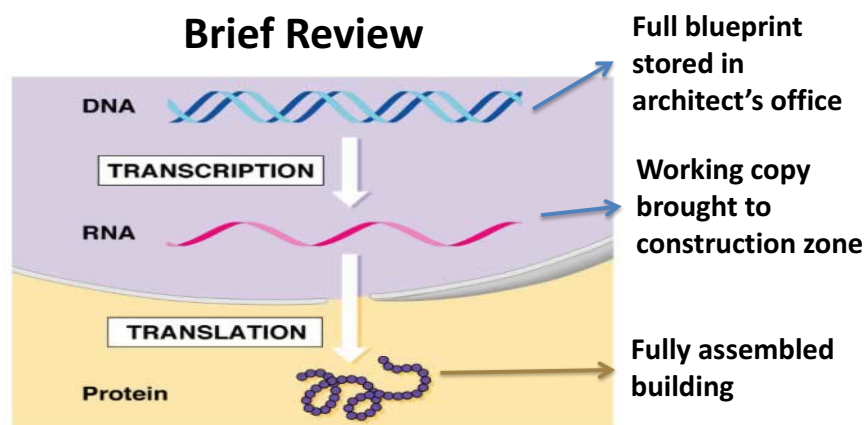


Integrative Approaches in Biobanks : Getting to Biological Mechanisms of Disease

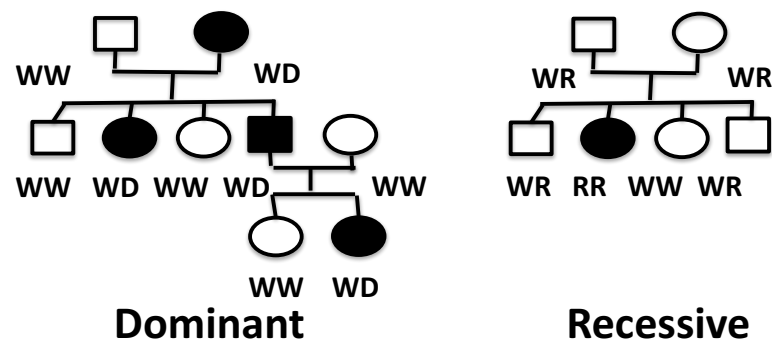
Nancy J. Cox, PhD
Vanderbilt Genetics Institute
Vanderbilt University Medical Center

But First Some Review

Brief Review



Rare Disease Transmitted in Families



Finding the Genetic Cause of a Rare Mendelian Disease

I am a cool dude.



Finding the Genetic Cause of a Rare Mendelian Disease

I am a coal dude.



Geneticists call this a “missense” mutation; still makes a protein, but the protein isn’t making as much sense.

Finding the Genetic Cause of a Rare Mendelian Disease

I am a cool rude.



This makes even less sense; it is a more deleterious missense mutation.

Finding the Genetic Cause of a Rare Mendelian Disease

I am a cmol dude.



This is closer to a “nonsense” mutation; mutation creates a protein that can’t go to completion.

Finding the Genetic Cause of a Rare Mendelian Disease

I am a **f**ool dude.



This might be considered a “gain-of-function” mutation. The protein is active – but is doing something entirely new.

Common Diseases

- Are familial, in that the risk of disease in a relative of an affected person is higher than the risk in the general population, but the transmission is not simple
- Many genetic and non-genetic risk factors contribute to common diseases
- Any disease that sends people to doctors or hospitals may have a genetic component

Finding Genetic Factors Contributing to a Common Disease...

Eating lots of sugar and fat is good for increasing your risk of diabetes. Instead eat lots of fruits and vegetables.



Finding Genetic Factors Contributing to a Common Disease...

Eating lots of sugar and fat is good! For increasing your risk of diabetes instead eat lots of fruits and vegetables.



Genomic Discoveries for Rare Mendelian Disease



Genomic Discoveries for Common Disease



Genomic Discoveries for Common Disease



G	C	A	C	G	G	T	T	G	T	T
T	C	C	C	A	G	C	T	A	G	C
G	G	T	T	C	G	T	A	T	C	T
G	C	A	C	G	G	T	T	G	T	T
T	T	A	A	A	C	T	T	C	C	C
C	C	C	T	G	G	G	C	G	T	T

Picking Mendelian Cherries



Relating Variation to Phenotype



Genotyping



Sequencing

Relating Variation to Phenotype



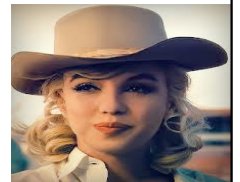
Common
Variants

+

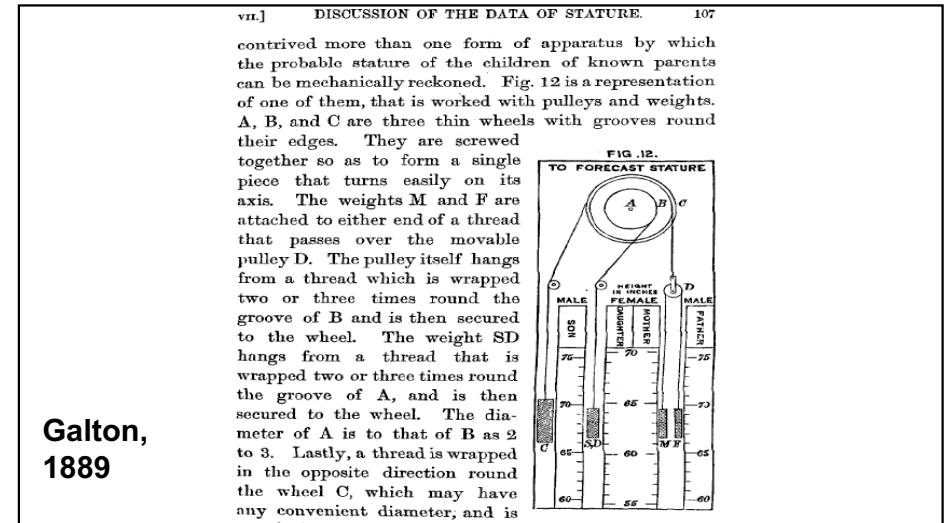
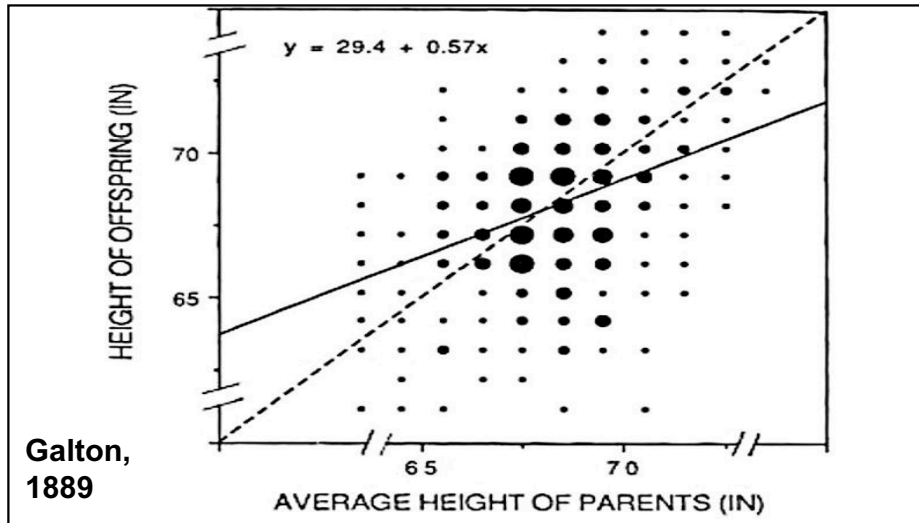


Rare
Variants

=



Genome
Interrogation



Estimating Heritability from SNPs

REPORT

GCTA: A Tool for Genome-wide Complex Trait Analysis

Jian Yang,^{1,*} S. Hong Lee,¹ Michael E. Goddard,^{2,3} and Peter M. Visscher¹

ARTICLE

Estimating Missing Heritability for Disease from Genome-wide Association Studies

Sang Hong Lee,¹ Naomi R. Wray,¹ Michael E. Goddard,^{2,3} and Peter M. Visscher^{1,*}

Table 2 Comparison of results of different polygenic methods across diseases

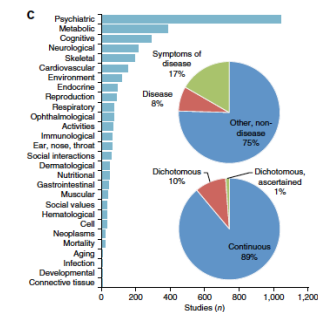
Disease	Prevalence (%)	Family based heritability ^a	Caused by common GWAS SNPs		
			LMM-based heritability (s.e.)	Polygenic modeling and Bayesian inference	
				Total variance explained (50% CI)	N SNPs (50% CI)
Rheumatoid arthritis	1	0.53–0.68 (–0.13 MHC) ^b	0.32 (0.037)	0.18 (0.15–0.20) (+0.04 known non-MHC) ^b	2,231 (1,588–2,740)
Celiac disease	1	0.5–0.87 (–0.35 MHC) ^b	0.33 (0.042)	0.44 (0.40–0.47)	2,550 (1,907–3,061)
MI/CAD	6	0.3–0.63	0.41 (0.067)	0.48 (0.43–0.54)	1,766 (1,215–2,125)
T2D mellitus	8	0.26–0.69	0.51 (0.065)	0.49 (0.46–0.53)	2,919 (2,335–3,442)

^aFamily based heritability estimates were taken from previous data for rheumatoid arthritis^{27,28}, celiac disease^{18,30}, MI/CAD^{31,32} and T2D^{33,34}. ^bWe excluded some loci in certain analyses: although the family based heritability estimates are based on the whole genome, the extended MHC region was removed from the common GWAS SNP analyses for rheumatoid arthritis and celiac disease, and validated non-MHC loci were further removed from the polygenic modeling analysis of the rheumatoid arthritis GWAS data. 50% CI, 50% credible interval; s.e., standard error.

