Applying Genomics in Transplantation

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Optum Health Education
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Disclosures

Consultant for UnitedHealthGroup R&D

Funding:

U01 AI152960 NIH/NIAID Keating (PI)
MHC & KIR Sequencing & Association Analyses in iGeneTRAiN

R01 AI144522 NIH/NIAID Keating (PI)
Multi-omic Biomarker Discovery & Validation in Heart Transplant Patients

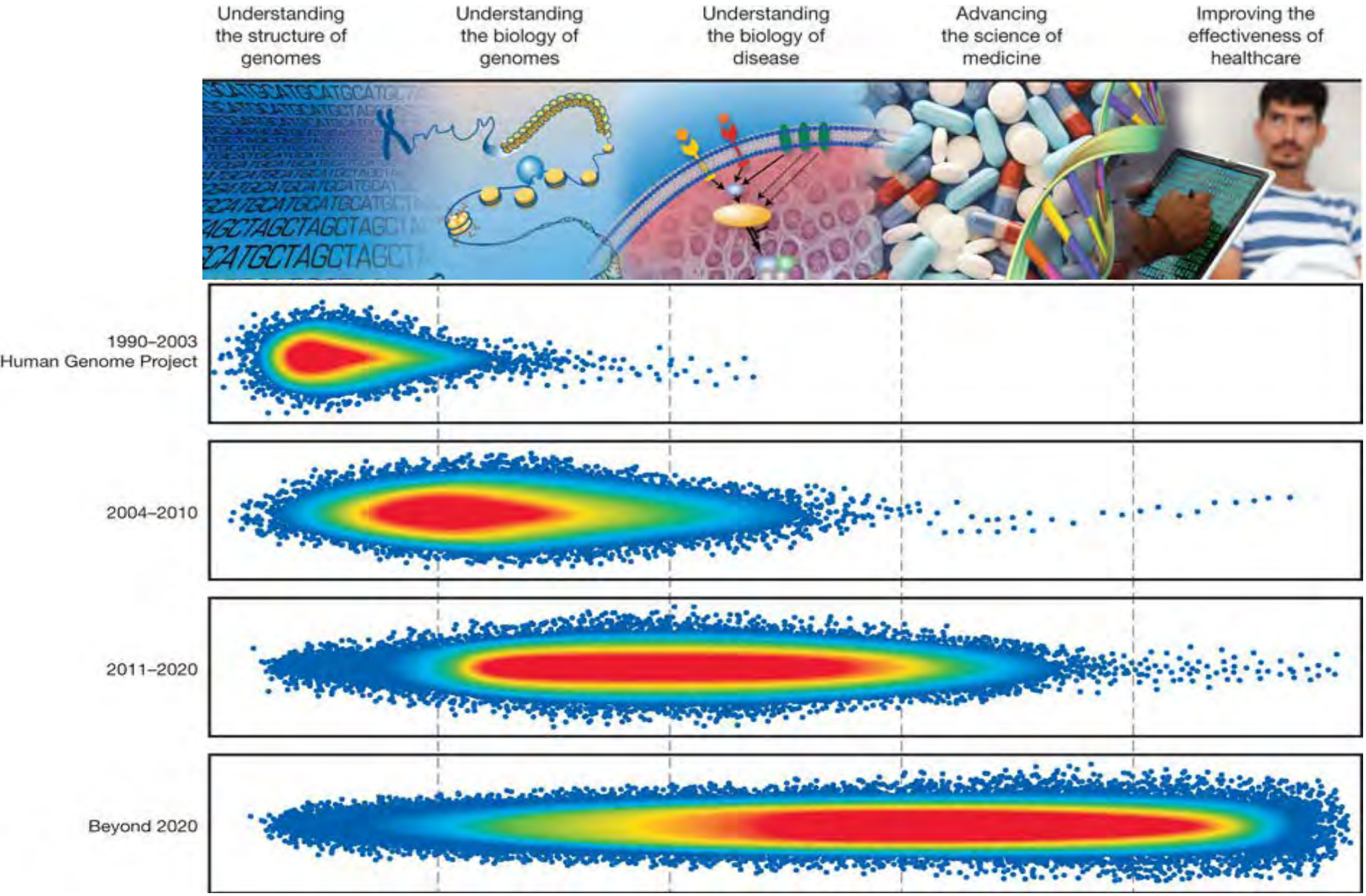
R01 HD091185 NIH/NICHD Keating (MPI)
Validating Injury to the Renal Transplant Using Urinary Signatures in Children

Biesecker Foundation Keating (PI)
Diagnoses and Prognostication of Rejection in Liver Transplant patients

Presentation Overview

- DNA biomarkers in Transplant
  - International Genetics & Translational Research in Tx Network (iGeneTRAiN)
    - Histocompatibility screening (HLA/non-HLA compatibility)
    - Pharmacogenomics (PGx)
    - Primary disease diagnoses
    - iGeneTRAiN-Industry studies

- Integrating DNA, RNA, proteomics & metabolomics
  - Kidney, Liver & Heart transplant multi-omics studies

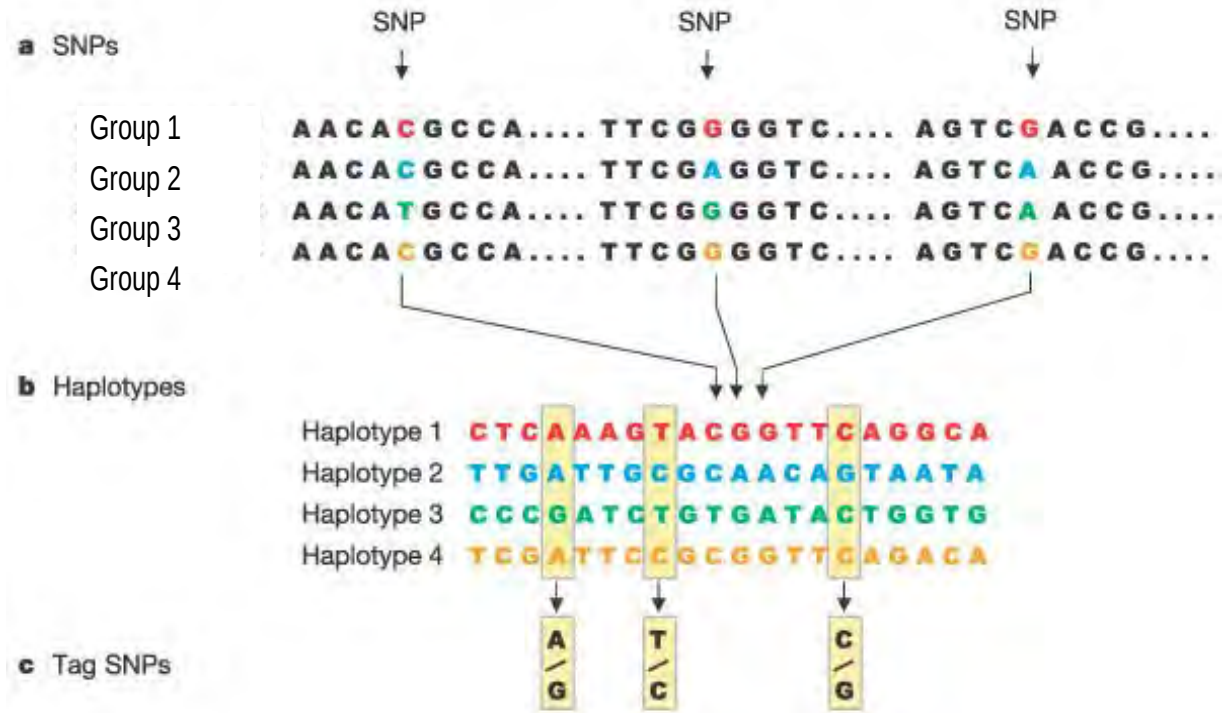


Eric Green *et al*. *Nature* **470**, 2011

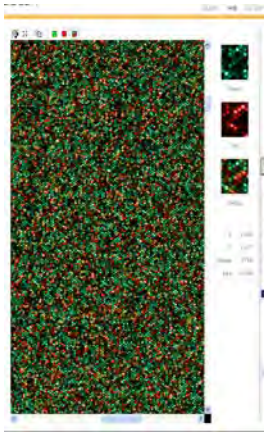
nature


Genomic DNA
GENE A
GENE B
GENE C
GENE D
A. Structural Variations
IND1
IND2
IND3
B. SNVs and InDels
CEU Common Ancestral Sequence
C G C C T T A C A T G C
IND1
IND2
IND3
IND4
IND5
IND6

Construction of Human Genome-wide Genetic Maps

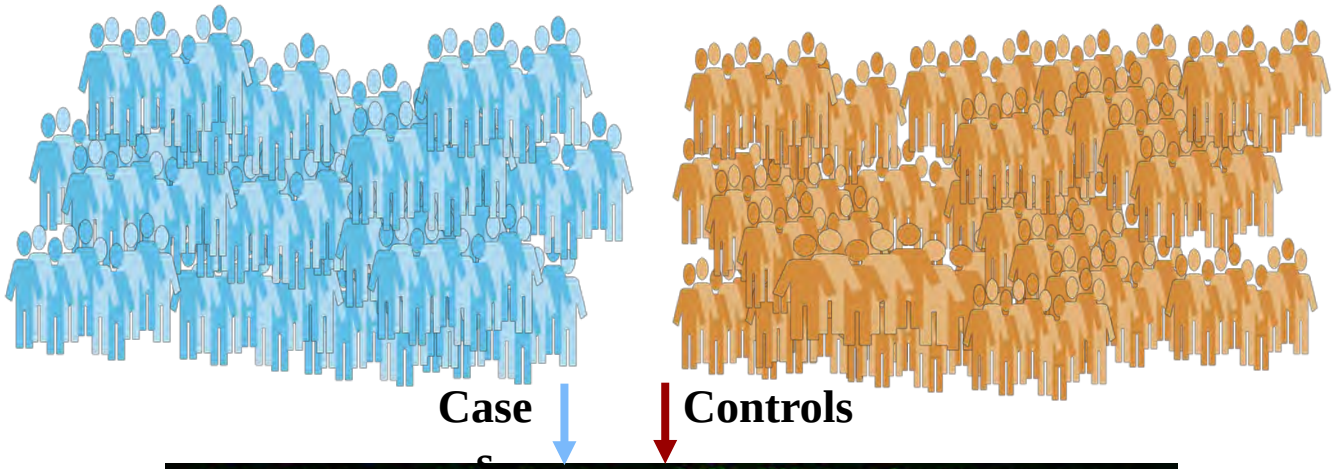


Human1M-Duo

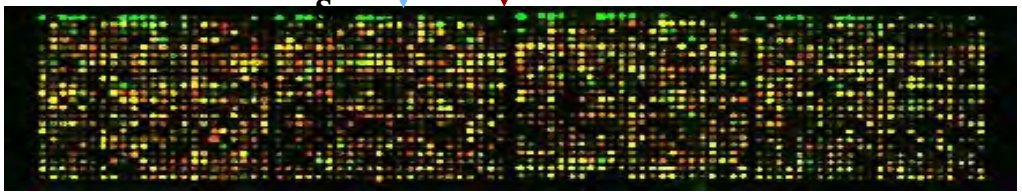


www.hapmap.org

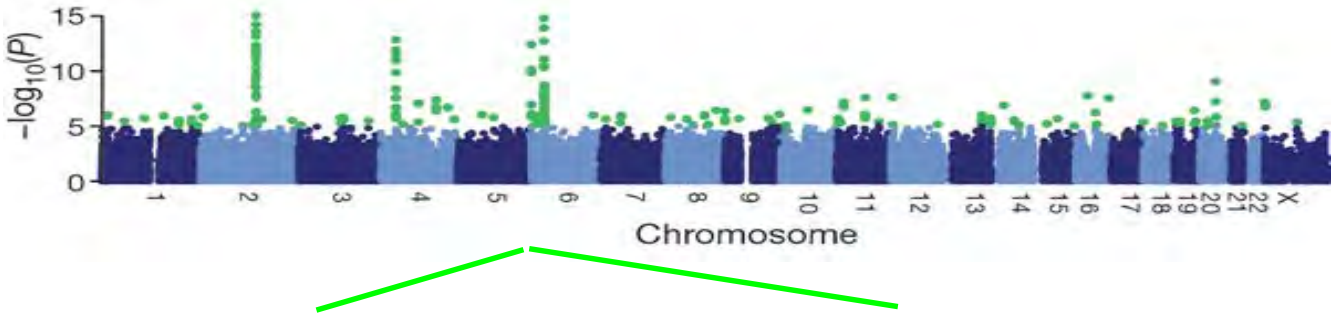
Genome-wide Association Studies : Basic Principles



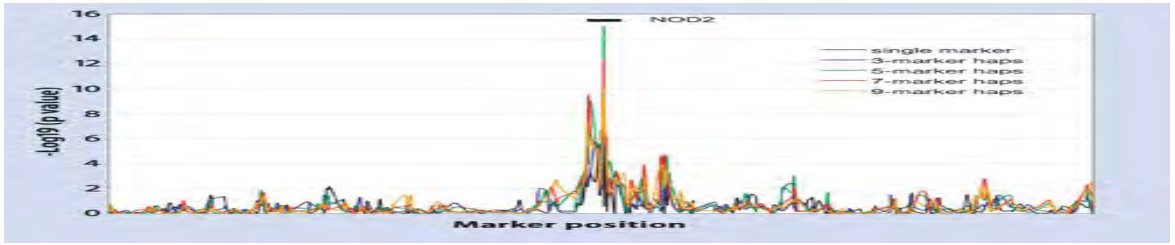
Family-based or case/control DNA samples



