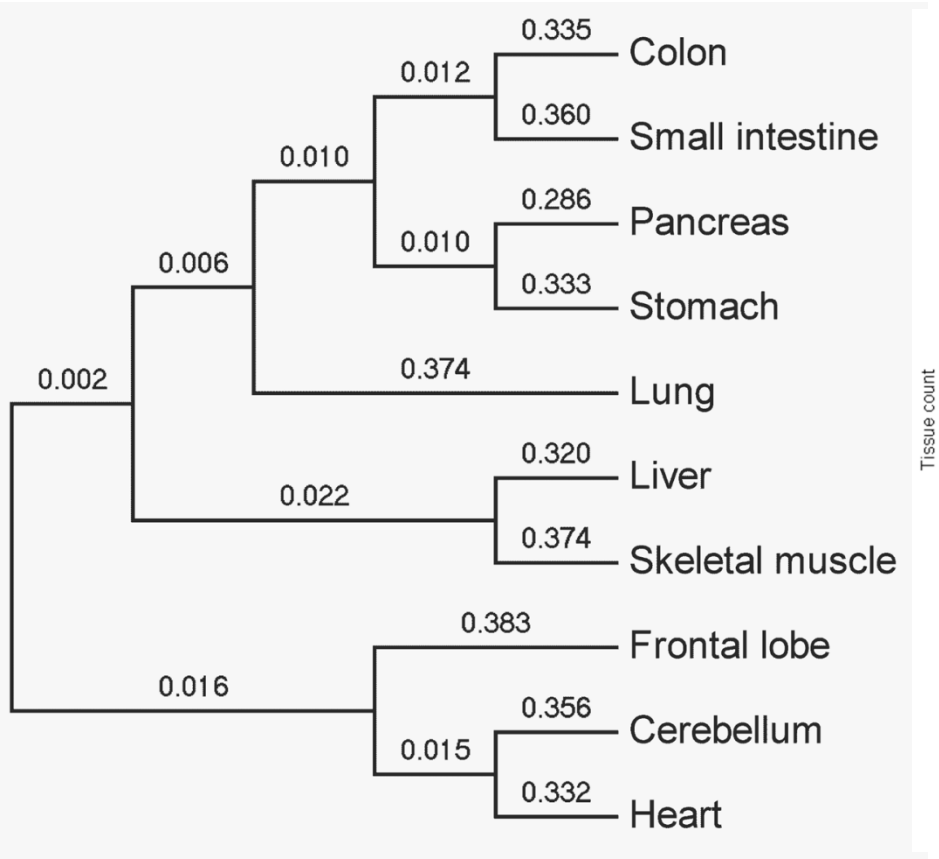
Predictive value of gene expression dependent on proximity to pathological tissue