Meta-analysis of the heritability of human traits based on fifty years of twin studies

VOLUME 47 | NUMBER 7 | JULY 2015 NATURE GENETICS

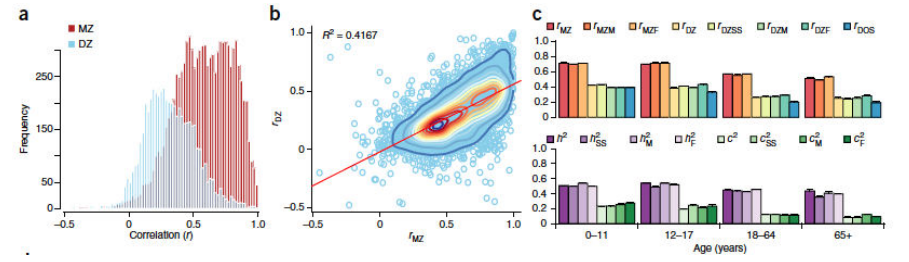
Tinca J C Polderman^{1,10}, Beben Benyamin^{2,10}, Christiaan A de Leeuw^{1,3}, Patrick F Sullivan⁴⁻⁶, Arjen van Bochoven⁷, Peter M Visscher^{2,8,11} & Danielle Posthuma^{1,9,11}



Meta-analysis of the heritability of human traits based on fifty years of twin studies

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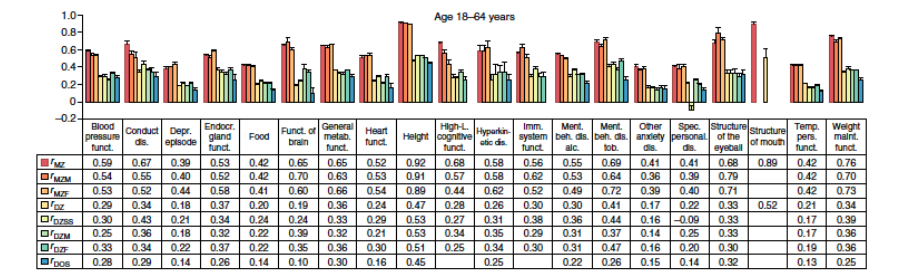
Tinca J C Polderman^{1,10}, Beben Benyamin^{2,10}, Christiaan A de Leeuw^{1,3}, Patrick F Sullivan⁴⁻⁶, Arjen van Bochoven⁷, Peter M Visscher^{2,8,11} & Danielle Posthuma^{1,9,11}



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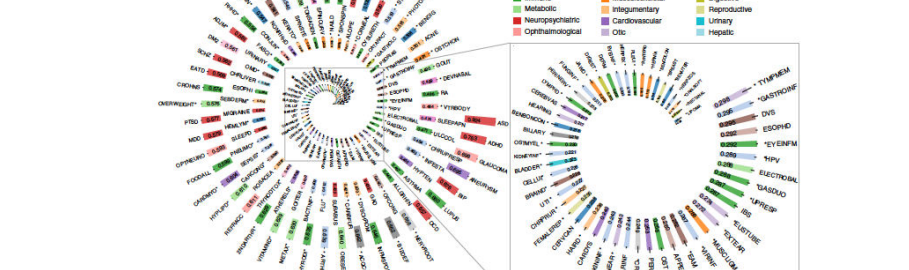
Tinca J C Polderman^{1,10}, Beben Benyamin^{2,10}, Christiaan A de Leeuw^{1,3}, Patrick F Sullivan⁴⁻⁶, Arjen van Bochoven⁷, Peter M Visscher^{2,8,11} & Danielle Posthuma^{1,9,11}



Classification of common human diseases derived from shared genetic and environmental determinants

VOLUME 49 | NUMBER 9 | SEPTEMBER 2017 NATURE GENETICS

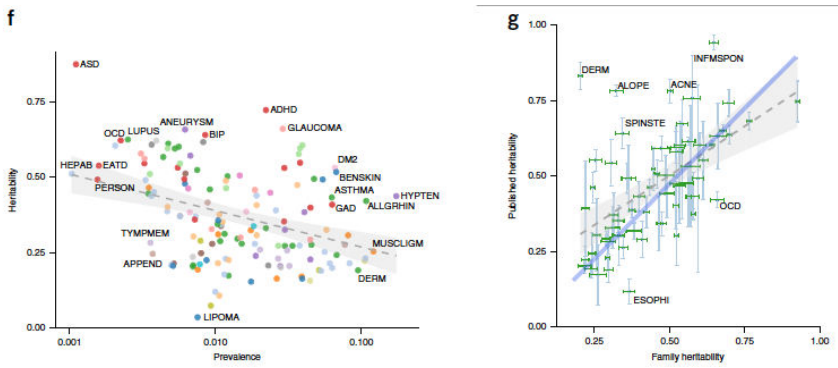
Kanix Wang^{1,2}, Hallie Gaitsch², Hoifung Poon³, Nancy J Cox⁴ & Andrey Rzhetsky^{2,5}



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Kanix Wang^{1,2}, Hallie Gaitsch², Hoifung Poon³, Nancy J Cox⁴ & Andrey Rzhetsky^{2,5}



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Kanix Wang^{1,2}, Hallie Gaitsch², Hoifung Poon³, Nancy J Cox⁴ & Andrey Rzhetsky^{2,5}

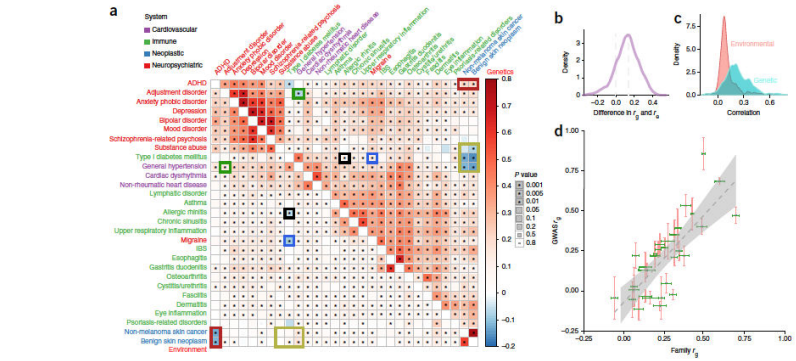
Table 1 Disease prevalence and heritability estimates for the 30 most prevalent diseases in our study

Disease	Prevalence	h^2	h^2 s.d.
Cardiac dysrhythmia	0.045	0.240	0.011
General hypertension	0.173	0.462	0.009
Esophageal disease	0.077	0.292	0.008
Functional digestive disorder	0.051	0.203	0.009
Type II diabetes mellitus	0.066	0.561	0.010
Allergic rhinitis	0.108	0.445	0.006
Asthma	0.063	0.437	0.008
Atopic contact dermatitis	0.099	0.202	0.006
Chronic sinusitis	0.047	0.523	0.008
Eye inflammation	0.045	0.292	0.009
Dileuoritis	0.068	0.256	0.012
Cellulitis	0.061	0.226	0.007
Ear infection	0.106	0.244	0.007
Eye infection	0.053	0.200	0.009
Fungal infection	0.083	0.211	0.007
UTI	0.083	0.227	0.007
Viral warts HPV	0.038	0.289	0.009
Acne	0.036	0.501	0.010
Keratosis	0.058	0.344	0.015
General spondylosis spine disorder	0.081	0.325	0.008
Muscle ligament disorder	0.121	0.268	0.006
Synovium tendon bursa disorder	0.039	0.180	0.009
Benign colon neoplasm	0.039	0.173	0.019
Benign skin neoplasm	0.067	0.547	0.007
Non-melanoma skin cancer	0.054	0.520	0.008
Anxiety phobic disorder	0.063	0.432	0.007
Depression	0.038	0.579	0.006
Substance abuse	0.045	0.422	0.010
Breast disorder	0.044	0.166	0.010
Disease of the female reproductive organs	0.105	0.235	0.009

Classification of common human diseases derived from shared genetic and environmental determinants

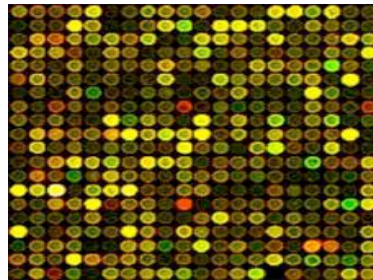
VOLUME 49 | NUMBER 9 | SEPTEMBER 2017 NATURE GENETICS

Kanix Wang^{1,2}, Hallie Gaitsch², Hoifung Poon³, Nancy J Cox⁴ & Andrey Rzhetsky^{2,5}



Transcriptomics Review

Measuring Transcript Levels



RNA-seq analysis workflow

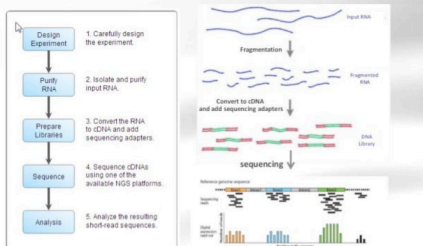
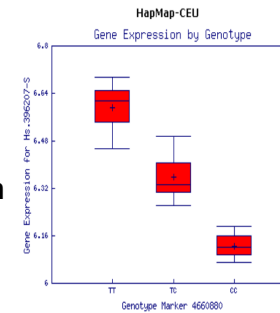


Illustration of eQTL (An integration: genome x transcriptome)