Whole genome genotyping

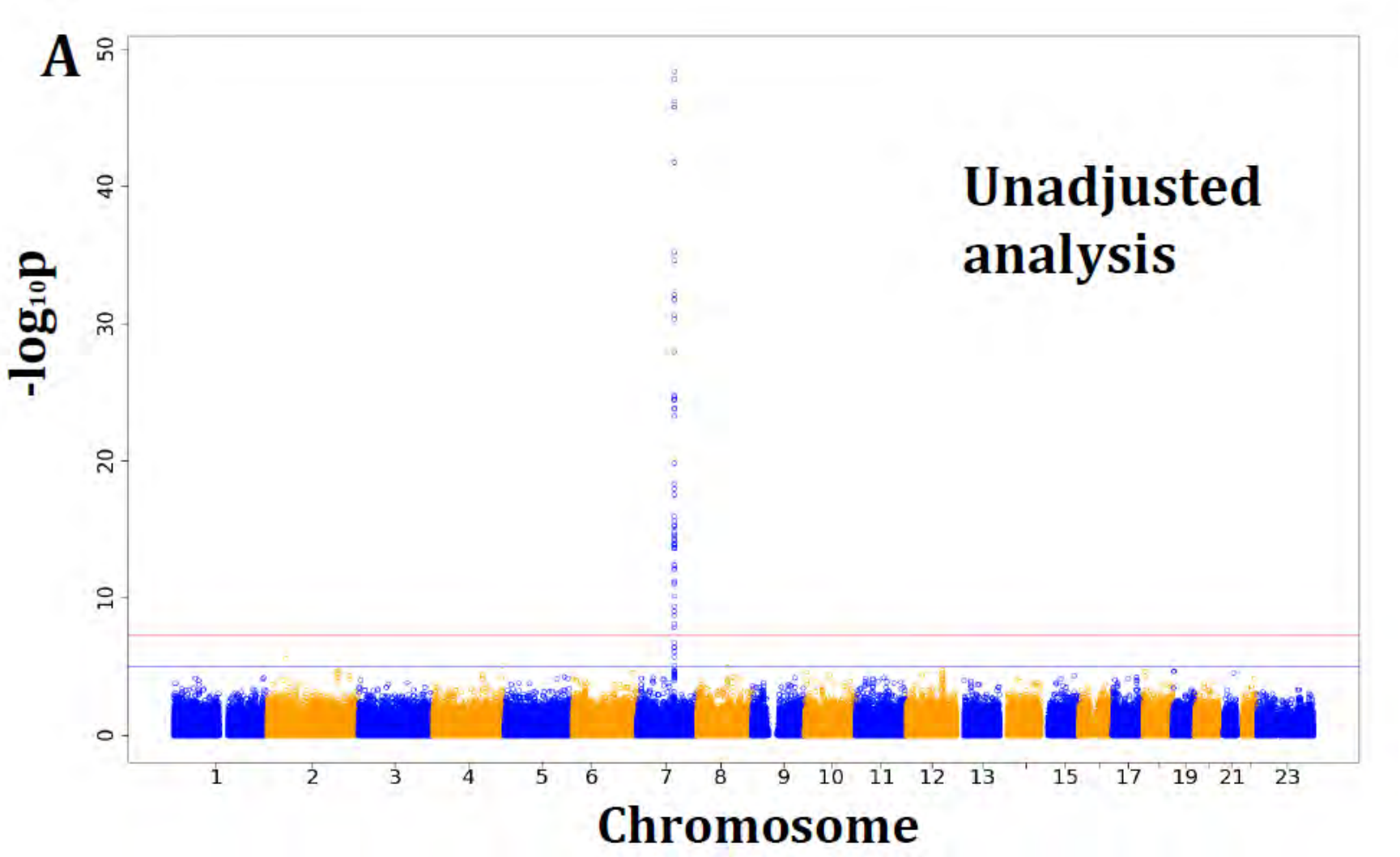


Genome-wide Association studies (GWAS)



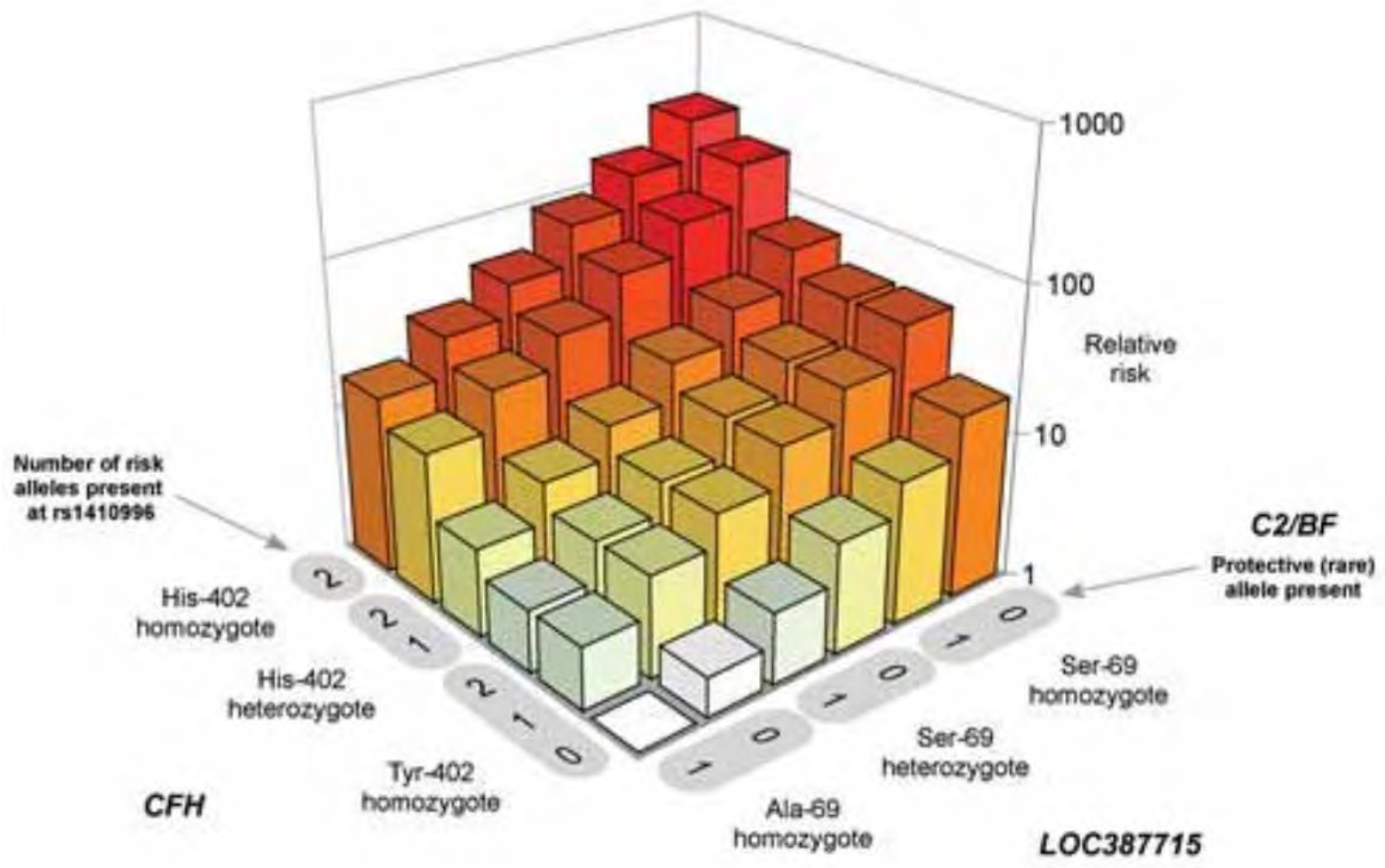
Replication & Fine mapping

Manhattan plots of association of tacrolimus dose normalized blood trough levels (n ~ 1500 Caucasians)



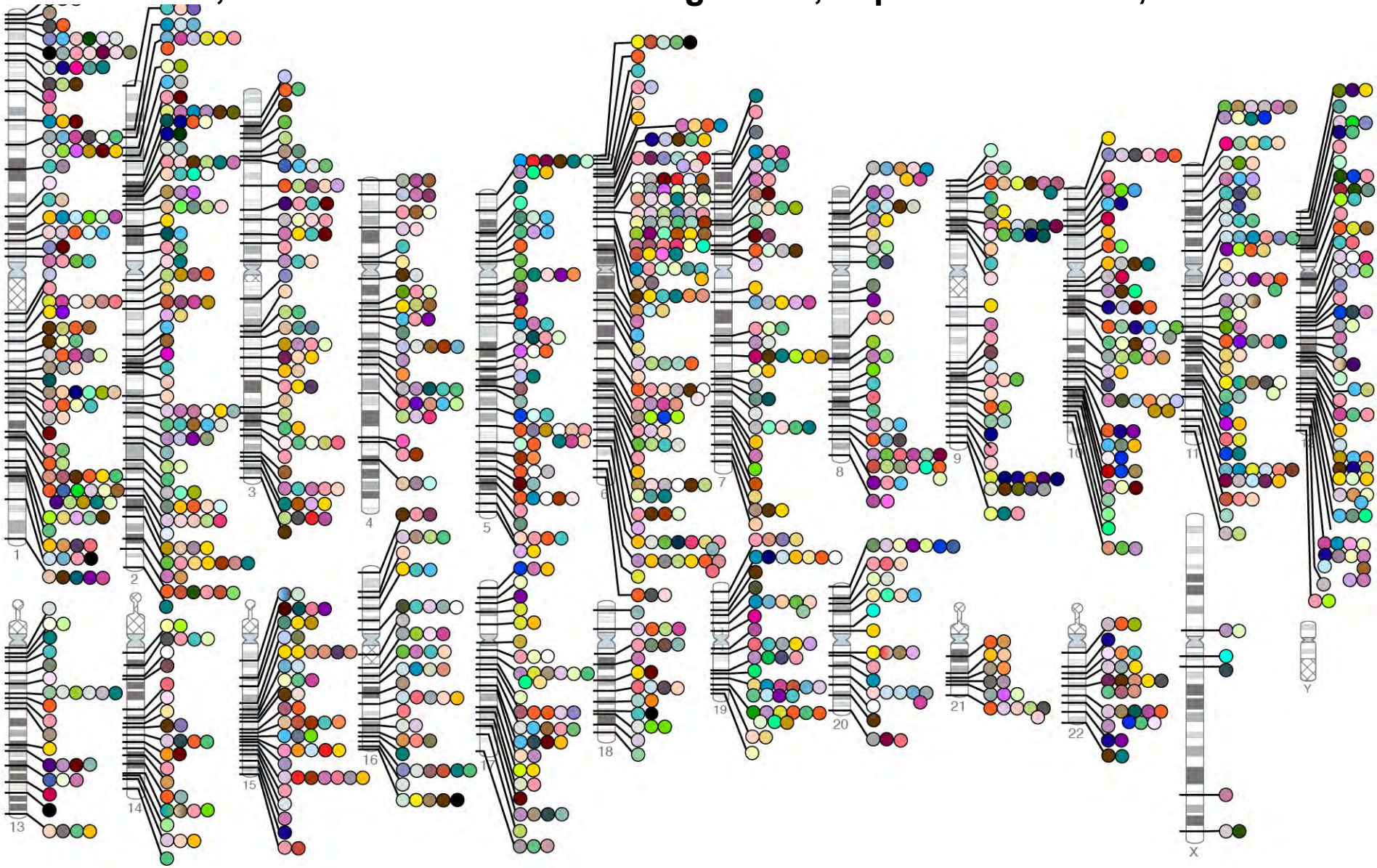
DeKAF Genomics

Interaction of risk variants for Age-related Macular Degeneration



Seddon *et al* 2008

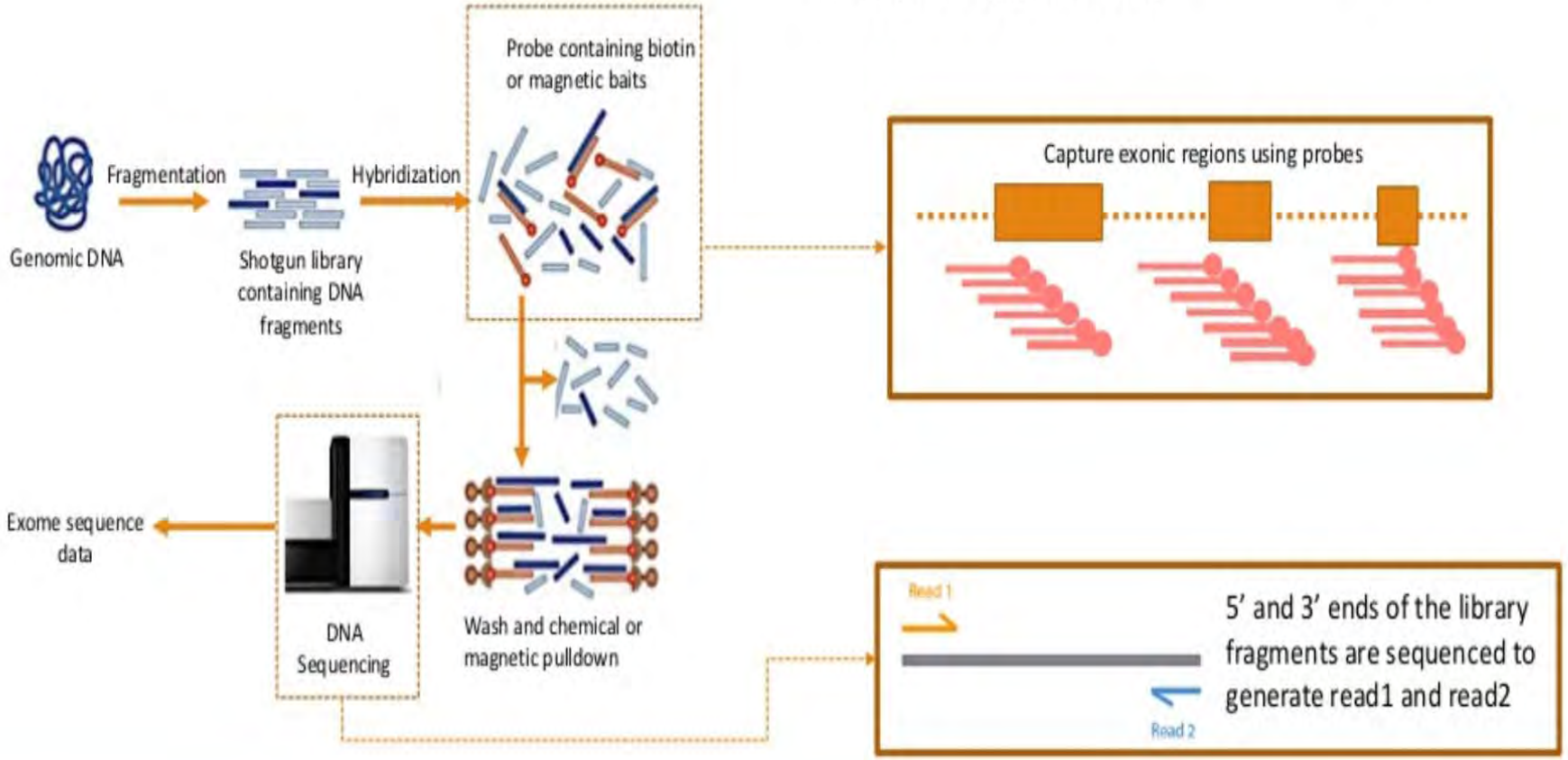
>5,000 Published GWAS through 2015, at $p \leq 5 \times 10^{-8}$ for >1,000 traits