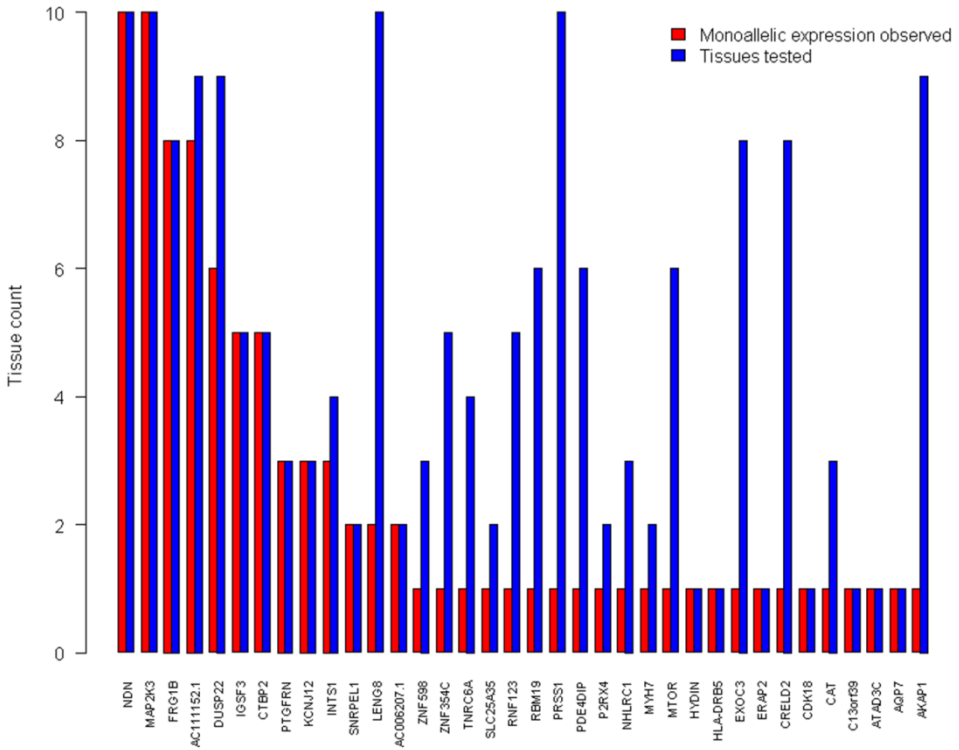


We have limited understanding of the Type I and II error rate

However, a lack of sharing may allow us to discover the pathological tissue

Tissue-specificity of allelic effects