Gene
Expression

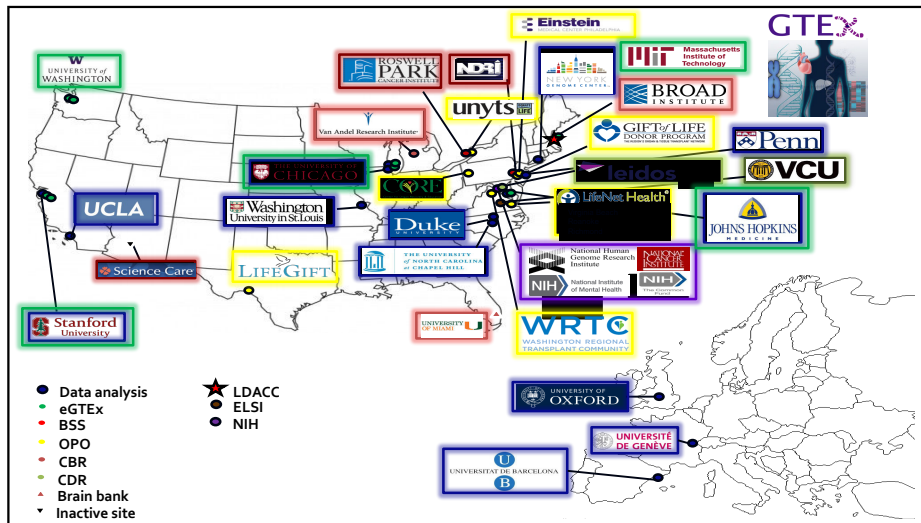
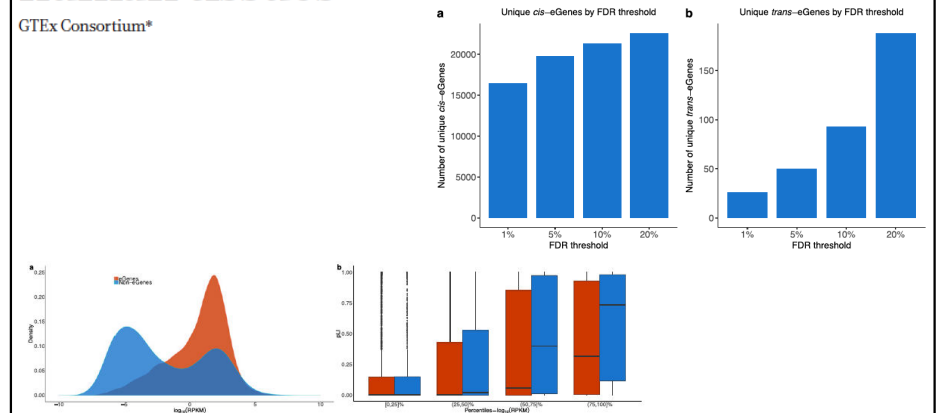


Genotype

Genetic effects on gene expression across human tissues

204 | NATURE | VOL 550 | 12 OCTOBER 2017

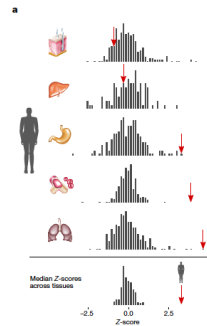
GTEx Consortium*



The impact of rare variation on gene expression across tissues

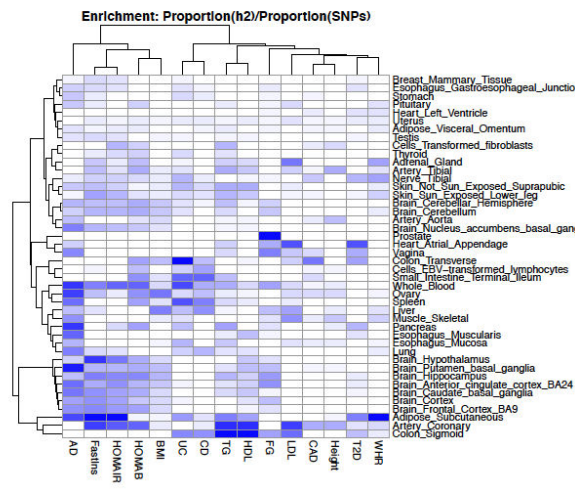
12 OCTOBER 2017 | VOL 550 | NATURE | 239

Xin Li^{1*}, Yungil Kim^{2*}, Emily K. Tsang^{1,3*}, Joe R. Davis^{1,4*}, Farhan N. Damani², Colby Chiang⁵, Gaelen T. Hess⁴, Zachary Zappala^{1,4}, Benjamin J. Strober⁶, Alexandra J. Scott⁵, Amy Li⁴, Andrea Ganna^{7,8,9}, Michael C. Bassik⁴, Jason D. Merker¹, GTEx Consortium[†], Ira M. Hall^{5,10,11}, Alexis Battle^{2§} & Stephen B. Montgomery^{1,4§}

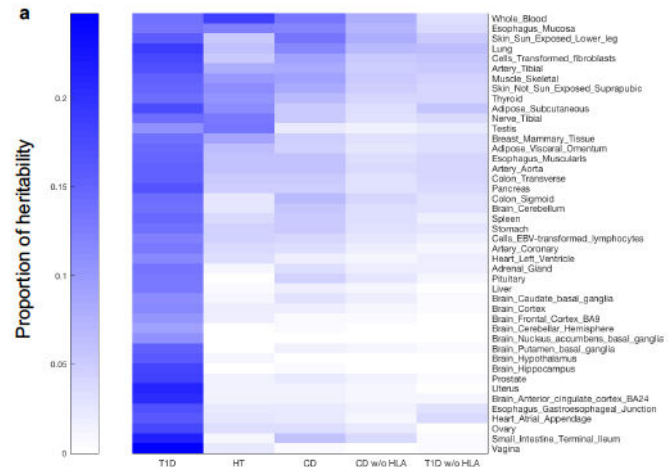


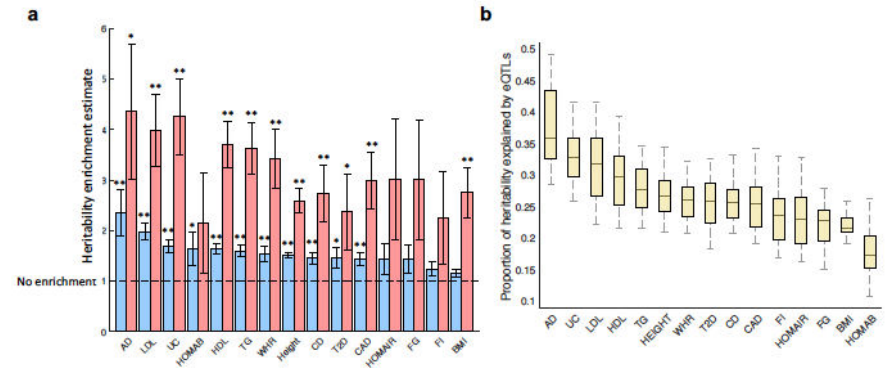
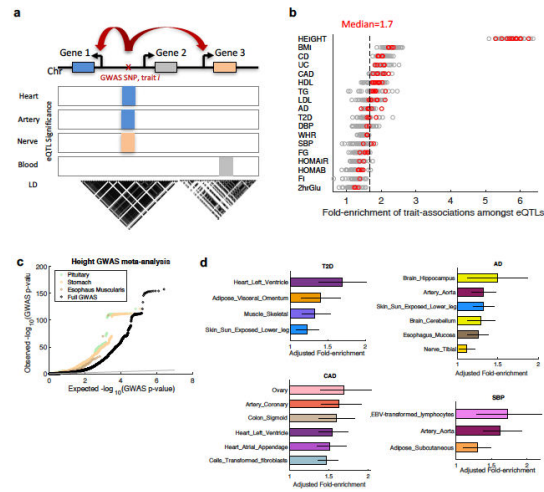
we observed that 58% of underexpression and 28% of overexpression outliers had rare variants near the relevant gene, compared to 8% for non-outliers (Fig. 3c).

GWAS and Transcriptomics

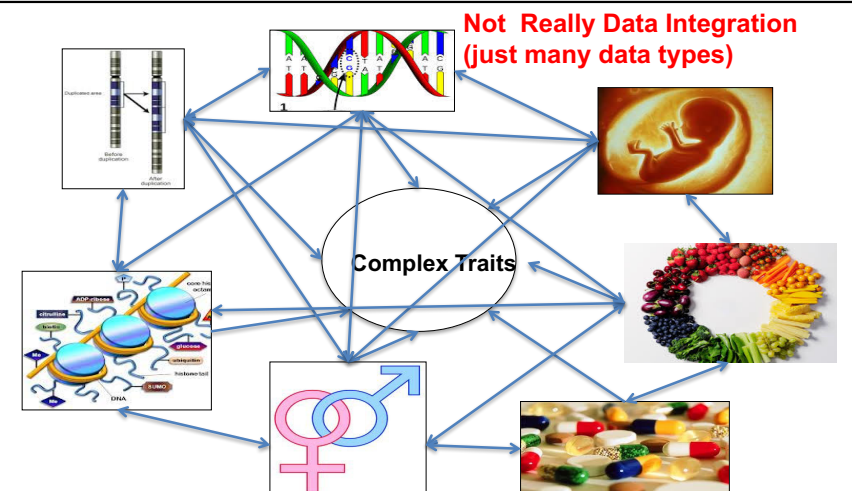


Fold enrichment (proportion of h^2 explained by eQTLs / proportion of total SNPs that were tissue-specific eQTLs)

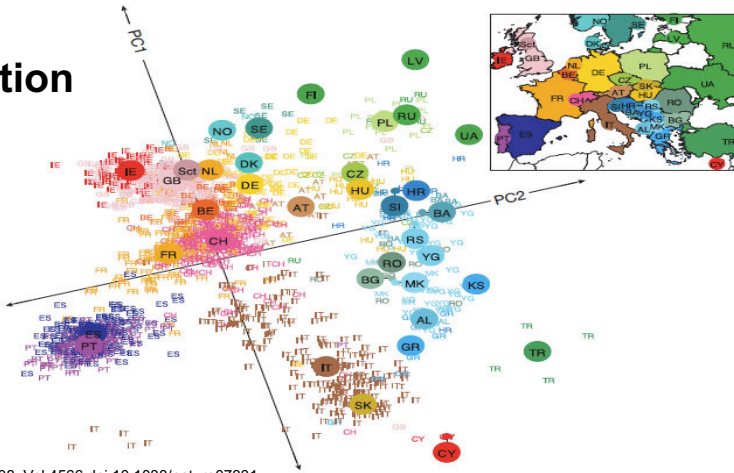




What is Data Integration?