NHGRI/EBI GWA Catalog www.genome.gov/GWAStudies

Exome sequencing workflow

1. Fragmentation and library preparation
2. Hybridization to exonic regions (regions in exome capture array)
3. Magnetic pull down and wash excess fragments
4. Sequencing (paired-end)



Bamshad et al. Nature Reviews Genetics 2011

Whole Exome Sequencing Discoveries

ORIGINAL ARTICLE

A Protein-Truncating *HSD17B13* Variant and Protection from Chronic Liver Disease

- Whole Exome Seq & EHR from 46,500 Geisinger patients were mined for associations with various lab values
- *HSD17B13* Loss-of-Function mutations associate with ALT & AST levels
- Associated with lower:
  - Alcoholic Liver disease (42% & 53% for 1 & 2 copies)
  - NAFLD (17% & 30% for 1 & 2 copies)
  - Progression from Steatosis to Steatohepatitis

N Engl J Med. 2018 PMID: 29562163

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- *HSD17B13* is druggable with using antibodies

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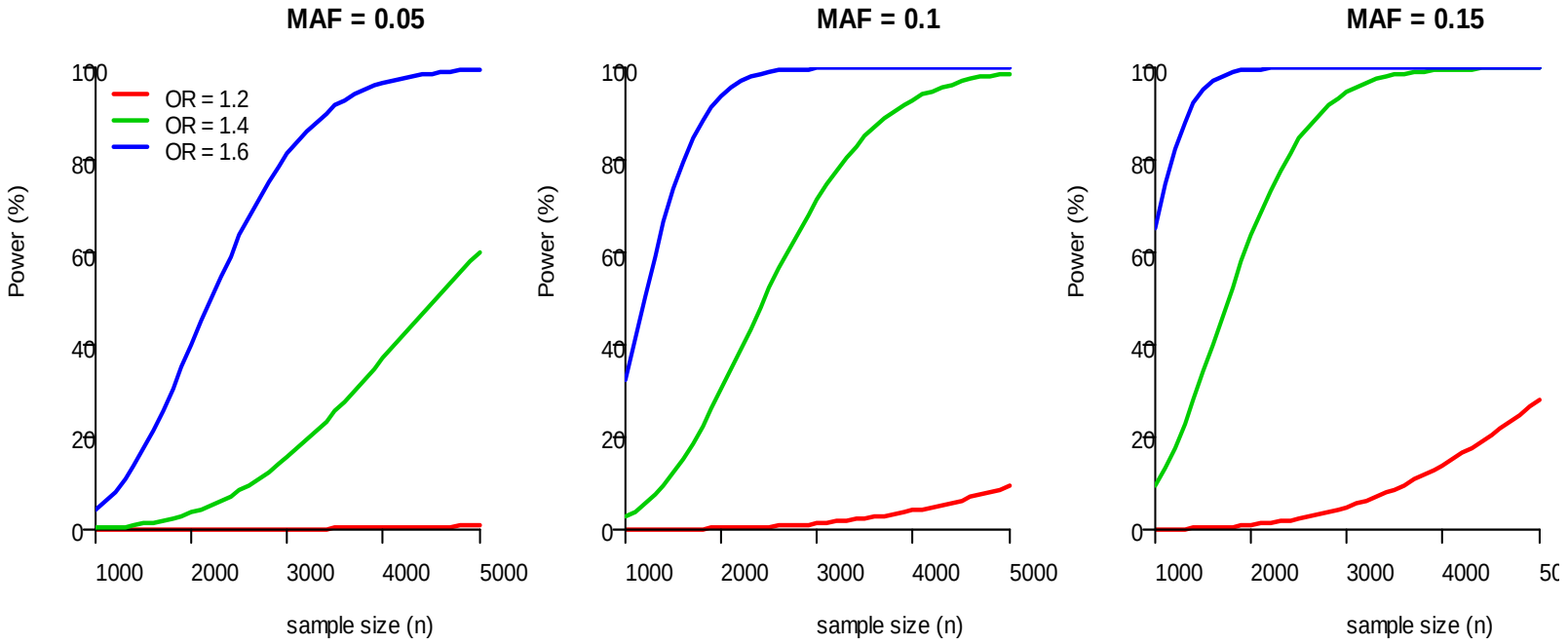
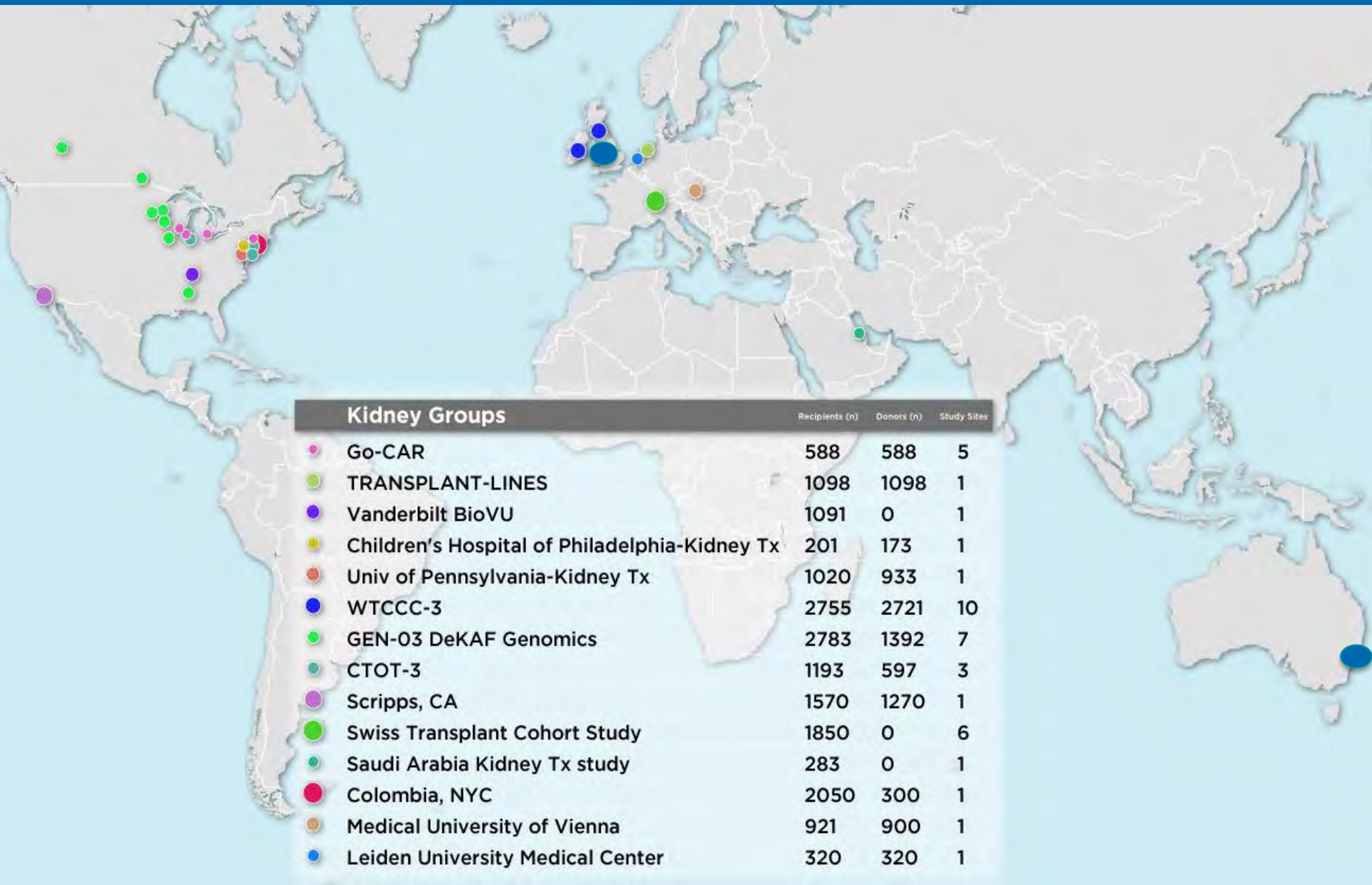
Genome-wide Statistical Power for Detecting Main Effects



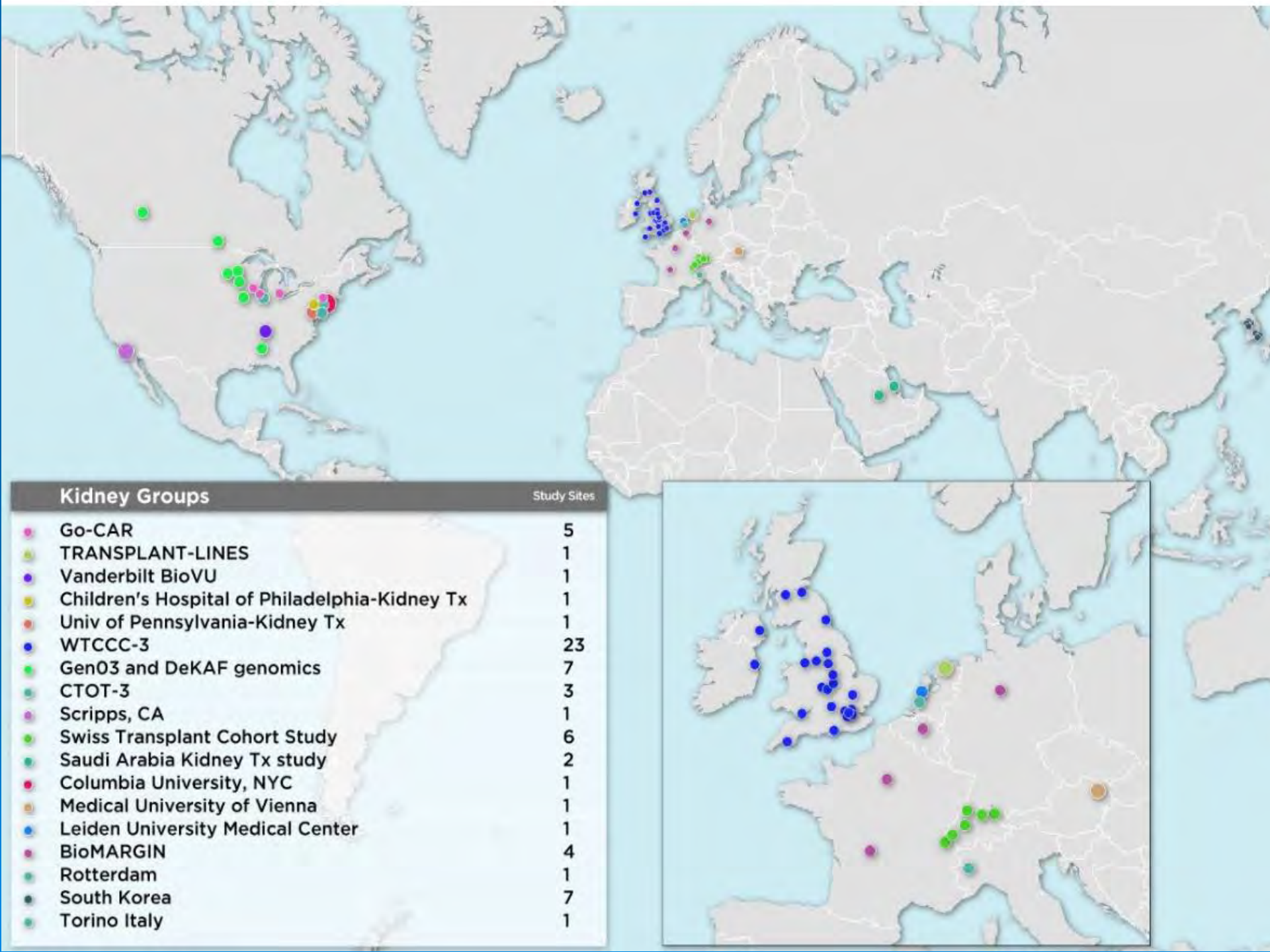
Power calculation based on *n* unrelated cases & controls. Disease model is multiplicative with disease minor allele frequencies (MAFs) of 0.05, 0.1 & 0.2 & OR of 1.2, 1.4 & 1.6. Significance assessed at 5% level using Bonferroni correction **assuming ~500K tests**