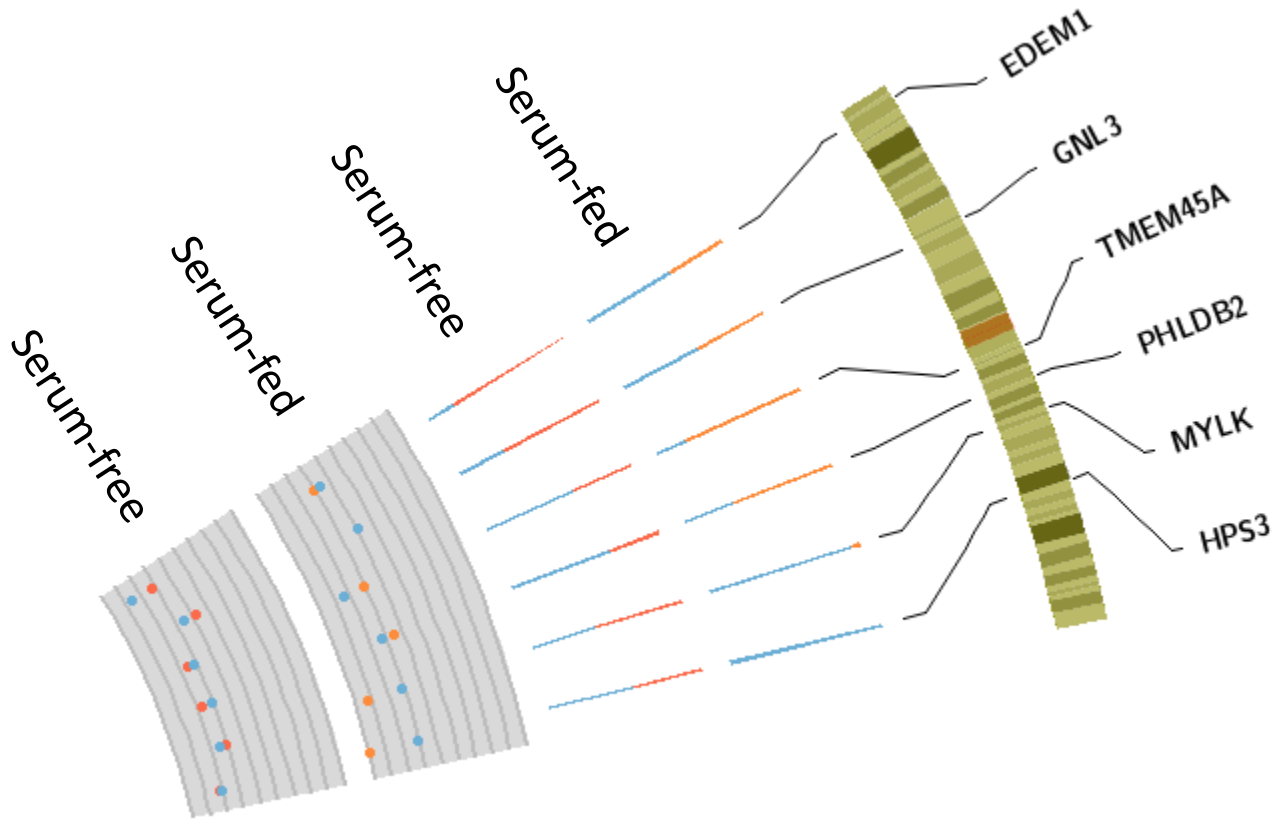




Kimberly Kukurba, Tracy Nance, Robert Piskol and Billy Li

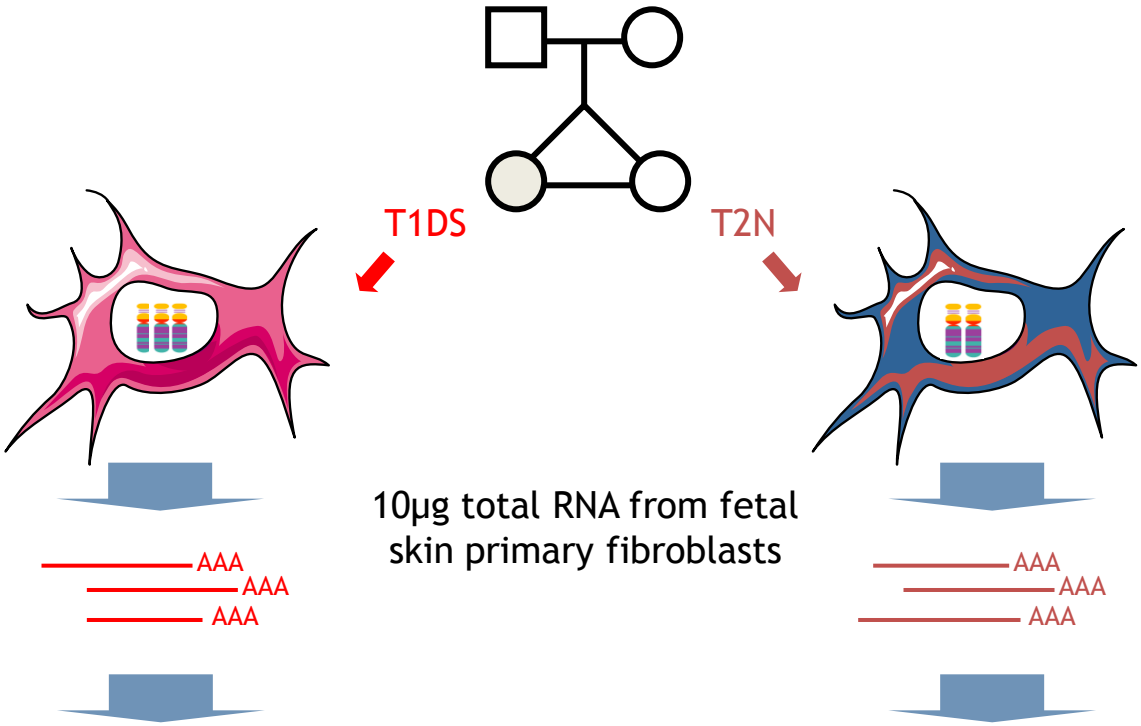
Using RNA-Sequencing to survey differential allelic expression in cardiovascular disease