Data Integration



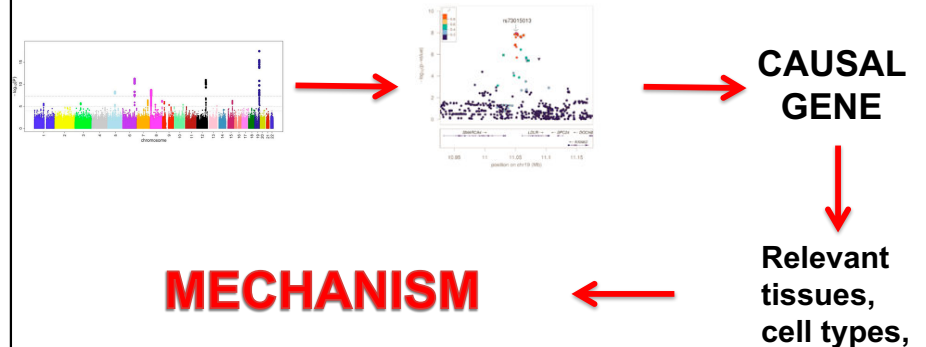
Novembre et al, 2008, Vol 4566 doi:10.1038/nature07331

Data Integration: Phenome X -OMICS

**What can we do uniquely
well in biobank data?**

**How can biobank strengths
improve understanding of
mechanisms of disease?**

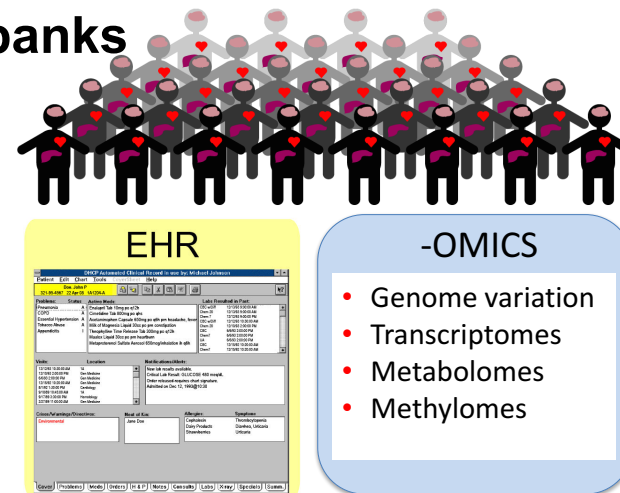
What Biobanks Do Uniquely Well?



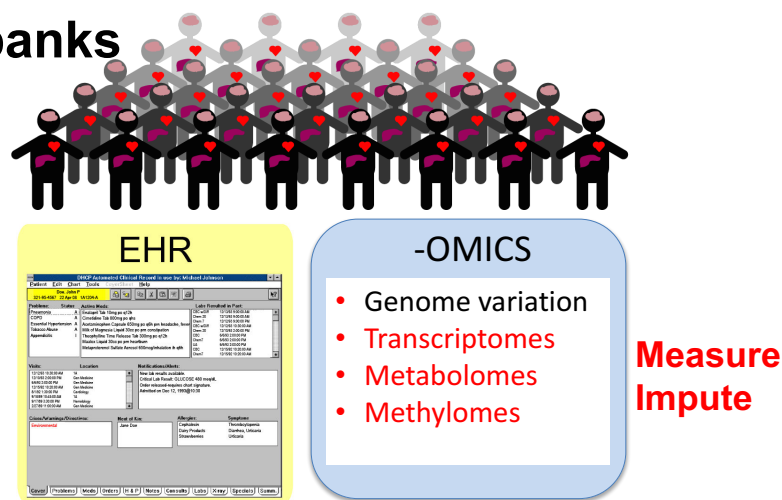
What Biobanks Do Uniquely Well?



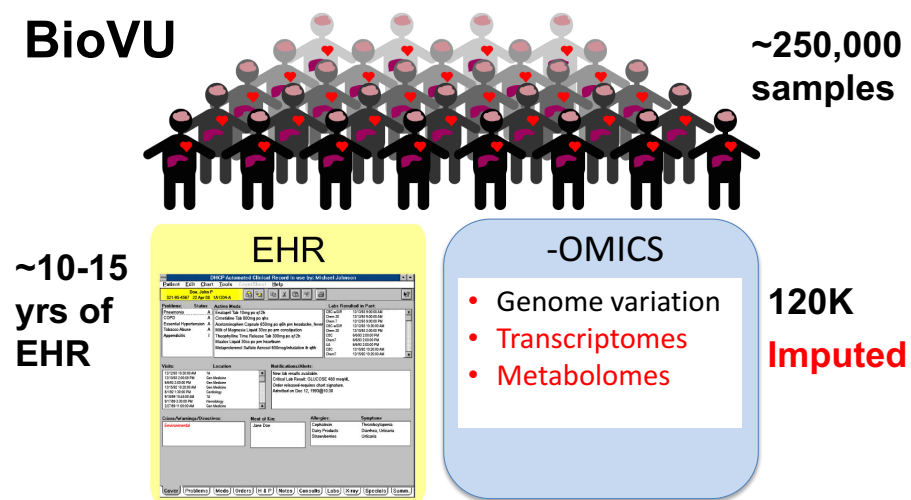
Biobanks

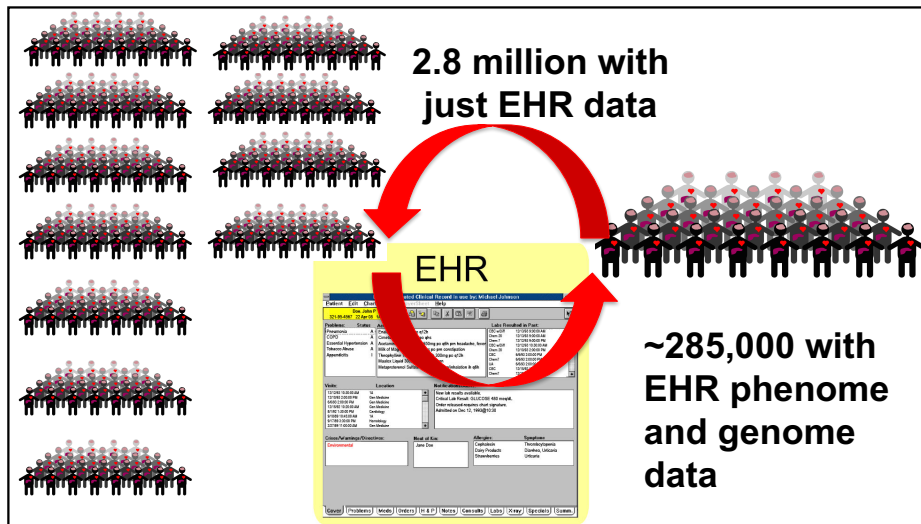


Biobanks

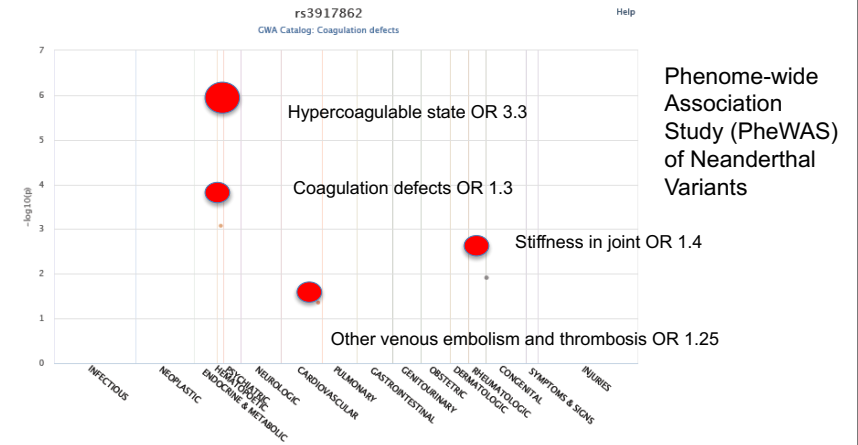


BioVU





What can we do uniquely well in BioVU?



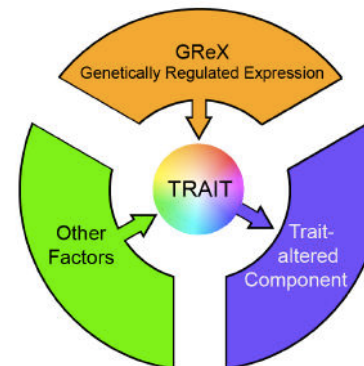
GWAS: What variants are associated with this phenotype?

PheWAS: What phenome is associated with this variant?