(Keating *et al* 2008: PMID: 18974833)

iGeneTRAiN Kidney Groups



Kidney Groups	Recipients (n)	Donors (n)	Study Sites
Go-CAR	588	588	5
TRANSPLANT-LINES	1098	1098	1
Vanderbilt BioVU	1091	0	1
Children's Hospital of Philadelphia-Kidney Tx	201	173	1
Univ of Pennsylvania-Kidney Tx	1020	933	1
WTCCC-3	2755	2721	10
GEN-03 DeKAF Genomics	2783	1392	7
CTOT-3	1193	597	3
Scripps, CA	1570	1270	1
Swiss Transplant Cohort Study	1850	0	6
Saudi Arabia Kidney Tx study	283	0	1
Colombia, NYC	2050	300	1
Medical University of Vienna	921	900	1
Leiden University Medical Center	320	320	1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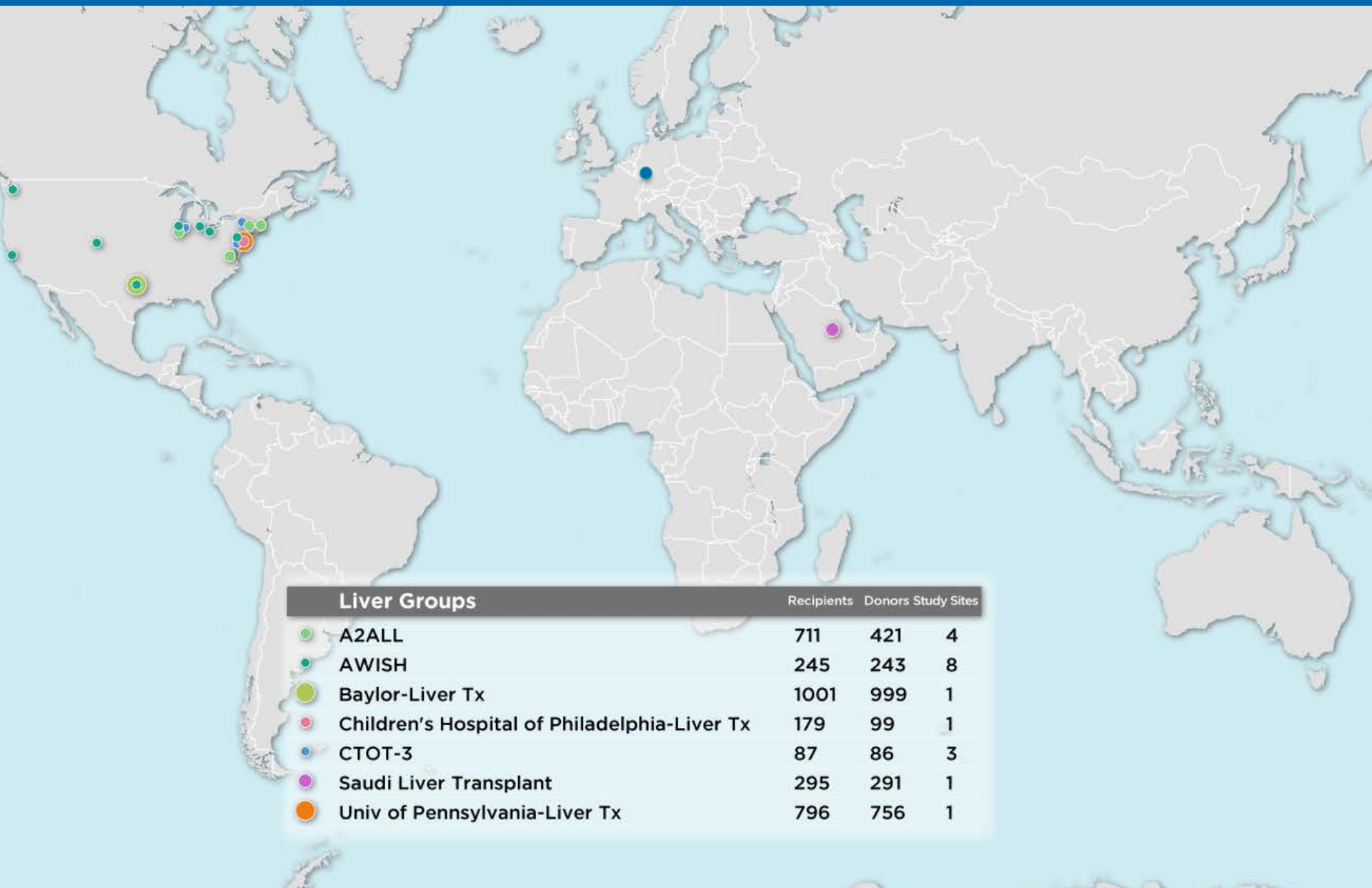
WTCCC-3 23
Gen03 and DeKAF genomics 7
CTOT-3 3
Scripps, CA 1
Swiss Transplant Cohort Study 6
Saudi Arabia Kidney Tx study 2
Columbia University, NYC 1
Medical University of Vienna 1
Leiden University Medical Center 1
BioMARGIN 4
Rotterdam 1
South Korea 7
Torino Italy 1

iGeneTRAiN Liver Groups



Liver Groups	Recipients	Donors	Study Sites
A2ALL	711	421	4
AWISH	245	243	8
Baylor-Liver Tx	1001	999	1
Children's Hospital of Philadelphia-Liver Tx	179	99	1
CTOT-3	87	86	3
Saudi Liver Transplant	295	291	1
Univ of Pennsylvania-Liver Tx	796	756	1

iGeneTRAiN Liver Groups

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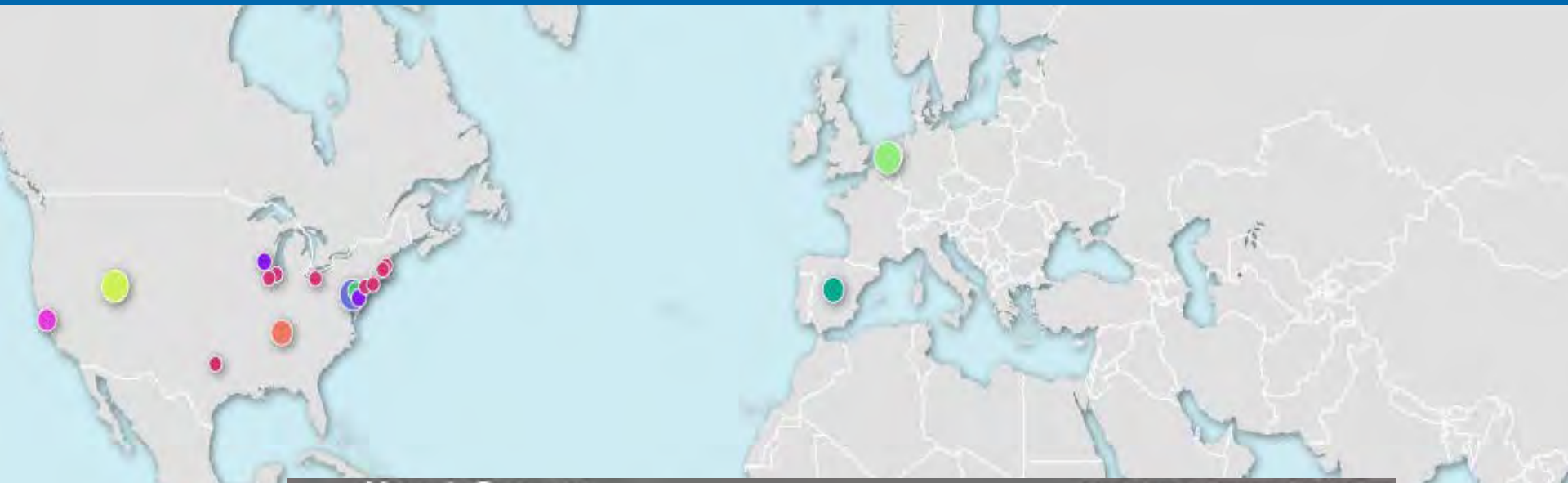
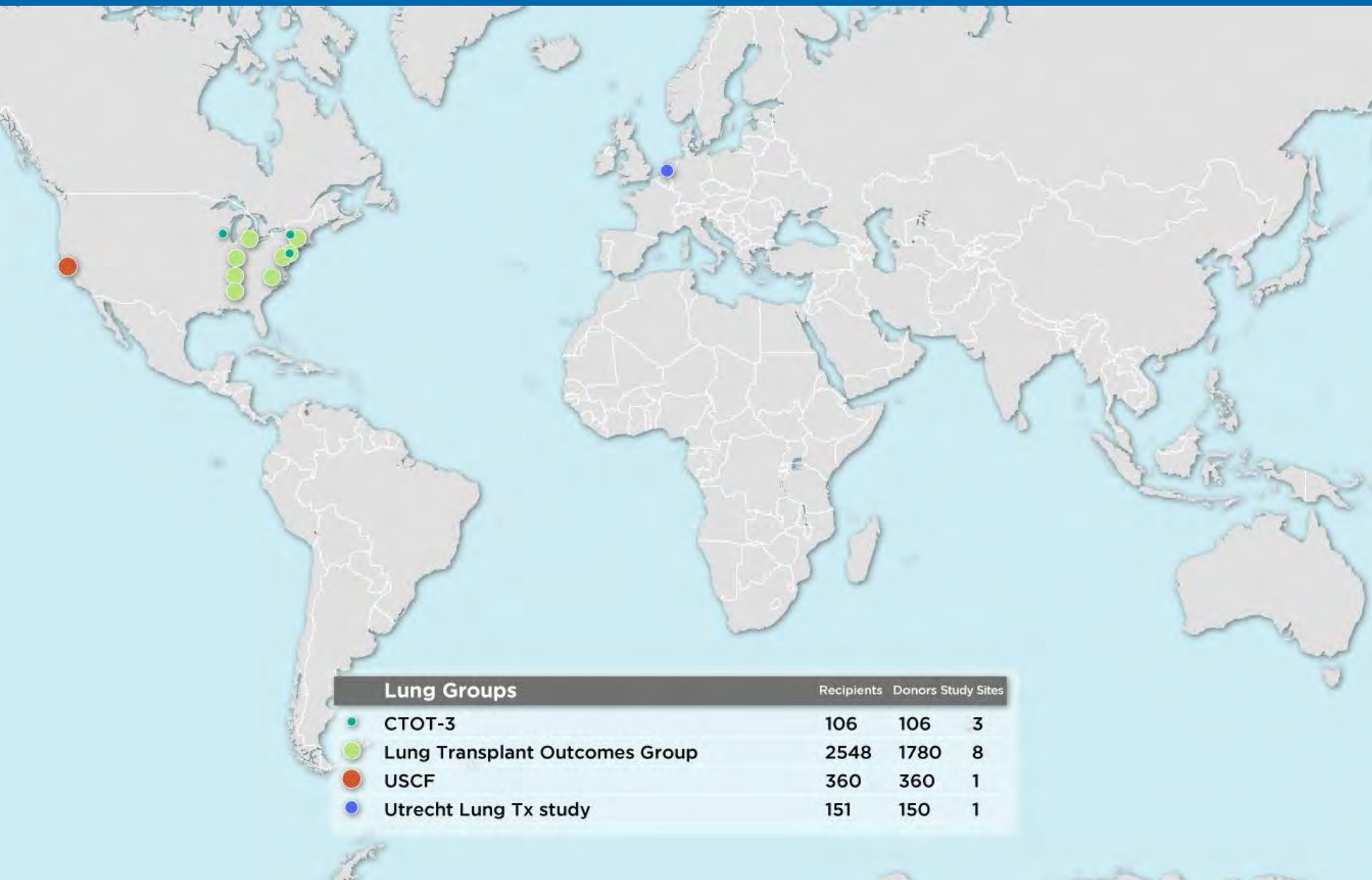
iGeneTRAiN Heart Groups



Heart Groups	Recipients	Donors	Study Sites
Children's Hospital of Philadelphia-Heart Tx	69	69	1
CTOT-3	52	46	2
CTOT-5	102	102	10
Intermountain Utah	320	0	1
Madrid Heart Tx Study	191	185	1
Rotterdam heart tx	324	275	1
Stanford/LPCH	137	137	1
Univ of Pennsylvania-Heart Tx	354	354	1
Utrecht Heart Tx study	178	178	1
Vanderbilt BioVU	184	0	1

iGeneTRAiN Lung Groups



Lung Groups	Recipients	Donors	Study Sites
CTOT-3	106	106	3
Lung Transplant Outcomes Group	2548	1780	8
USCF	360	360	1
Utrecht Lung Tx study	151	150	1

International Genetics & Translational Research in Transplantation Network (iGeneTRAiN)

- Stage 1
  - Heart, Kidney, Liver, Lung & Stem-Cell Tx groups
    - n > 51,000 GWAS'd (most using Tx specific array with 780,000 variants)
    - Core pipelines for genome-wide and HLA & KIR imputation

www.igenetrain.org + Keating *et al.* PMID: 26479416 *Transplantation* Nov 2015

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  - Harmonize existing phenotypes, GWAS & whole exome seq datasets
    - mHA/Loss-of-function pipelines
  - Meta-analyses within organ & cross solid-organ for:
    - Acute rejection, graft/patient survival, NODAT, skin cancer, PGx outcomes

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  - Meta-analyses within organ & cross solid-organ for:
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- Stage 2
  - Standardization & harmonization of biopsies/bio-specimens/biomarkers across studies

www.igenetrain.org + Keating *et al.* PMID: 26479416 *Transplantation* Nov 2015

Clinical Trials in Organ Transplantation (CTOT)