Compared serum-starved and serum-fed coronary artery smooth muscle cells



Azad Raiesdana and Thomas Quertermous

Differential allelic expression in Down's syndrome

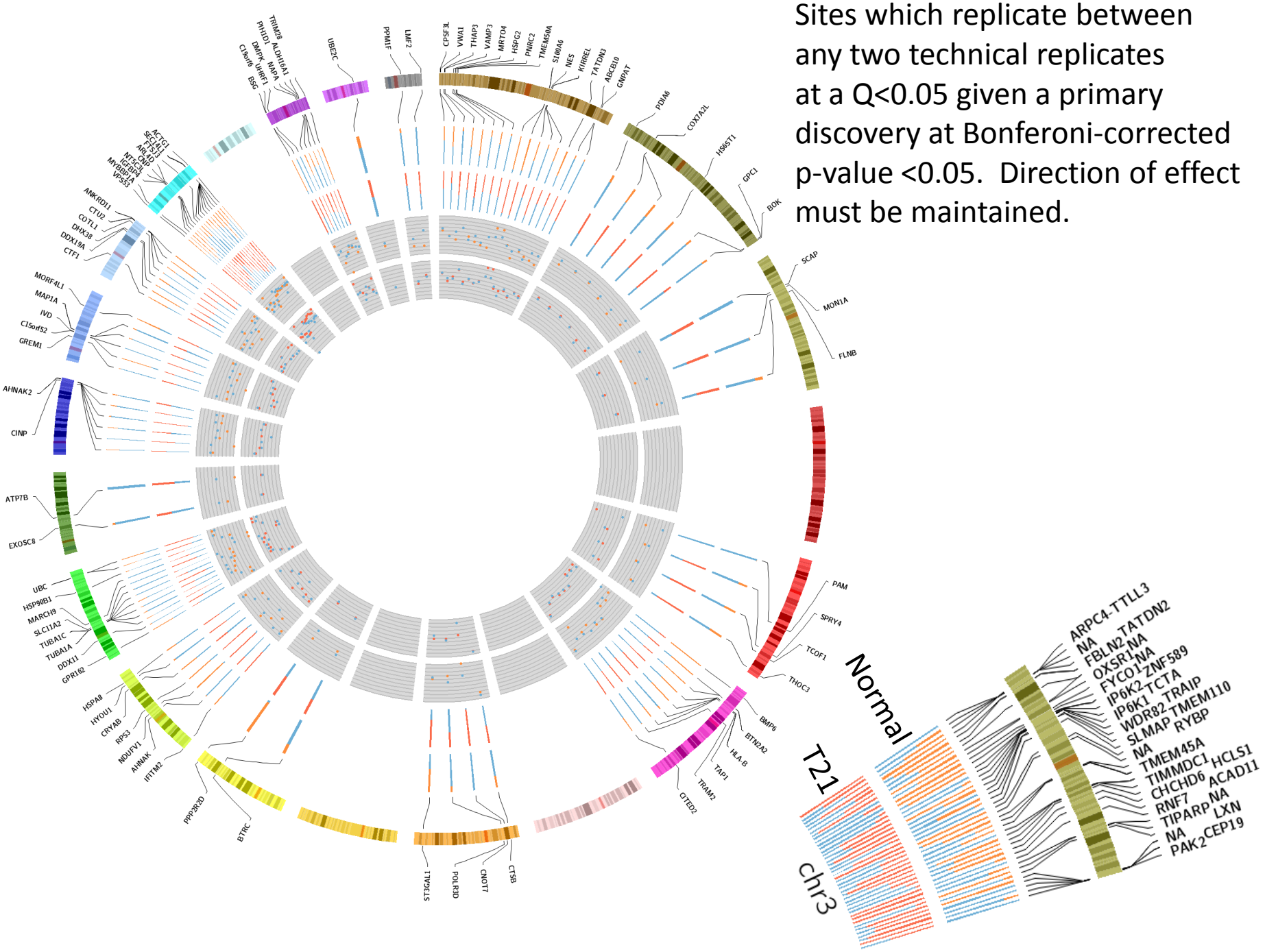


Gene expression sequencing

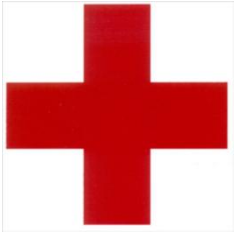
Regulatory variants influenced by extra copy of chromosome 21 indicated involvement of:

BACE2, COL6A1, COL6A2

Sites which replicate between any two technical replicates at a Q<0.05 given a primary discovery at Bonferroni-corrected p-value <0.05. Direction of effect must be maintained.



How will gene expression influence decisions in the clinic?