PrediXcan (Gamazon et al., 2015, Nat Genet)

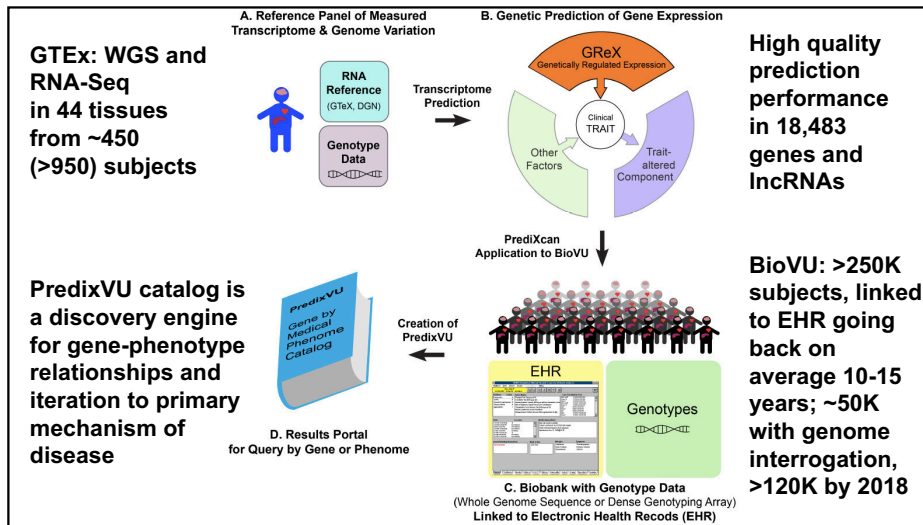
<https://github.com/hakyimlab/PrediXcan>

Reference panel: GTEx



$$T = \sum_k \omega_k X_k + \varepsilon$$

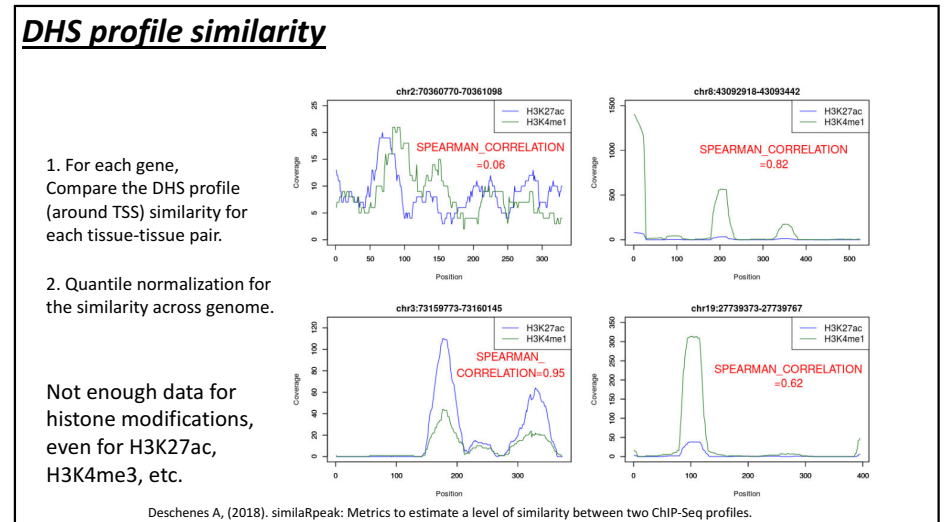
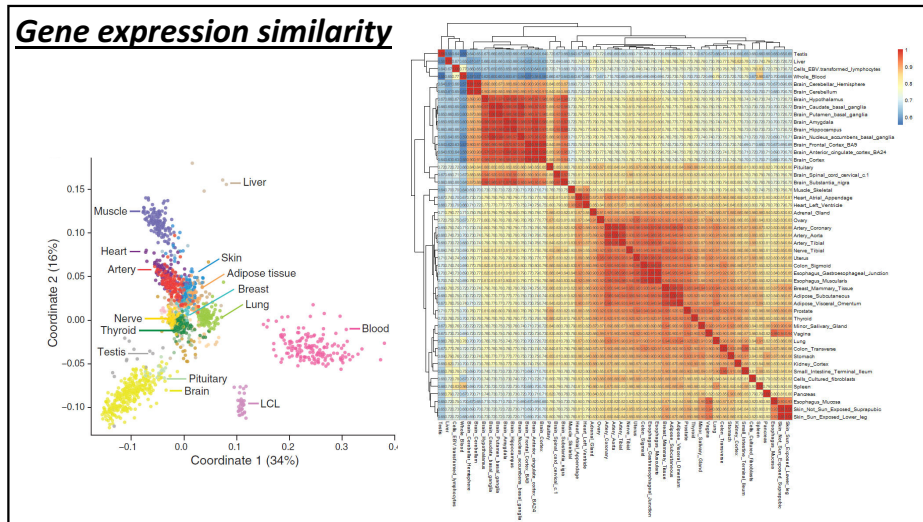
GReX – Genetically Regulated Expression



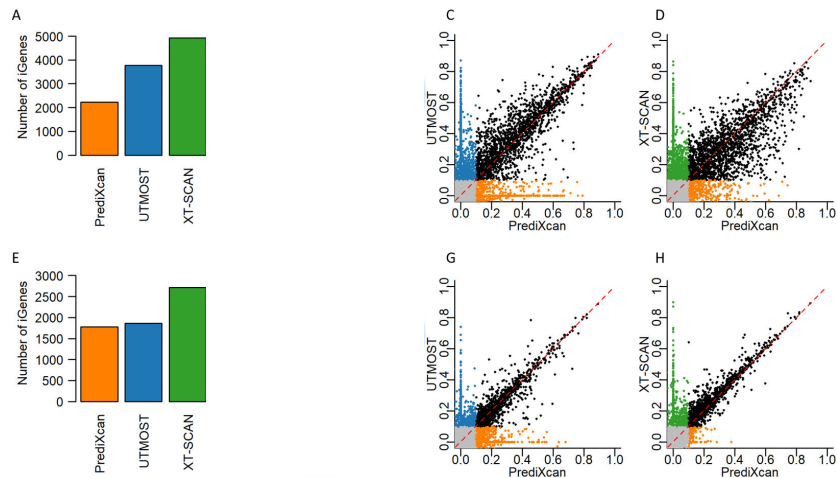
<u>Comparison</u>	PrediXcan	UTMOST	XT-SCAN
Cross-tissue	No	Yes	Yes
Prediction Model	Elastic Net	Sparse Group LASSO	Weighted Elastic Net
Incorporate regulatory elements	No	No	Yes (DHS)
Incorporate expression similarity	No	No	Yes

Eric Gamazon

Dan Zhou



Performance comparison among PrediXcan, UTMOST, and XT-SCAN



What Discoveries Do Biobanks Uniquely Enable?

*Tennessee Biobanks
Special Recipe:*

**Genes (with
known
function)**



Phenome!

Gene-based PheWAS

What does this gene do?

**What does the natural variation
in the expression of this gene
associate with across the
medical phenome?**

The Full Medical Phenome!

EHR

Patient: John P. [ID: 321-45-678] 22 Apr 19, 161204A

Problems:

- Essential Hypertension
- Chronic Kidney Disease
- Appendicitis

Medications:

- Enalapril Tab 10mg po qd
- Acetaminophen Caplets 500mg po q6h prn headache, fever
- Milk of Magnesia Liquid 300mg po qd
- Theophylline Time Release Tab 300mg po q12h
- Mallory Liquid 300mg po qd
- Metoprolol Succinate Release 500mg po qd

Lab Results:

- CBC w/Plt: 12/15/12 10:00 AM
- Che7: 12/15/12 10:00 AM
- Che7: 12/15/12 10:00 AM
- Che7: 12/15/12 10:00 AM
- Che7: 12/15/12 10:00 AM
- Che7: 12/15/12 10:00 AM
- Che7: 12/15/12 10:00 AM

Visit: 12/15/12 10:00 AM

Location: 1A

Medication: 1A

Notes: New lab results available. Critical Lab Result: GLUCOSE 400 mg/dL. Order released-requires chart signature. Admitted on Dec 12, 1998@10:30

Diagnosis/Procedures: Environmental

Medication: 1A

Notes: 1A

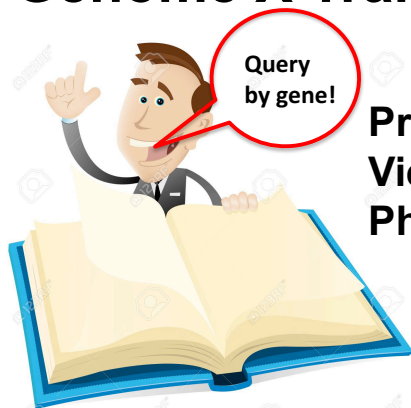
Diagnosis/Procedures: Environmental

Medication: 1A

Notes: 1A

- Characterize larger-scale consequences of genes
- More/better ways to mechanism
- PheRS larger, richer targets for gene discovery

Genome X Transcriptome X EHR



Query
by gene!

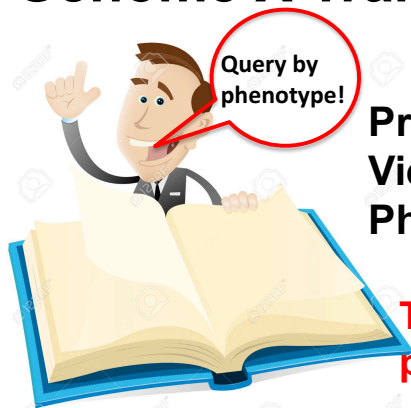
**PredixVU: A Catalog for
Viewing Gene-Based
Phenome-Wide Association**

... or gene set, pathway,
network ...

ZNF577

Trait	R ²	OR per unit SD	P-value	Cases	Controls
Viral hepatitis C	0.37	0.84	6.21E-07	808	20904
Viral hepatitis	0.37	0.86	4.38E-06	937	20904
Malignant neoplasm of liver, primary	0.37	0.81	1.61E-04	267	22080
Cancer of liver and intrahepatic bile duct	0.37	0.82	2.43E-04	318	22080
Precordial pain	0.37	1.22	4.34E-04	374	12283

Genome X Transcriptome X EHR



Query by
phenotype!

**PredixVU: A Catalog for
Viewing Gene-Based
Phenome-Wide Association**

Test pleiotropy, investigate
phenome relationships, ...