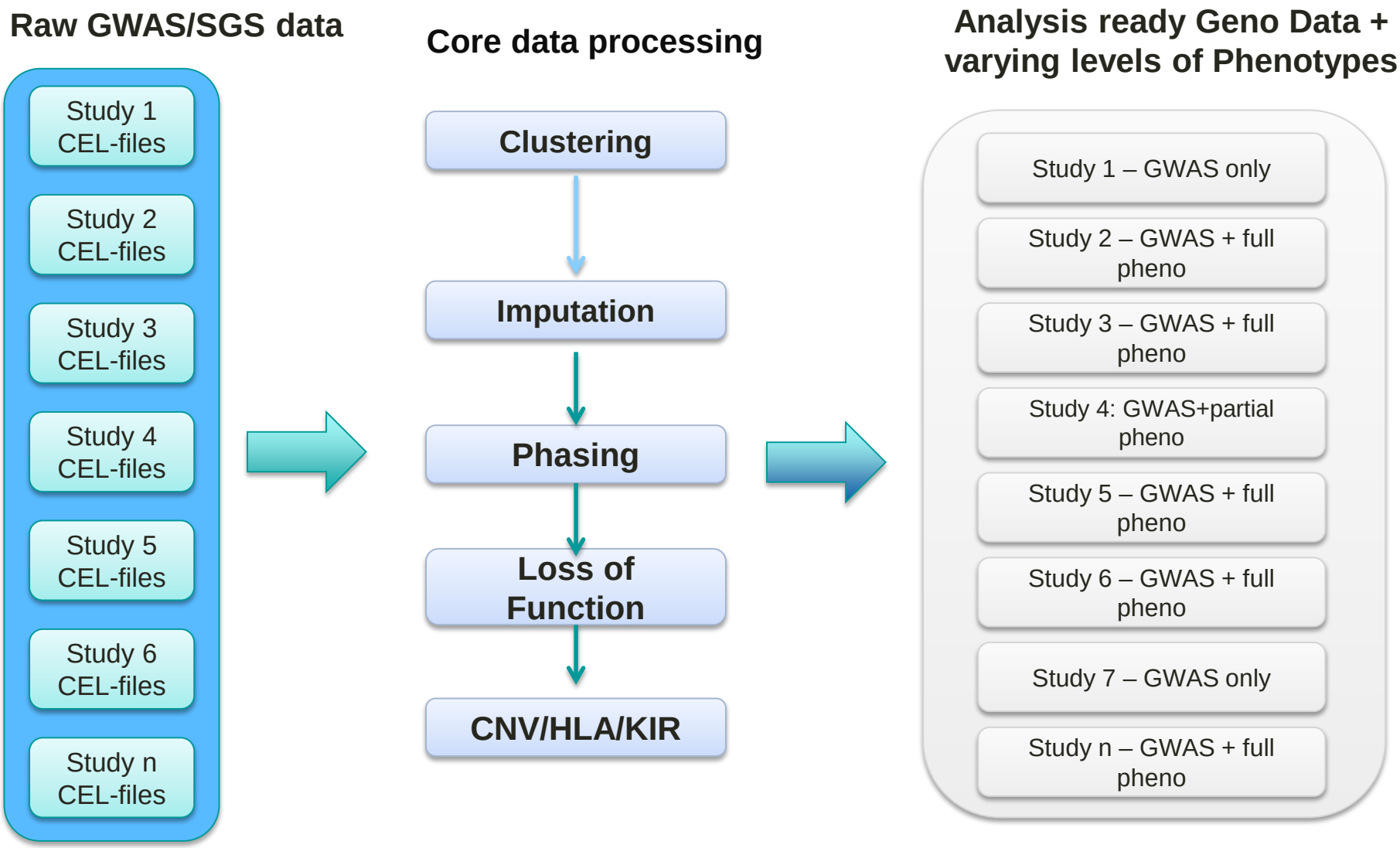
Public

Click here to log

Approved Studies

Protocol	Study Status	Title	Principal Investigator
CTOT-01	Closed	Noninvasive Monitoring to Predict Outcome in de novo Kidney Transplant Recipients	Peter Heeger, MD
CTOT-02/CCTPT-02	Closed	B-Cell Depletion by Anti-CD20 in Renal Allograft Recipients who Develop de novo Anti HLA Alloantibodies	Mohamed Sayegh, MD and Anil Chandraker, MD
CTOT-03	Closed	Correlation of Donor Proinflammatory mRNA Profiles with Early Outcomes of Thoracic and Abdominal Transplantation	Abraham Shaked, MD
CTOT-04	Closed	Noninvasive Diagnosis of Renal Allograft Rejection by Urinary Cell mRNA Profiling	Abraham Shaked, MD
CTOT-05	Closed	Observational Study of Alloimmunity in Cardiac Transplant Recipients	Peter Heeger, MD; Mohamed Sayegh, MD; and Anil Chandraker, MD
CTOT-06	Closed	A Mechanistic Substudy of the Bristol-Myers Squibb Sponsored Trial "Belatacept Conversion Trial in Renal Transplantation	Mohamed Sayegh, MD
CTOT-07	Closed	Development of Genomic Signatures for the Prediction, Diagnosis and Prognostication of Liver Allograft Rejection and recurrent Hepatitis C Disease	Abraham Shaked, MD, PhD

Ovation: HIPAA Compliant-Environment for genomic pipelines/analyses



1,400,000,000,000 Genotypes (direct & imputed)

Published/Ongoing iGeneTRAiN GWAS analyses

- Kidney Primary Association analyses
  - Rejection, graft survival (time to event/ rejection Y/N)
  - Phase 1: DeKAF, TxLINES, Vanderbilt, UKIRTC, Leiden, Scripps, Vienna
  - Phase 2: Phase 1 + UPenn, Columbia, BioMARGIN, STCS, S.Korea

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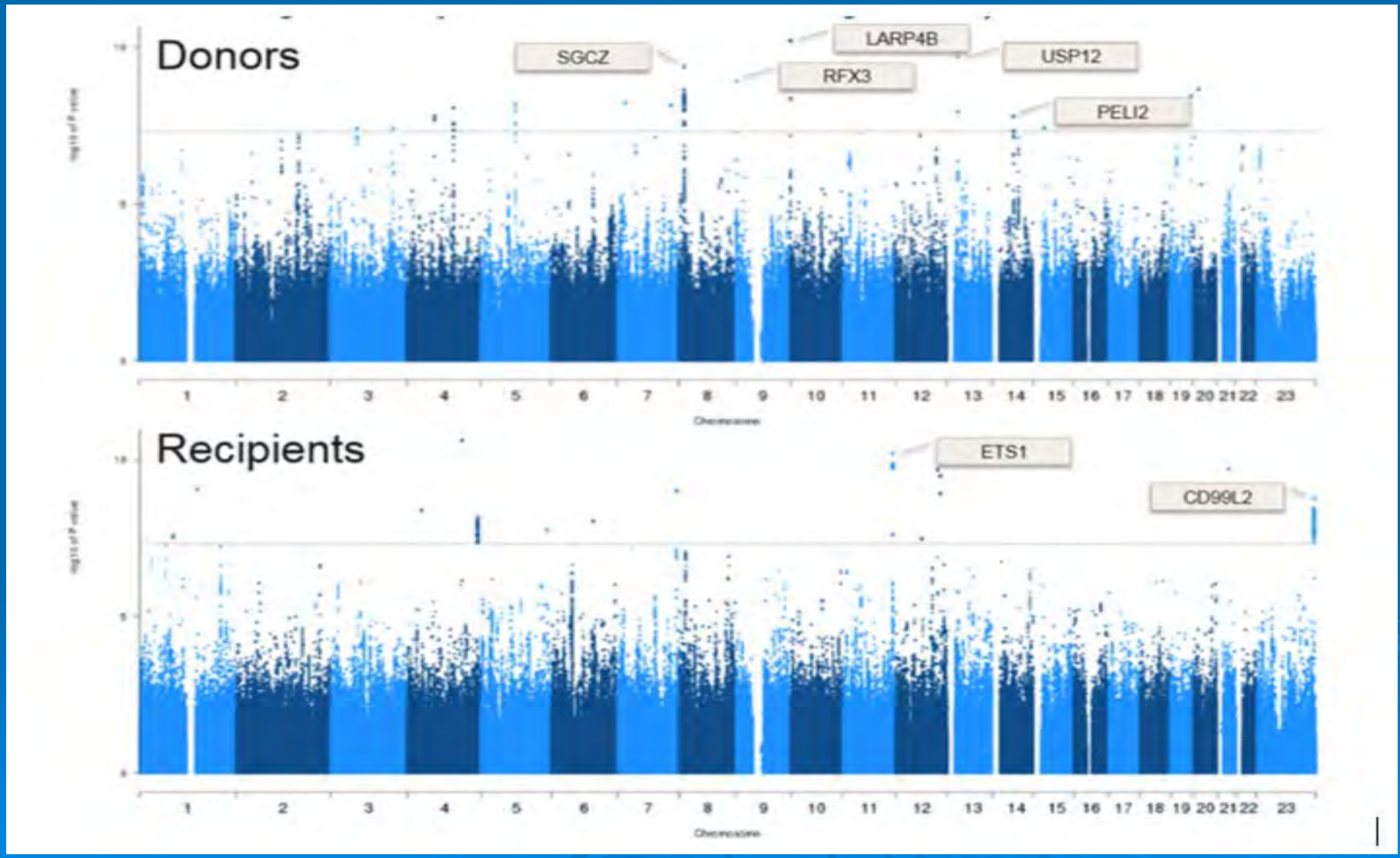
- Heart Transplant Studies used:
  - UPenn, CTOT-05, Stanford/LP, Madrid, Rotterdam, Utrecht
  - Primary Phenotypes: BPR in year 1 (Grade 2R & clinically treated)
    - Death/Graft Loss (which ever occurred first)
  - Meta-analyses performed using standard GWAS models
    - Covariates: D-R age & gender, Year of Tx, PCs

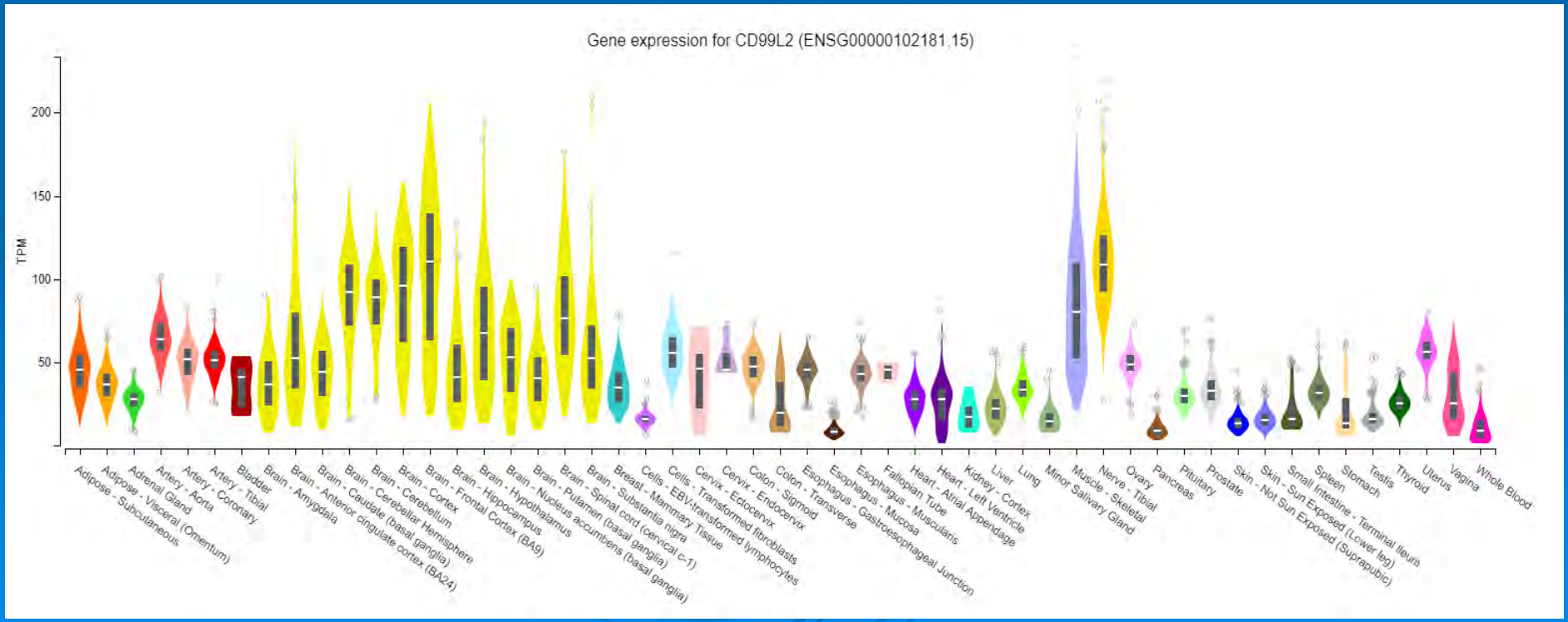
iGeneTRAiN Heart donor-recipient pairs: Time-to-Rejection



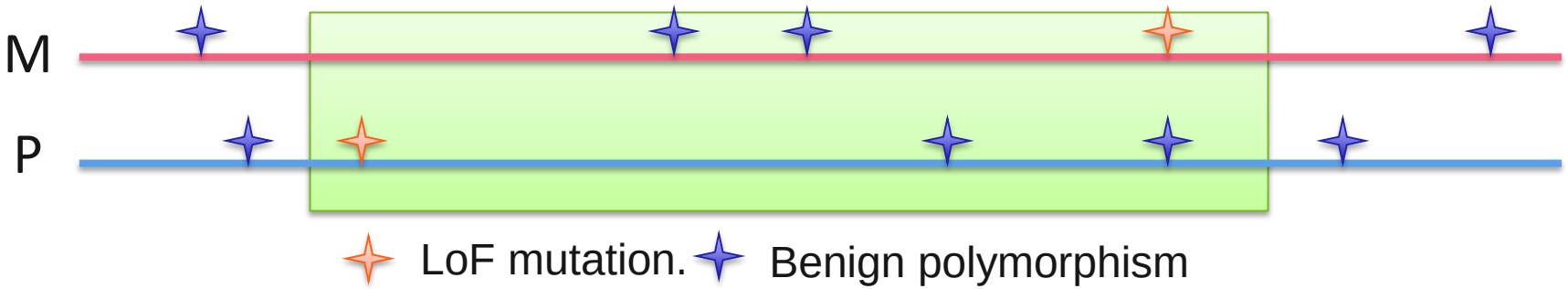
Top Heart GWAS Signal ($p < 1 \times 10^{-9}$)

- Vascular adhesion molecule:
  - Gene expression ubiquitous including heart
  - Role in late leukocyte extravasation to overcome endothelial B Membrane
  - Potentially plays an important role in graft rejection (PMID:1384180)

    - Not druggable, but it is a possible biopharmaceutical target

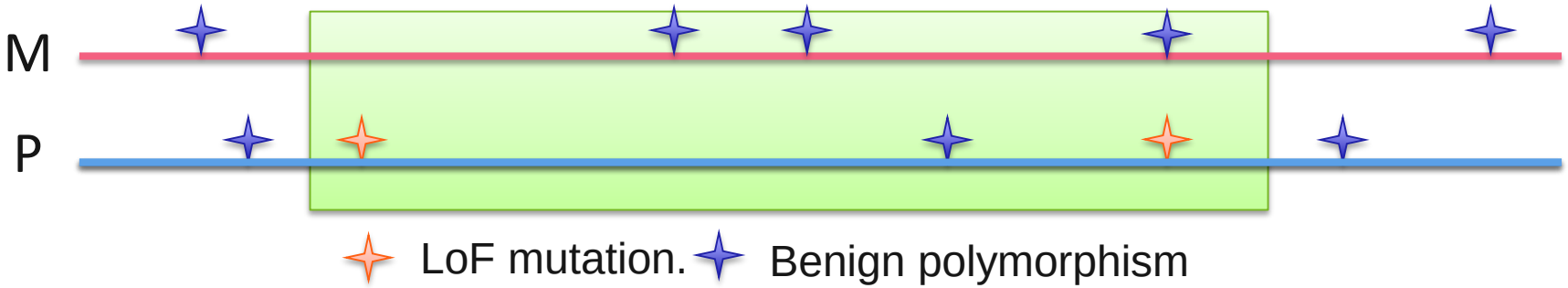


Analysis pipeline: Loss-of-Function mutations



- Step 1: Identify Loss-of-Function mutations on a genome-wide scale
- Step 2: Calculate for each individual which genes are inactive in 1 or 2 copies
- Step 3: Identify genes that are inactive in the recipient but active in the donor