Build cellular models of disease

Survey diagnostic responses to treatments

Identify diverse disease mechanisms; move us beyond protein coding mutations alone

Identify pathological tissues

Allow us to identify effects (or transferability) in different populations

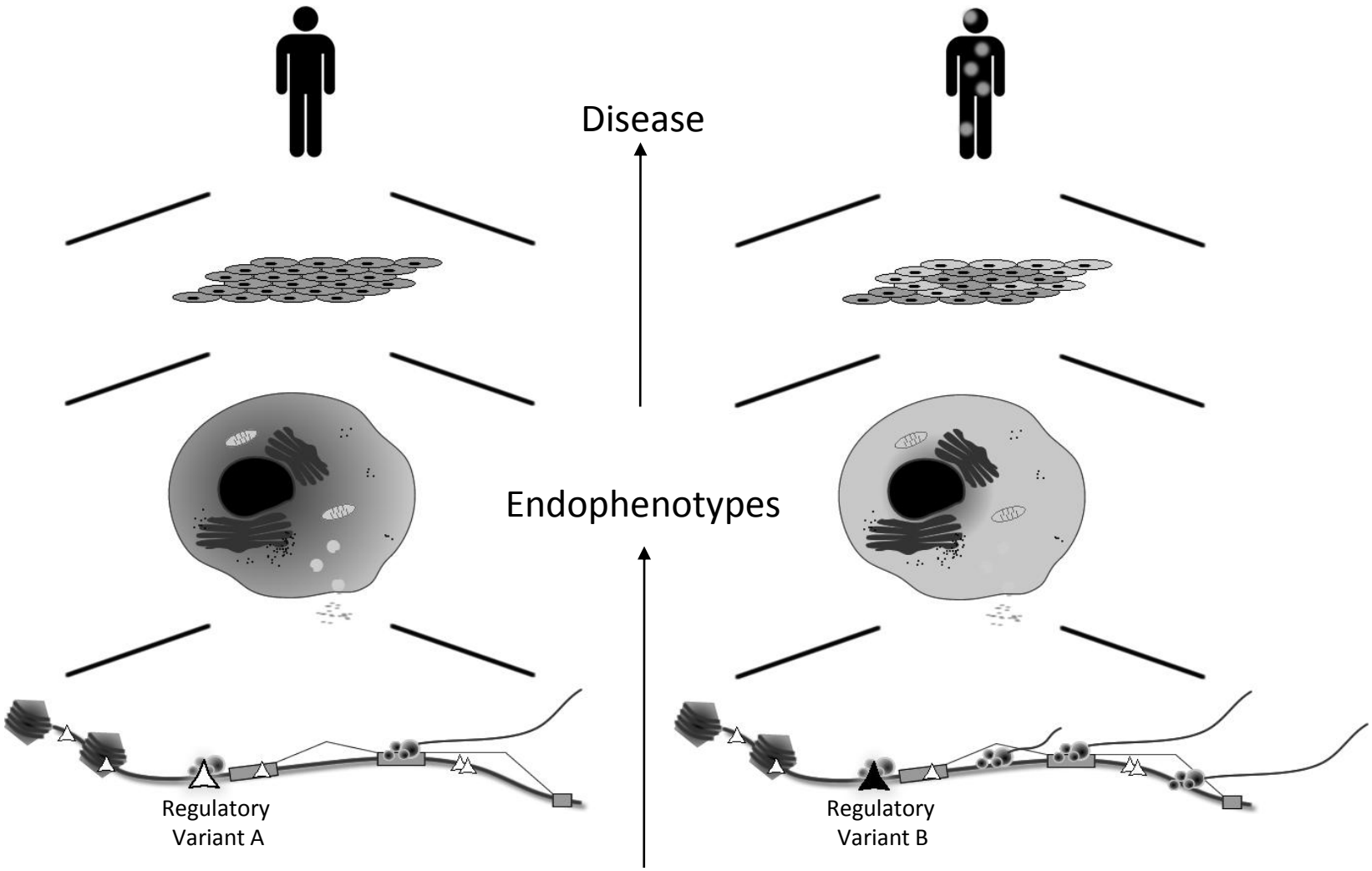
Classify undiagnosed conditions

Cost-effective

"**The field will transition from doing primarily association work to figuring out what implicated variants do biologically**."

David Goldstein, Director of the Center for Human Genome Variation, Duke University, *Nature*, Feb 2012

Increase value of investment in genetic studies
Determine what is best to assay to predict disease risk



Montgomery et al., *Human Mol Genetics*, 2009

montgomerylab.stanford.edu

Further recommended reading:

1) Genome, epigenome and RNA sequences of monozygotic twins discordant for multiple sclerosis (2010, Nature)

2) 9p21 DNA variants associated with coronary artery disease impair interferon-γ signalling response (2011, Nature)