Viral Hepatitis C

Gene	R ²	OR per unit SD	P-value	Cases	Controls
ZNF577	0.37	8.44E-01	6.21E-07	808	20904
ZNF649	0.12	8.48E-01	1.85E-06	808	20904
SPAG1	0.22	8.63E-01	2.98E-05	808	20904
KLRC1	0.42	8.57E-01	4.31E-05	808	20904
CST2	0.14	8.64E-01	4.42E-05	808	20904

Intestinal Infection

Gene	R ²	OR per unit SD	P-value	Cases	Controls
NDUFA4	0.03	1.16	1.83E-09	1608	24187
C10orf120	0.006	1.13	4.92E-08	1608	24187
TJP1	0.066	1.12	5.27E-06	1608	24187
YEATS2	0.012	0.89	6.33E-06	1608	24187
DGAT2	0.12	1.12	1.11E-05	1608	24187
GNA12	0.23	1.12	1.32E-05	1608	24187
ZNF525	0.13	0.89	1.96E-05	1608	24187
ALLC	0.15	0.89	2.08E-05	1608	24187
HDGFRP2	0.005	0.90	3.45E-05	1608	24187

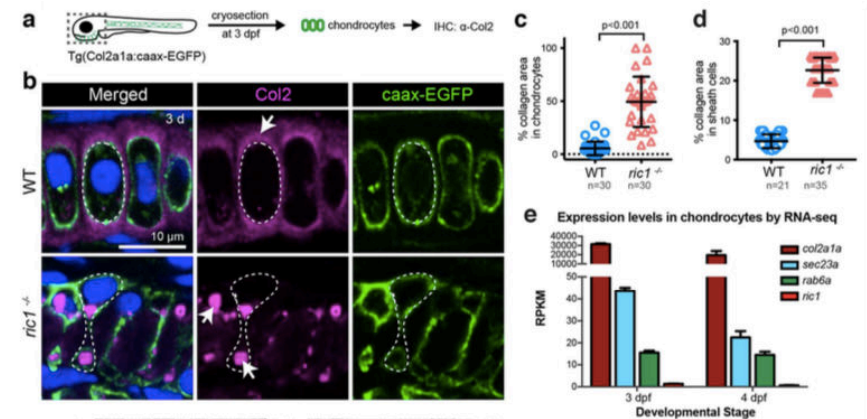
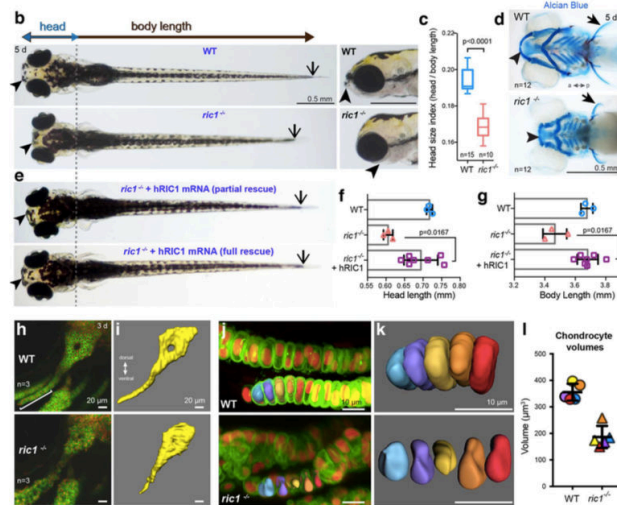
A Fish(ing) Story

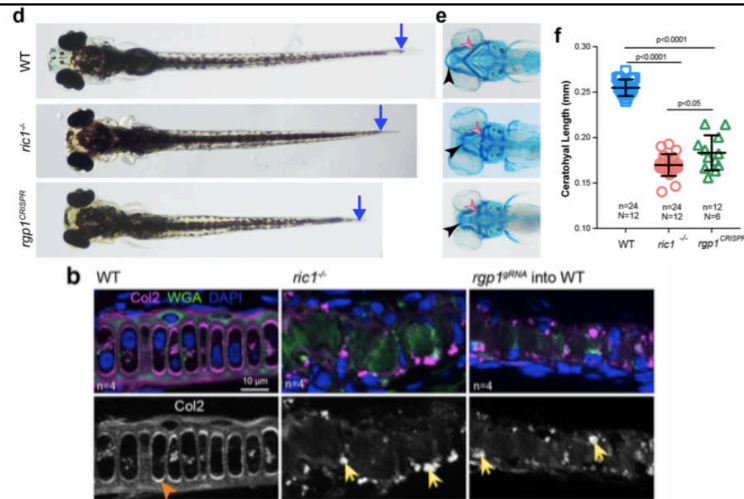
Tennessee Biobanks
Special Recipe:

RIC1
Mechanism:
Moves fibrillar
collagens out
of cells



Phenome?

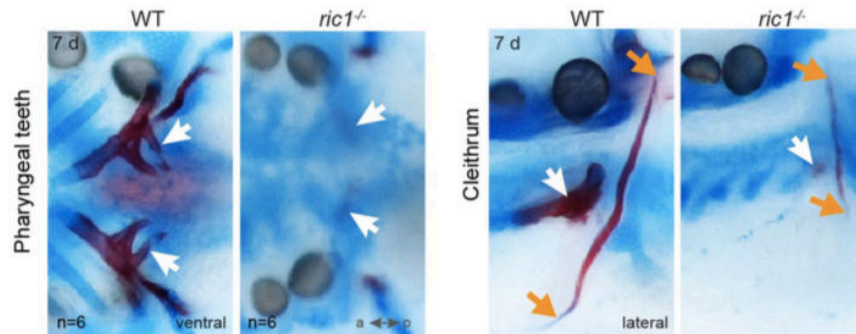




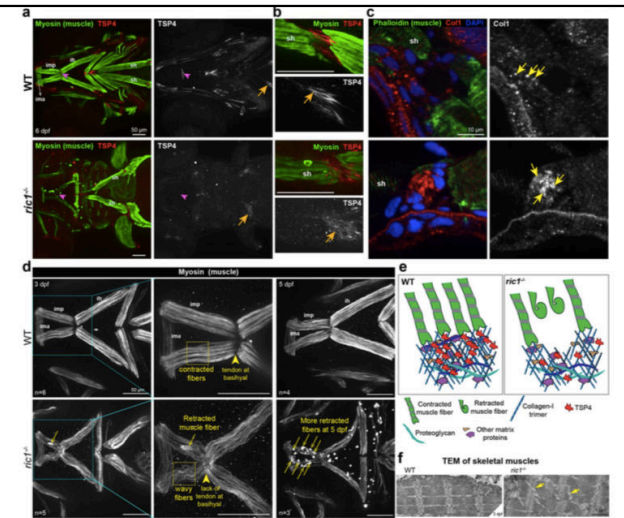
BioVU Phenome for *RIC1/RGP1*

- Bone, fracture, and connective tissue disorders
- Tooth development and eruption
- Gait disorders
- Asthma, heart valve defects and replacement
- Esophageal, gut and digestive disorders
- Strabismus, amblyopia
- Neurological and neuropsychiatric disorders

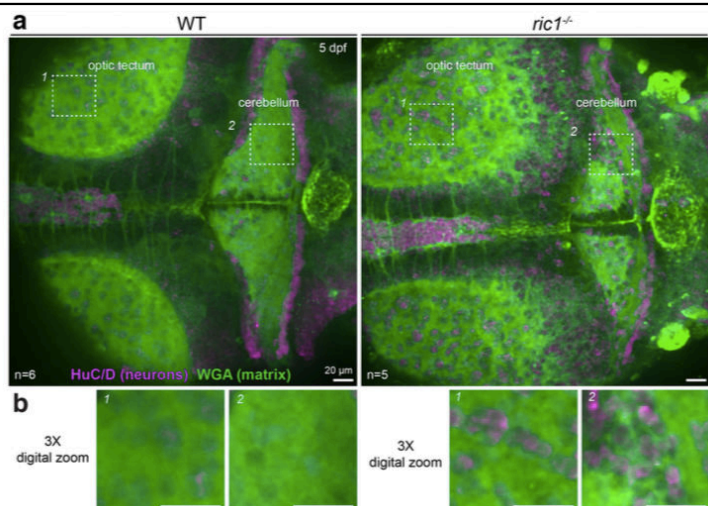
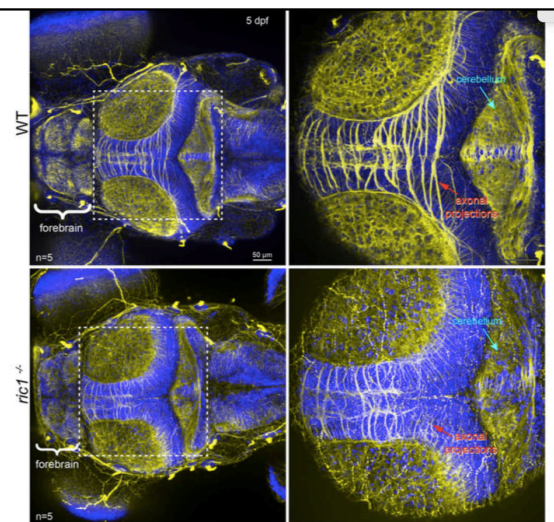
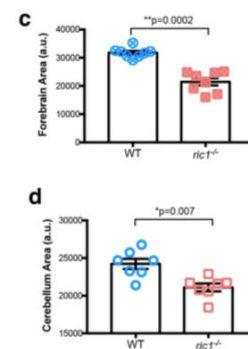
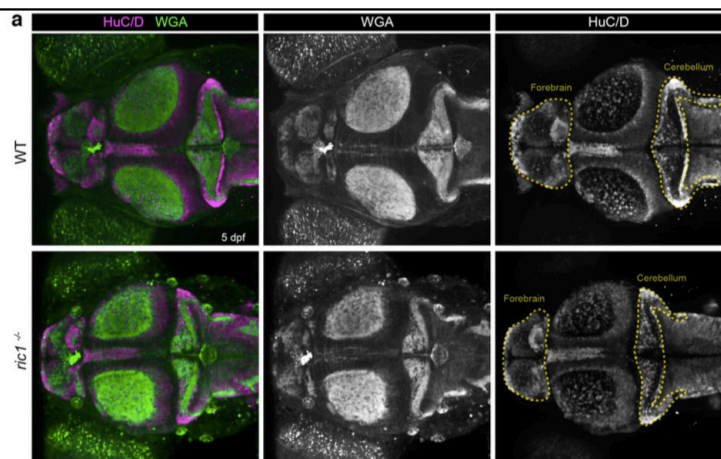
Results of BioVU studies prompted zebrafish studies of tooth development...