Analysis pipeline: Loss-of-Function mutations



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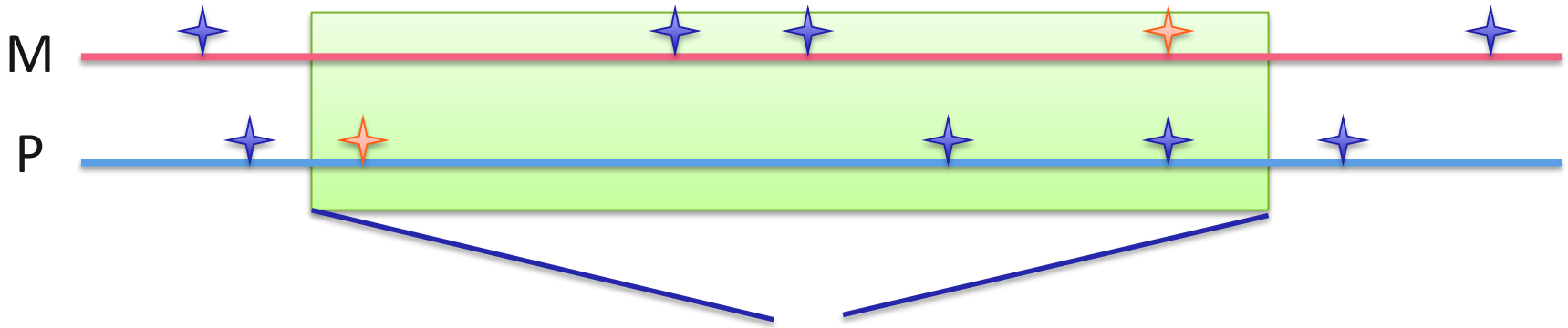
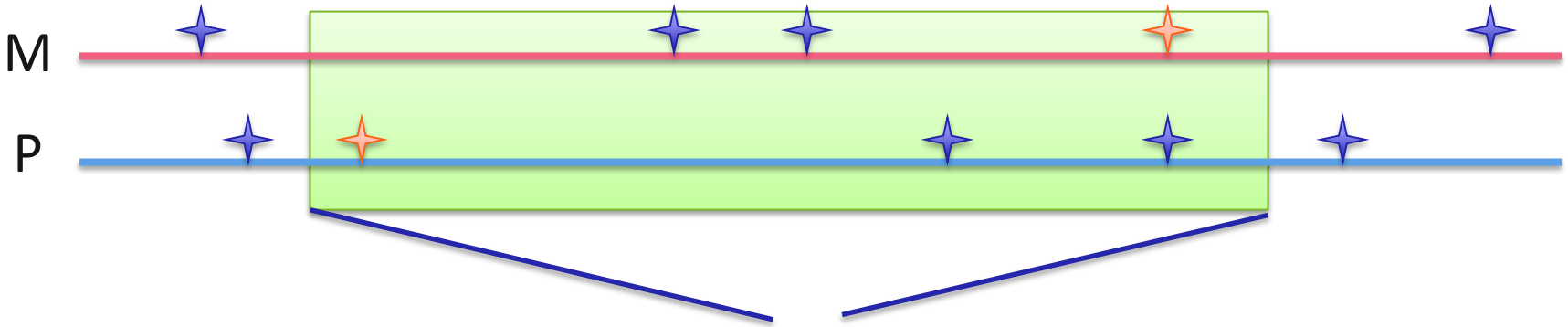
Assessing inactive genes in recipients but active in donors



	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Gene 6	Gene 7	Gene …	Gene 21,000
Donor 1	0	0	0	0	0	0	1	0	0
Recipient 1	0	1	0	0	0	0	0	0	0
Donor 2	0	0	0	0	0	0	0	0	0
Recipient 2	0	0	0	2	0	0	1	0	0
Donor 3	0	0	0	0	0	0	0	0	0
Recipient 3	0	0	0	0	0	1	0	0	0
Donor …	0	2	0	0	0	0	0	0	0
Recipient …	0	0	0	0	0	0	0	1	0
Donor 888	0	0	0	1	0	0	0	0	0
Recipient 888	0	0	0	0	0	0	2	0	0

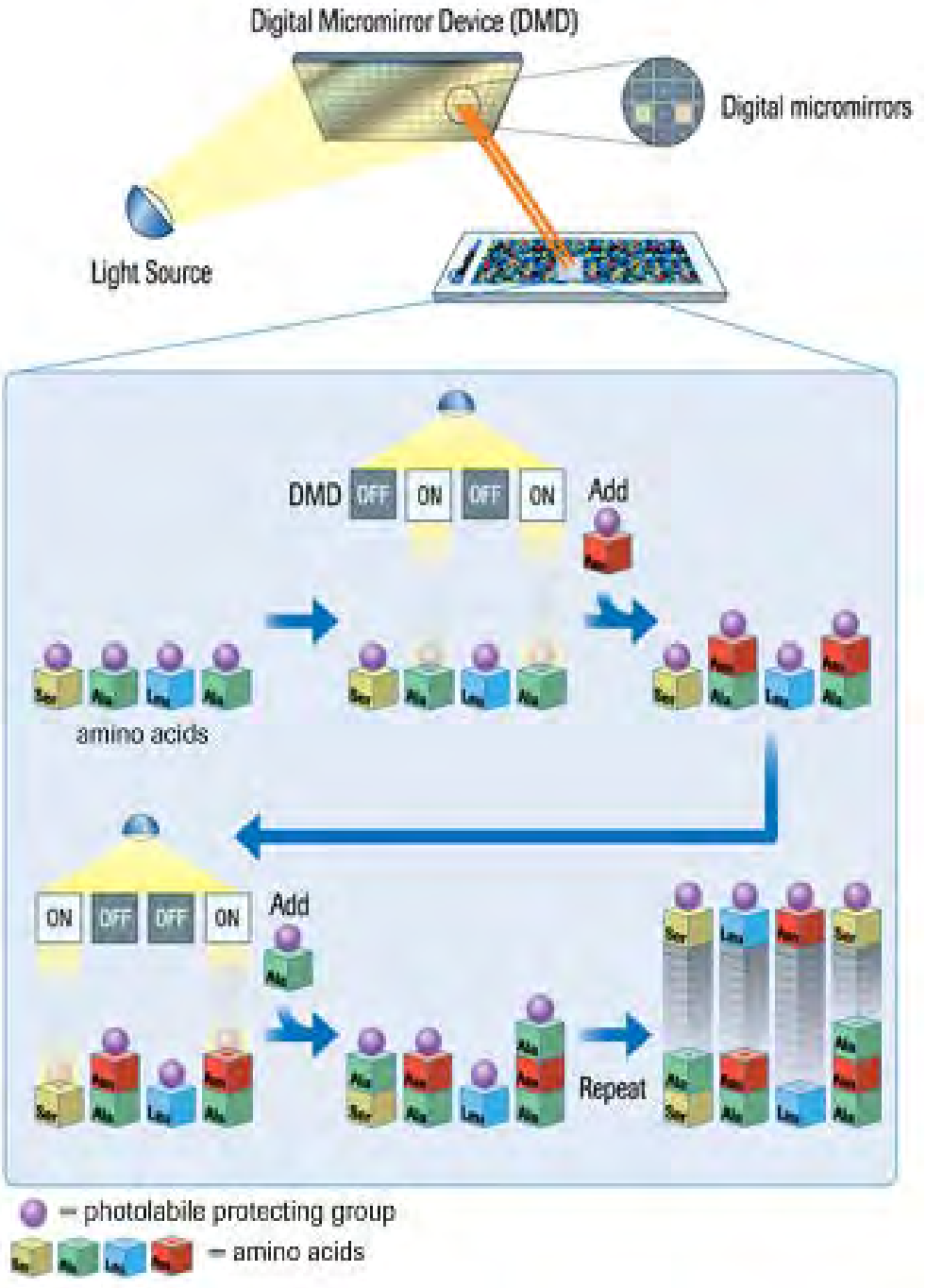
Assessing inactive genes in recipients but active in donors



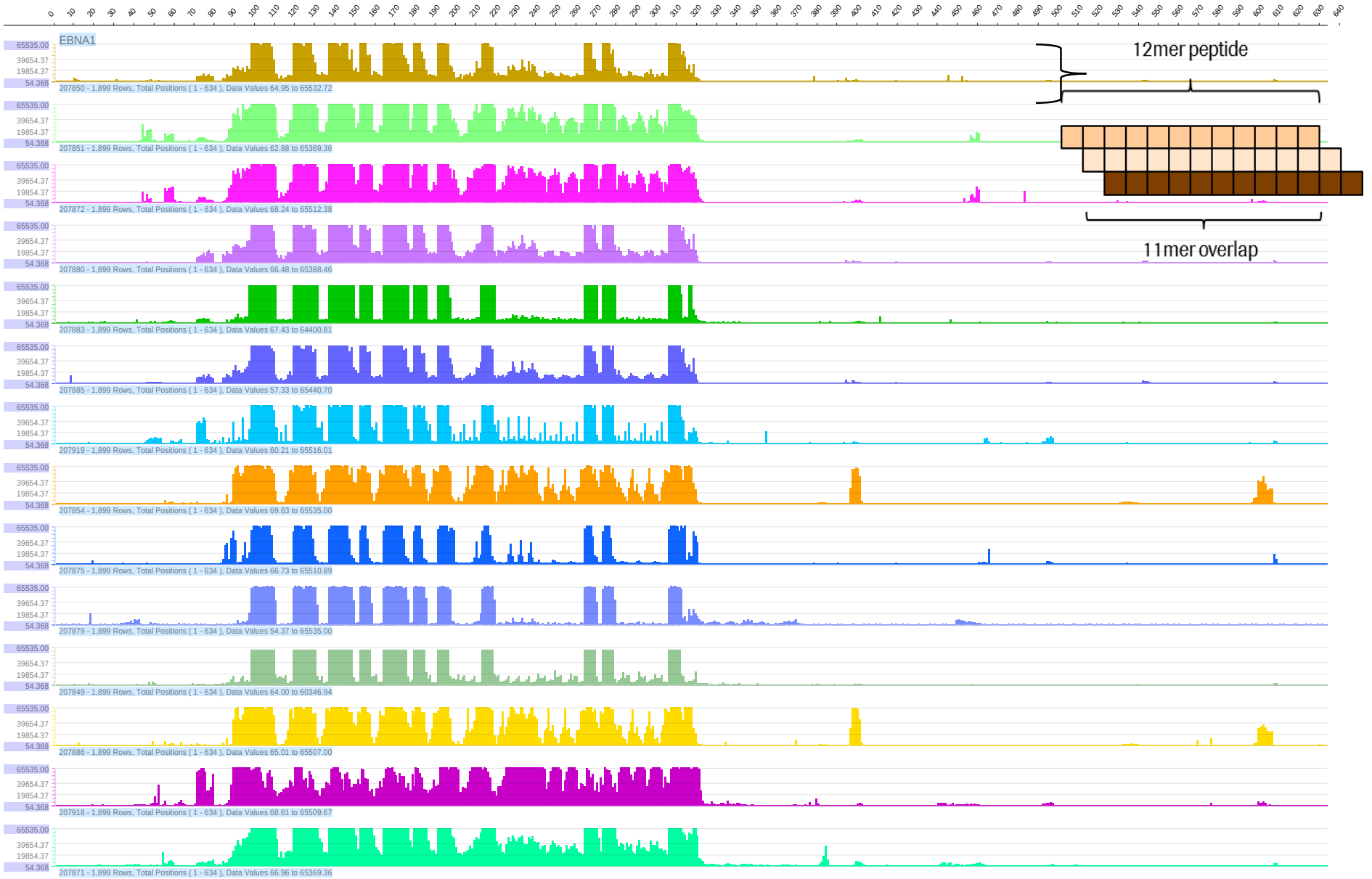
	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Gene 6	Gene 7	Gene …	Gene 20,000
Donor 1	0	0	0	0	0	0	1	0	0
Recipient 1	0	1	0	0	0	0	0	0	0
Donor 2	0	0	0	0	0	0	0	0	0
Recipient 2	0	0	0	2	0	0	1	0	0
Donor 3	0	0	0	0	0	0	0	0	0
Recipient 3	0	0	0	0	0	1	0	0	0
Donor …	0	2	0	0	0	0	0	0	0
Recipient …	0	0	0	0	0	0	0	1	0
Donor 888	0	0	0	1	0	0	0	0	0
Recipient 888	0	0	0	0	0	0	2	0	0

Roche NimbleGen Tx Peptide Array v1

- Utilize 20+ amino acids with linker on surface
- In-situ synthesis - creates 12-16-mer peptides
- Synthesize up to 2.9M unique peptides per array
- Enables full proteome scale single array analyses



Reactivity to peptides for EBNA-1 protein



Published/Ongoing iGeneTRAiN GWAS analyses

- Primary Association analyses
  - Rejection, graft survival/death (time to event/ rejection Y/N)
    - KIDNEY, HEART, LIVER individual studies → CROSS ORGAN META-ANALYSES
  - Allogenicity/graft outcomes (Lancet Feb 2019, NEJM May 2019)

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Lancet Feb 2019

- Genome-wide mismatches in 59,268 nsSNPs in transmembrane proteins
  - 477 kidney tx recipient-donor pairs
- mismatches associated with graft loss in multivariable model
  - adjusted for HLA eplet mismatch (HLA-A,-B, -C, -DP, -DQ & -DR)
- 5-year death censored graft survival:
  - 98% in 1st quartile (lowest mismatch)
  - 91% in 2nd quartile
  - 89% in 3rd quartile
  - 82% in 4th quartile (p=0·003, log-rank test).

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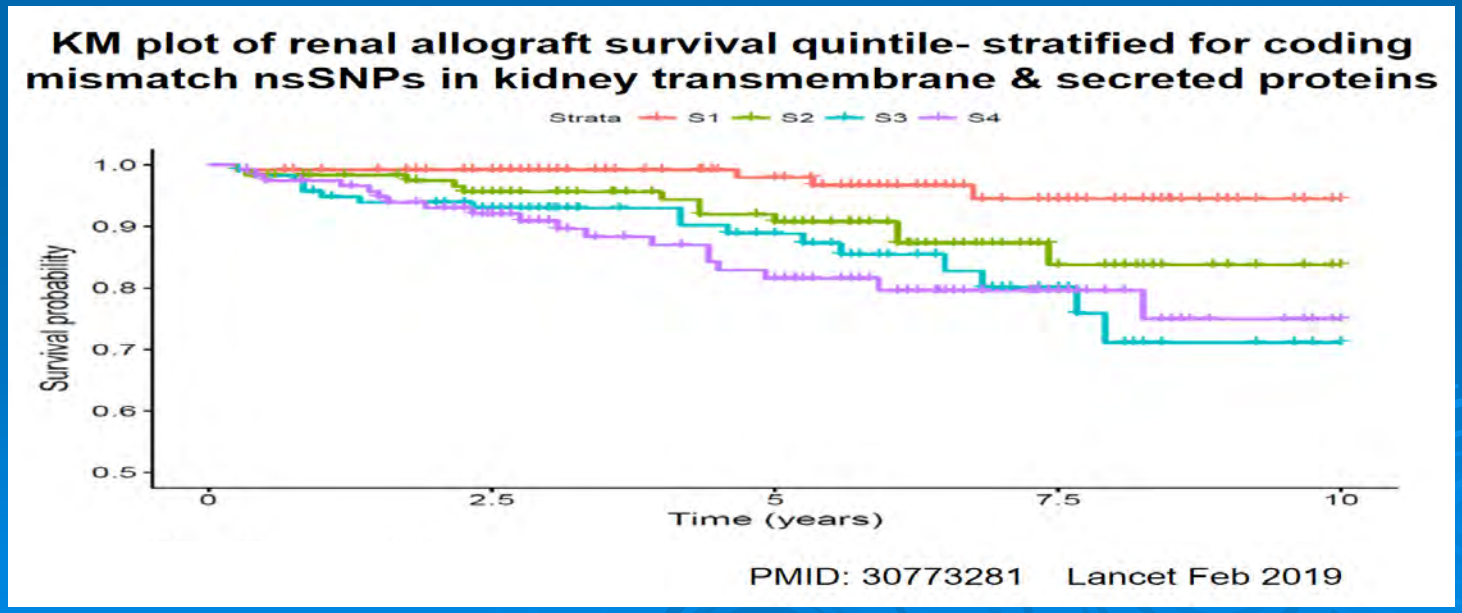

- Ab's against mismatched epitopes in patients sera with bx-confirmed rejection

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    - KIDNEY, HEART, LIVER individual studies → CROSS ORGAN META-ANALYSES
  - Allogenicity/graft outcomes (Lancet Feb 2019, NEJM May 2019)

NEJM May 2019

- Significant association with allograft rejection discovered in *LIMS1* region
  - rs893403 HR 1.84; 95%CI, 1.35-2.50; $P=9.8\times10^{-5}$
- Replicated under D-R model in 3 independent cohorts (2,004 D-R pairs)
  - HR, 1.55; 95% CI, 1.25-1.93; $P=6.5\times10^{-5}$
- Combined analysis risk genotype was associated with rejection
  - (**HR, 1.63**; 95% CI, 1.37-1.95; $P=4.7\times10^{-8}$) with alloantibodies evident

PMID: 31091373

Published/Ongoing iGeneTRAiN GWAS analyses

- Primary Association analyses
  - Rejection, graft survival/death (time to event/ rejection Y/N)
    - KIDNEY, HEART, LIVER individual studies → CROSS ORGAN META-ANALYSES
  - Allogenicity/graft outcomes (Lancet Feb 2019, NEJM May 2019)
- Other post-tx phenotypes
  - Decline eGFR over 5 years (donor & recipient genotypes) (AJT 2019)
  - DILI, Nephrotoxicity, Early graft dysfunction (AJT 2019)
  - HCC development (in preparation)
  - Polygenic risk score for non-melanoma skin cancer risk (AJT 2019)
  - New onset of Diabetes after transplant (NODAT) (in preparation)
  - Genetics of Tacrolimus Trough levels and Metabolism (AJT 2019)

Published/Ongoing iGeneTRAiN GWAS analyses

- Primary Association analyses
  - Rejection, graft survival/death (time to event/ rejection Y/N)
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  - Allogenicity/graft outcomes (Lancet Feb 2019, NEJM May 2019)
- Other post-tx phenotypes
- Donor-Recipient interaction analyses
  - Loss-of-Function (LoF) pipeline
    - e.g. stop-gained, splice-disrupting, or frame-shift mutations)
  - HLA, KIR focused analyses:
    - Amino acid imputation (using SNP2HLA, HLA*IMP & KIR*IMP)
    - D-R interaction models: Eplet, PIRCHE-II, SFVT
- Primary Disease; Nephronophthisis (JASN 2018)

Assessing genetics of cause-of-transplant

- Nephronophthisis, medullary cystic kidney disease: most common genetic disorder causing pediatric ESRD
- Often caused by homozygous *NPHP1* full gene deletions
  - Autosomal recessive inheritance

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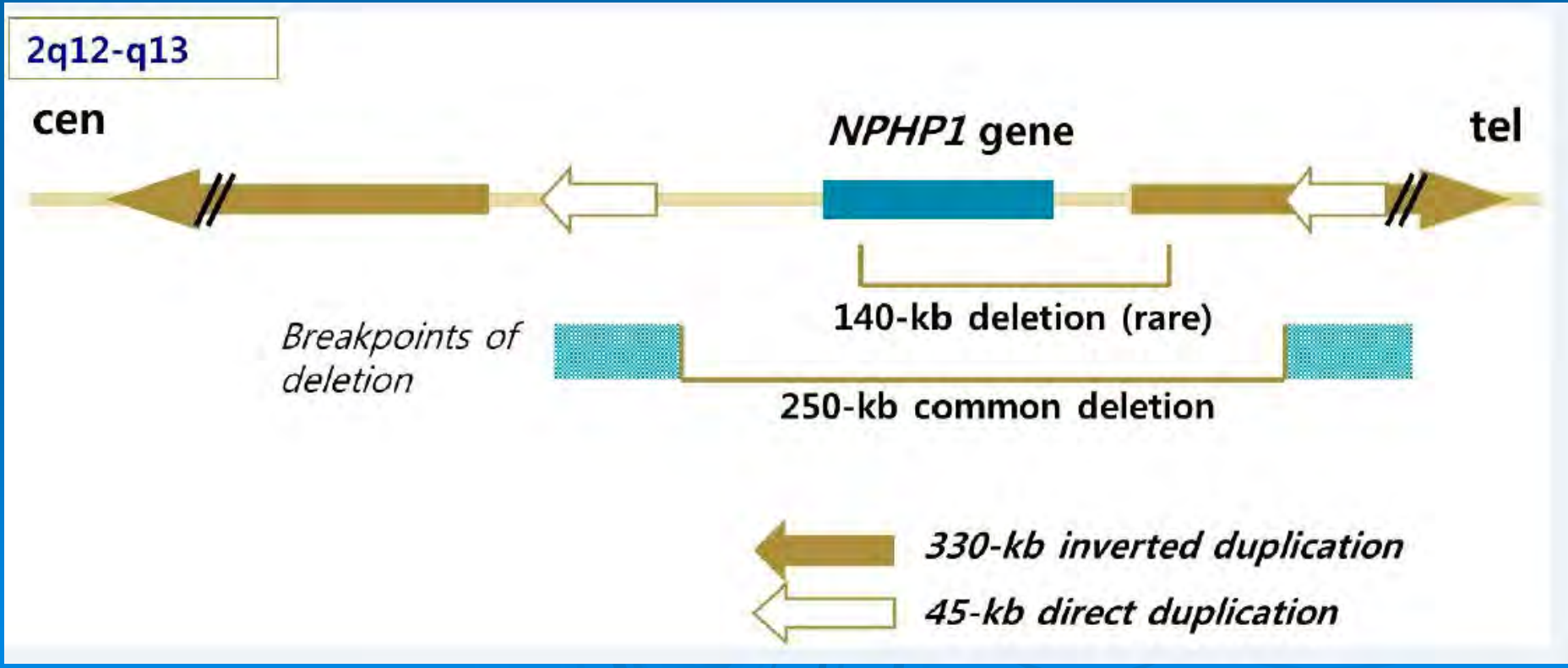

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PMID: 29654215

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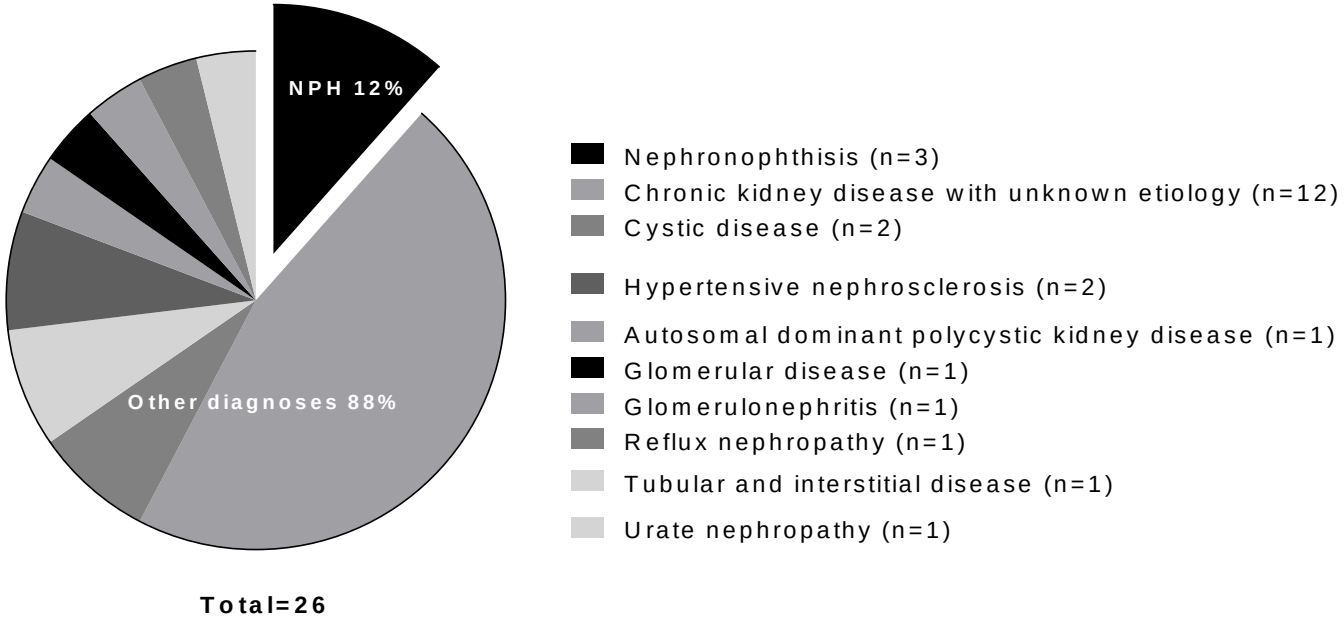
Diagnoses prior to Transplant



JASN 2018
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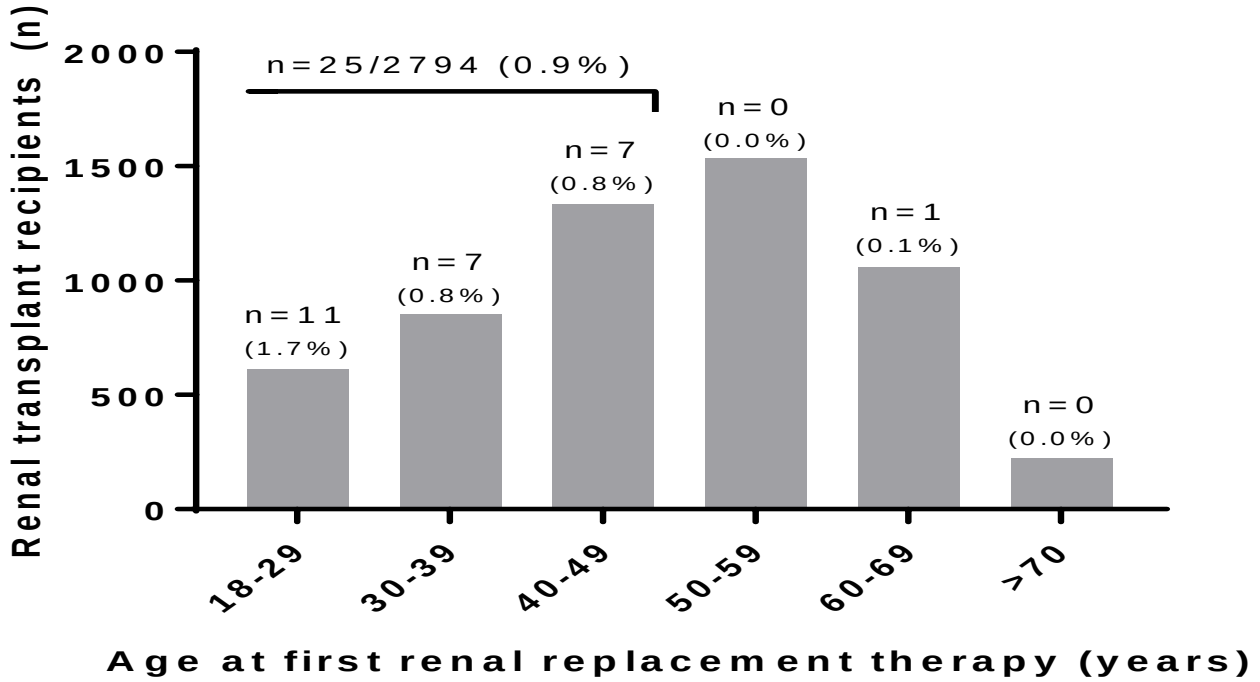
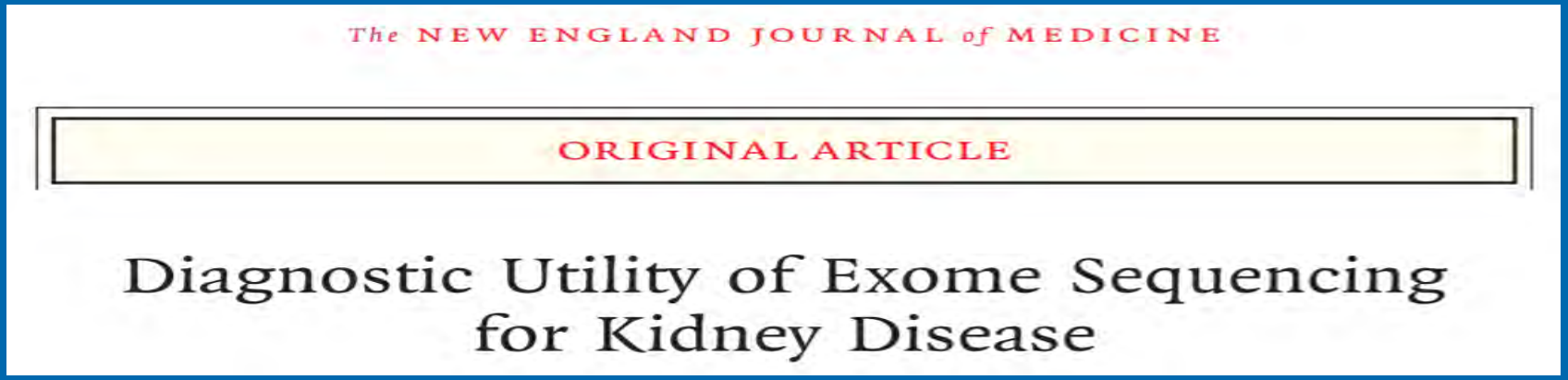