Muscle attachment studies in small (eye) and large muscles...

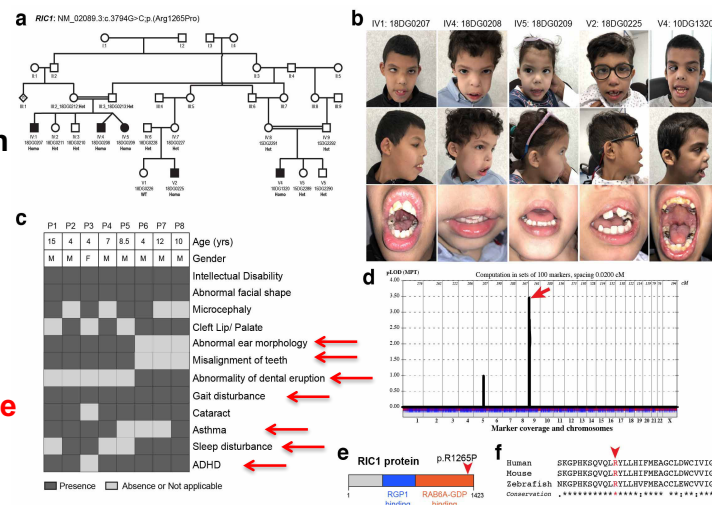


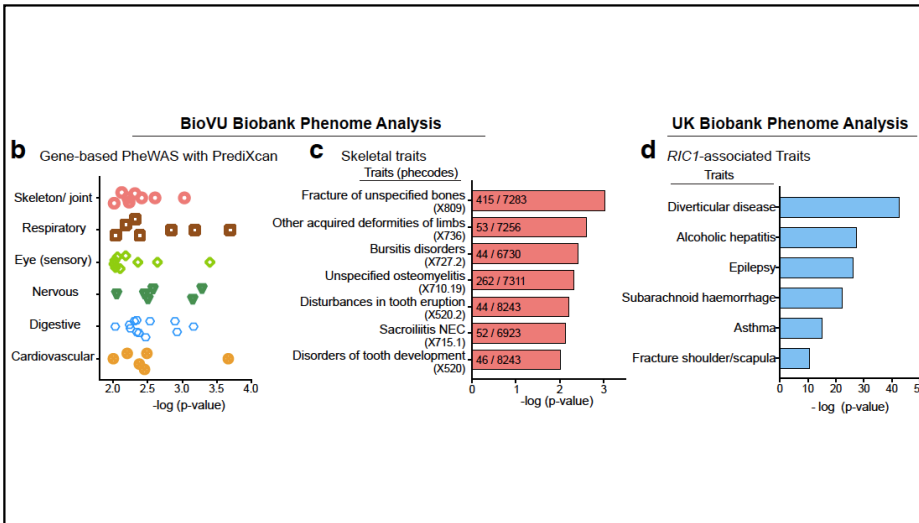
And brain studies



BioVU results used to guide the re-evaluation of patient phenomes

From Moms:
Gut Phenome





Pleiotropy:

Failure of basement membrane:
digestive disorders, vitamin /
mineral deficiencies, malabsorption

RIC1, *RGP1* fail to traffic fibrillar collagens – the most abundant extracellular matrix proteins in vertebrates, providing tissues and organs with form, stability and connectivity

Inadequate ECM, failure to produce cartilage:
asthma, organ prolapse, connective tissue disorders, valve defects, fracture, tooth defects

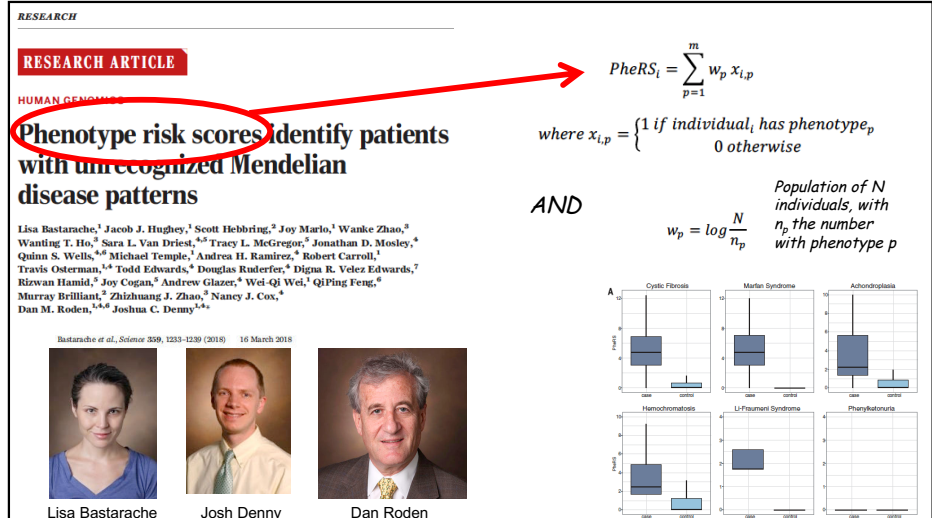
Failure of muscle attachment:
strabismus, amblyopia, gait disorders

Inadequate brain ECM:
Epilepsy, pervasive developmental delay, intellectual disability, ADHD, alcohol related disease, restless legs

Phenome Fishing to Find More Genes in the *RIC1/RGP1* Space



Use a Phenome Risk Score (PheRS) as the phenotype (rather than an individual disease code)



Genes with Significant Association to *RIC1/RGP1* PheRS

COL14A1, COL3A1, COL5A2, COL9A3

Exchanges bound GDP for free GTP – a key part of the *RIC1/RGP1* machine for collagen transport (Duh!)

FAM124A, FGD6, EPC2, EPPK1, ADAMTSL4, ATP5H, EDEM2, CPT1A

↓ ER-associated degradation of misfolded glycoproteins
↓
Attaches to fibrillin and fibrillar collagens to stabilize extracellular matrix

Creating a Poly-gene Score for ECM Strength

- Directions of effects consistent across the genes functioning to strengthen ECM
- Sum predicted expression across gene set to probe phenome associations to ECM strength

Pleiotropy is ubiquitous.

Continuum from Mendelian to Complex

Continuum from LOF to deleterious to ↓ expression

GREX associated with VACTERL PheRS

Gene	p	Tissue
SNX25	8.39E-18	ArteryAorta
UBE2W	8.39E-18	Brain, Cerebellum
RPS17	8.39E-18	Brain, NucleusAccumbensBasalGanglia
MYBL1	8.39E-18	Liver
HLA_DQA1	8.39E-18	Vagina
PCSK4	8.39E-18	WholeBlood
HLA_DQA2	4.90E-16	Spleen
HLA_DQA1	1.27E-13	Vagina
DHODH	2.27E-11	Prostate
ACTA2	3.60E-09	SkinNotSunExposed
POLQ	1.56E-07	ArteryAorta
VSK1	1.56E-07	Brain, Cerebellum
ZFYVE1	1.56E-07	Brain, NucleusAccumbensBasalGanglia
LILRB4	1.56E-07	WholeBlood
ACSL1	2.24E-07	Adrenal

J Biol Chem, 2016 Feb 5;291(6):3026-42. doi: 10.1074/jbc.M115.676601. Epub 2015 Nov 24.

Loss of the Ubiquitin-conjugating Enzyme UBE2W Results in Susceptibility to Early Postnatal Lethality and Defects in Skin, Immune, and Male Reproductive Systems.

Yiwa B¹, Seifried SP¹, Vincent MP¹, Foster AS¹, Bissler SJ¹, Narasimhan SP², Zeng L¹, Eickholt-Johnson K¹, Miller RA¹, Jinn DP¹, Chappas AE¹, Schmitt BP¹, Sotgiro KM¹, Paulson JS¹.

¹ Author Information

Abstract

UBE2W ubiquitinates N-termini of proteins rather than internal lysine residues, showing a preference for substrates with intrinsically disordered N termini. The in vivo functions of this intriguing E2, however, remain unknown. We generated Ube2w germ line KO mice that proved to be susceptible to early postnatal lethality without obvious developmental abnormalities. Although the basis of early death is uncertain, several organ systems manifested changes in Ube2w KO mice. Newborn Ube2w KO mice often show altered epidermal maturation with reduced expression of differentiation markers. Mirroring higher UBE2W expression levels in testis and thymus, Ube2w KO mice showed a disproportionate decrease in weight of these two organs (~50%), suggesting a functional role for UBE2W in the immune and male reproductive systems. Indeed, Ube2w KO mice displayed sustained neutrophilia accompanied by increased G-CSF signaling and testicular vacuolization associated with decreased fertility. Proteomic analysis of a vulnerable organ, presymptomatic testis, showed a preferential accumulation of disordered proteins in the absence of UBE2W, consistent with the view that UBE2W preferentially targets disordered polypeptides. These mice further allowed us to establish that UBE2W is ubiquitously expressed as a single isoform localized to the cytoplasm and that the absence of UBE2W does not alter cell viability in response to various stressors. Our results establish that UBE2W is an important, albeit not essential, protein for early postnatal survival and normal functioning of multiple organ systems.

Andrology, 2019 Feb;31(1):14-13171. doi: 10.1111/andr.13171. Epub 2018 Oct 15.

Paternal factors and embryonic development: Role in recurrent pregnancy loss.

Chawen V¹, Kumar M¹, Datta CP¹, Mahotra N², Singh N², Dadwan V², Datta R¹.

¹ Author Information

Abstract

The events occurring at the maternal-fetal interface define a successful pregnancy but the current paradigm has shifted towards assessing the contribution of spermatozoa for embryogenesis. Spermatozoa with defective DNA integrity may fertilize the oocyte but affect subsequent embryonic development. The present case-control study was conducted in male partners of couples experiencing recurrent pregnancy loss (RPL) to assess the gene expression of spermatozoa FOXG1, SOX3, OGG1, PARP1, RPS8, RBM9, RPS17 and RPL29. This was correlated with reactive oxygen species (ROS) levels and DNA Fragmentation Index (DFI). Semen samples were obtained from 60 cases and 30 fertile controls. Gene expression was done by qPCR analysis, and relative quantification was calculated by the 2^{-ΔΔCt} method. Chemiluminescence and the sperm chromatin structure assay were used to measure the ROS and DFI levels respectively. FOXG1, OGG1, RPS8 and RBM9 were seen to be upregulated, while SOX3 and PARP1 were downregulated. Relative expression of SOX3, OGG1, RPS8 and RPS17 showed a significant difference between patients and controls ($p < 0.05$). RPL patients were seen to have high ROS ($p < 0.001$) and DFI ($p < 0.001$) with respect to controls. Sperm transcript dysregulation and oxidative DNA damage can be "carried over" after implantation, thus affecting embryogenesis and health of the future progeny.

GReX Associated with VACTERL PheRS

Gene	p	Tissue
SNX25	8.39E-18	ArteryAorta
UBE2W	8.39E-18	Brain, Cerebellum
RPS17	8.39E-18	Brain, NucleusAccumbensBasalGanglia
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ACSL1	2.24E-07	Adrenal

Mol Genet Metab, 2016 Sep;119(1-2):83-90. doi: 10.1016/j.mggm.2016.06.006. Epub 2016 Jun 14.

Elevated plasma dihydroorotate in Miller syndrome: Biochemical, diagnostic and clinical implications, and treatment with uridine.

Dutay JA¹, Herman MG², Carpenter KH³, Barnhart MA⁴, Marshall SA⁵, Ode CL⁶, Winkler B⁷, Primer JH⁸.

¹ Author Information

Abstract

BACKGROUND: Miller syndrome (post-axial acrofacial dysostosis) arises from gene mutations for the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH). Nonetheless, despite demonstrated loss of enzyme activity dihydroorotate (DHO) has not been shown to accumulate, but paradoxically urine orotate has been reported to be raised, confusing the metabolic diagnosis.

METHODS: We analysed plasma and urine from a 4-year-old male Miller syndrome patient. DHODH mutations were determined by PCR and Sanger sequencing. Analysis of DHO and orotic acid (OA) in urine, plasma and blood-spot cards was performed using liquid chromatography-mass spectrometry. In vitro stability of DHO in distilled water and control urine was assessed for up to 60h. The patient received a 3-month trial of oral uridine for behavioural problems.

RESULTS: The patient had early liver complications that are atypical of Miller syndrome. DHODH genotyping demonstrated compound-heterozygosity for frameshift and missense mutations. DHO was grossly raised in urine and plasma, and was detectable in dried spots of blood and plasma. OA was raised in urine but undetectable in plasma. DHO did not spontaneously degrade to OA. Uridine therapy did not appear to resolve behavioural problems during treatment, but it lowered plasma DHO.

CONCLUSION: This case with grossly related plasma DHO represents the first biochemical confirmation of functional DHODH deficiency. DHO was also easily detectable in dried plasma and blood spots. We concluded that DHO oxidation to OA must occur enzymatically solved the biochemical conundrum in previous reports of Miller syndrome patients, and opened the

Front Immunol, 2017 Aug 24;8:1019. doi: 10.3389/fimm.2017.01019. eCollection 2017.

Decidual Macrophage Functional Polarization during Abnormal Pregnancy due to Toxoplasma gondii: Role for LILRB4.

Li J¹, Chen H¹, Li L¹, Zhang X², Liu X², Jiang Y², Zhang H², Chu X².

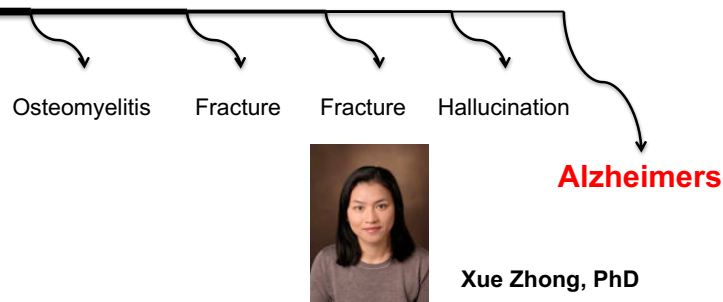
¹ Author Information

Abstract

During gestation, *Toxoplasma gondii* infection produces a series of complications including stillbirths, abortions, and congenital malformations. The inhibitory receptor, LILRB4, which is mainly expressed by professional antigen-presenting cells (especially macrophages and dendritic cells) may play an important immune-regulatory role at the maternal-fetal interface. To assess the role of LILRB4 during *T. gondii* infection, LILRB4⁺ and *T. gondii* infected pregnant mouse models were established. Further, human primary decidual macrophages were treated with anti-LILRB4 neutralizing antibody and then infected with *T. gondii*. These in vivo and in vitro models were used to explore the role of LILRB4 in *T. gondii*-mediated abnormal pregnancy outcomes. The results showed that abnormal pregnancy outcomes were more prevalent in LILRB4⁺ infected pregnant mice than in wild-type infected pregnant mice. In subsequent experiments, expression levels of LILRB4, M1, and M2 membrane-functional molecules, arginine metabolic enzymes, and related cytokines were assessed in uninfected, infected, LILRB4-neutralized infected, and LILRB4⁺ infected models. The results demonstrated *T. gondii* infection to downregulate LILRB4 on decidual macrophages, which strengthened M1 activation functions and weakened M2 tolerance functions by changing M1 and M2 membrane molecule expression, synthesis of arginine metabolic enzymes, and cytokine secretion profiles. These changes contributed to abnormal pregnancy outcomes. The results of this study provide not only a deeper understanding of the immune mechanisms operational during abnormal pregnancy, induced by *T. gondii* infection, but also identify potential avenues for therapeutic and preventive treatment of neonatal toxoplasmosis.

Using pleiotropy to aid discovery...

No gene exists simply to cause human disease...



Phenome Associations to GReX of PSEN1

Trait	OR per unit SD	p-value	Cases	Controls
Unspecified osteomyelitis	8.38E-01	4.03E-05	524	19865
Fracture of upper limb	8.95E-01	6.03E-05	1355	21096
Acute osteomyelitis	7.79E-01	7.06E-05	231	19865
Fracture of humerus	8.41E-01	7.18E-05	504	21096
Bacterial enteritis	1.15E+00	7.67E-05	948	Fractures: patella, radius, ulna, vertebral column Brain: intracranial hemorrhage, subdural hemorrhage, cerebral aneurism, Alzheimers
Intestinal infection due to C. difficile	1.15E+00	1.10E-04	894	
Heart failure with reduced EF	9.15E-01	1.10E-04	2262	
Osteomyelitis	8.70E-01	1.41E-04	735	
Osteomyelitis, periostitis, and other	8.80E-01	2.14E-04	835	
Congenital anomalies of limbs	6.75E-01	2.34E-04	76	

TREM2 GReX Associations

Nasu-Hakola Disease

Alzheimers and other dementias

Trait	OR per unit SD	p-value	Cases	Controls
Chronic osteomyelitis	8.18E-01	1.40E-05	344	19865
Acute osteomyelitis	7.93E-01	1.97E-05	231	19865
Cerebral laceration and contusion	7.34E-01	5.55E-05	Fractures: patella, radius, ulna, vertebral column Brain: Delirium, psychogenic disorder, dementia with cerebral degenerations	
Unspecified osteomyelitis	8.59E-01	1.17E-04		
Osteomyelitis	8.79E-01	1.51E-04		
Diverticulitis	8.47E-01	4.98E-04		
Hallucinations	7.44E-01	7.18E-04		

Osteoclasts and microglia share a developmental lineage...

- Substantial overlap in genes expressed, and the genetic architecture of their gene regulation
- Additional of the genes implicated in Alzheimers show similar patterns of phenome association

In 2.8M Subjects with EHR...

- Age at diagnosis for osteomyelitis is 15-20 years earlier than diagnosis of dementia
- Subjects with osteomyelitis diagnosed before 60 have 4-6 X increased risk of dementia diagnosis after age 70

In Silico Drug Trials

- Already conducting in silico drug trials: Do patients taking a drug for one indication have altered risk or age at onset for another disease (e.g. AD)? **YES**
- *Pleiotropic phenome* discovered through genetics creates **a larger target** for discovery and validation for *in silico* and later clinical trials

Translation Today



**“Traditional”
genetics/genomics**

Translation Tomorrow



**“Traditional”
genetics/genomics**

**The 90% Under the Surface –
Computation and Decision Support**

Improving Commonly Used Biomarkers

Genetics

Environment

Biomarkers

**Metabolome
proxies of
coding
variants**

LDL

Cystatin C

New –Omics Biomarkers

**Discovery of New
Biomarker Relationships**

Ranges and Thresholds

- Some biomarker measurements are adjusted for sex, age, **race/ethnicity**
- It is not about race/ethnicity! It is about DNA variation with frequency differences by historical geographic ancestry

Predictive Modeling

- Done extensively in learning healthcare systems now
- Where can we add genetics to the modeling and get improvement?
- For the most expensive conditions, even marginal improvements in prediction yield better care, lower costs

The IOTA Concept:

When we already have the genome information, even an iota of improvement over current practice (or existing predictive models) will have value for the most expensive and common conditions