JASN 2018
PMID: 29654215

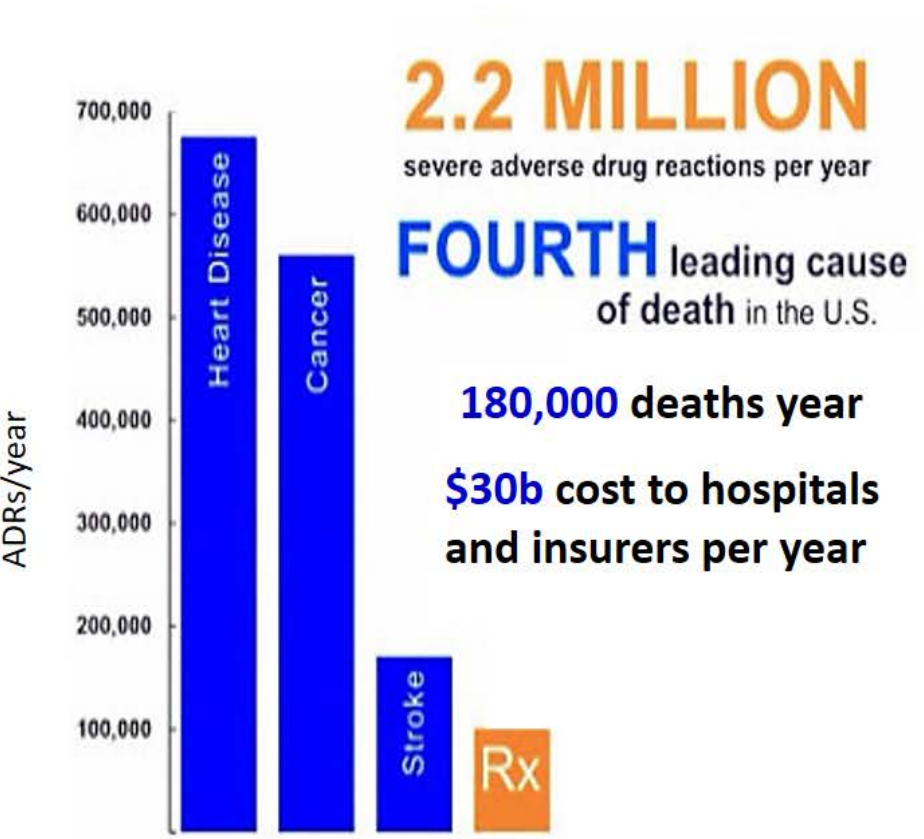
Using Whole Exome Sequencing (WES) to diagnoses CKDs



- Assessed diagnostic yield of WES in >3315 CKD patients
  - Diagnostic variants found in 307 patients (9.3%)
  - Encompasses 66 different monogenic disorders
- Diagnostic variants detected across all clinical categories
  - e.g. congenital or cystic renal disease: 127 of 531 patients (23.9%)
  - Nephropathy of unknown origin: 48 of 281 patients (17.1%)
- 34 of 2187 patients had actionable findings for medical disorders
  - Led to additional referrals & leading to more informed renal management

NEJM 2019 PMID: 30586318

“Trial & error” prescribing --> highly variable outcomes



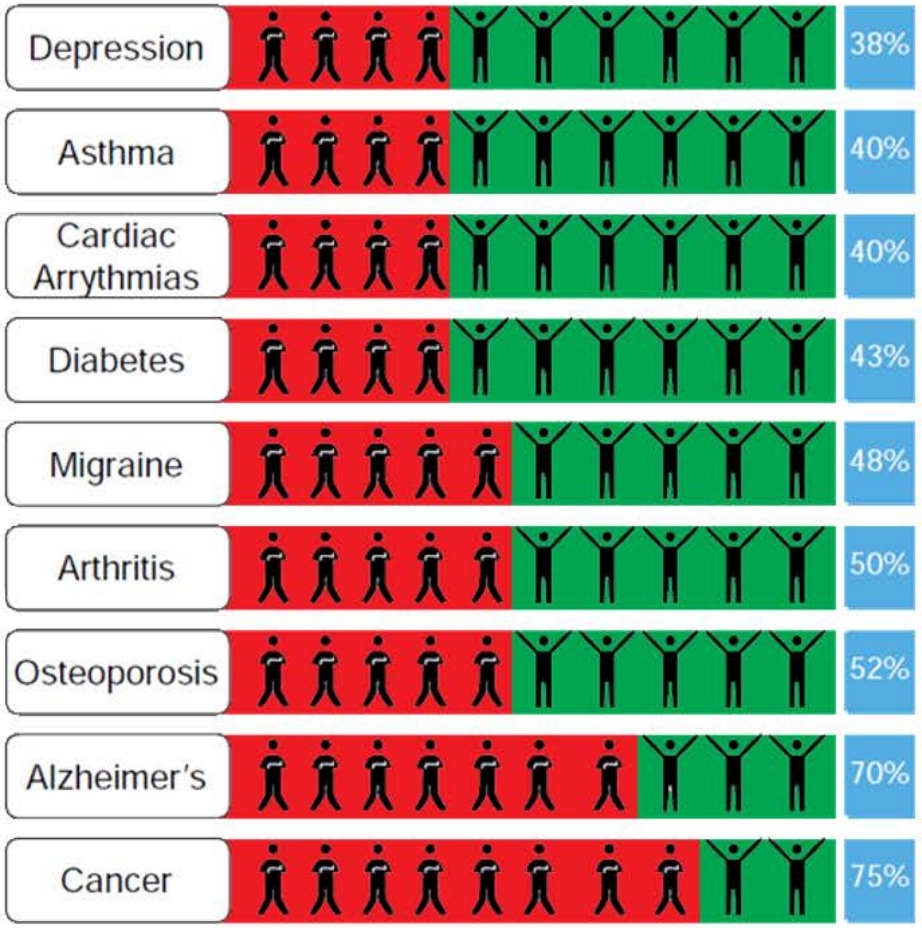
2.2 MILLION
severe adverse drug reactions per year

FOURTH leading cause of death in the U.S.

180,000 deaths year

$30b cost to hospitals and insurers per year

Adverse drug reactions are deadly/costly



50M Polypharmacy

patients on 5+ meds, will double by 2040

Sources: *U.S. Census, Annals of Pharmacotherapy 2018, Vol. 52(9) 829–837,*

Drug failure/poor efficacy is common

FDA. *Paving the way for personalized medicine*

Pharmacogenomics Payer Collaboration

CPIC®
Clinical Pharmacogenetics Implementation Consortium

What is CPIC?

The Clinical Pharmacogenetics Implementation Consortium (CPIC®) is an international consortium of individual volunteers and a small dedicated staff who are interested in facilitating use of pharmacogenetic tests for patient care.

One barrier to implementation of pharmacogenetic testing in the clinic is the difficulty in translating genetic laboratory test results into actionable prescribing decisions for affected drugs.

PG KB

PHARMGKB

Table 1. Commonly used drugs in transplantation and their pharmacogenes of importance.

Drug class(es)	Drug	Gene(s)	FDA Label for PGx testing	PharmGKB level of evidence	CPIC level (s)
Immunosuppression	Azathioprine	*TPMT, NUDT15*	Testing recommended (TPMT)	1A, 1B	A, A/B
	Cyclophosphamide	*TP53, SOD2, GSTP1, MTHFR*		2B, 2B, 2A, 2A	D, D, C/D, C
	Cyclosporine	*CYP3A4/CYP3A5*		2B	C
	Mercaptopurine	*TPMT, NUDT15*	Testing recommended (TPMT)	1A, 1B	A, A/B
	Methotrexate	*MTRR, ATIC, ABCB1, SLCO1B1, MTHFR*		2B, 2B, 2A, 2A, 2A	D, D, C/D, C, C
	Mycophenolic acid	*HPRT1*	Actionable PGx		B
	Sirolimus	*CYP3A5*		2A	C
	Tacrolimus	*CYP3A4/CYP3A5*		2A, 1A	C, A
Statins	Atorvastatin	*LDLR, KIF6, COQ2, APOE*	Actionable PGx (LDLR)	-, 2B, 2B, 2A	D, D, D, C
	Cerivastatin	*SLCO1B1*		2A	B
	Pravastatin	*KIF6, SLCO1B1*		2B, 2A	D, C
	Simvastatin	*ABCB1, SLCO1B1*		2A, 1A	C/D, A
Anti-Diabetic	Metformin	*SLC47A2, C11orf65*		2B, 2B	D, D
Anti-arrhythmic	Propafenone	*CYP2D6*	Actionable PGx	2A	C
Anti-hypertensive	Ace Inhibitors	*KCNIP4, ACE*		2A	D
	Digoxin	*ABCB1*		2A	C/D

Keating B. *et al* Transplantation 2018

Table 1. Commonly used drugs in transplantation and their pharmacogenes of importance.

Drug class(es)	Drug	Gene(s)	FDA Label for PGx testing	PharmGKB level of evidence	CPIC level (s)
Anti-hypertensive/ Beta blocker	Propranolol	*CYP2D6*	Informative PGx	4	C
Diuretic	Spironolactone	*ADD1*		2B	D
Blood thinner	Acenocoumarol	*CYP4F2, CYP2C9*		2B, 2A	B, B
	Aspirin	*PTGS1, LTC4S, GP1BA, HLA-DPB1*		2B, 2B, 2B, 2B	D, D, D, C
	Clopidogrel	*CYP2C19, CES1*	Actionable PGx (CYP2C19)	1A, 2B	A, C/D
	Phenprocoumon	*CYP4F2*		2A	B
	Warfarin	*PROS1, PROC, VKORC1, CYP2C9, GGCX, CALU, CYP4F2*	Actionable PGx (PROS1, PROC, VKORC1, CYP2C9)	-, -, 1A, 1A, 2B, 2B, 1B, 2B	D, D, A, A, D, D, A
Osteoporosis	Bisphosphonates	*FDPS*		2B	D
Antibiotic	Nalidixic acid	*G6PD*	Actionable PGx		B
	Nitrofurantoin	*G6PD*	Actionable PGx	3	B
	Norfloxacin	*G6PD*	Actionable PGx		B
	Sulfadiazine	*G6PD*	Actionable PGx		B

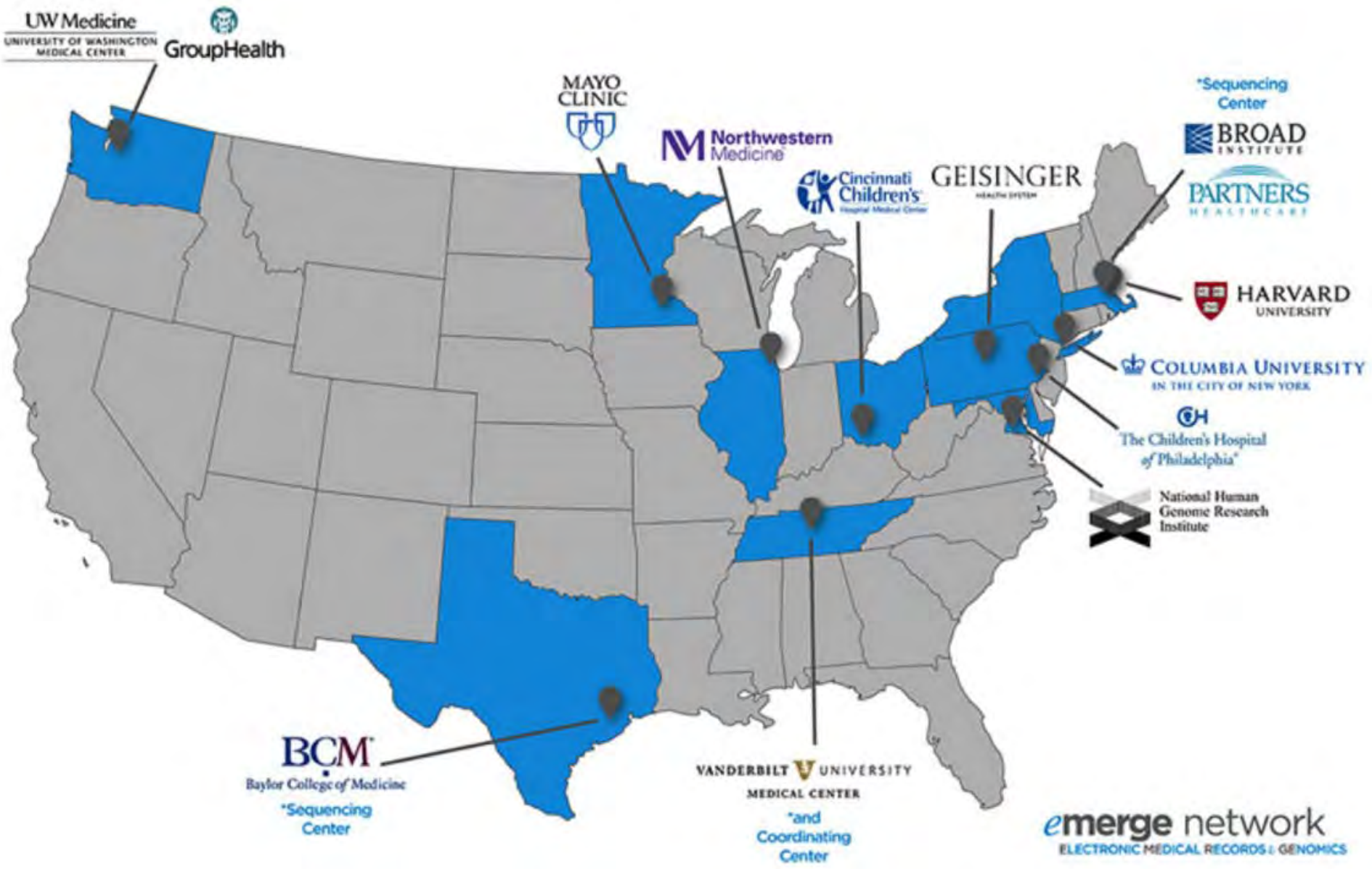
Keating B. *et al* Transplantation 2018

Table 1. Continued.

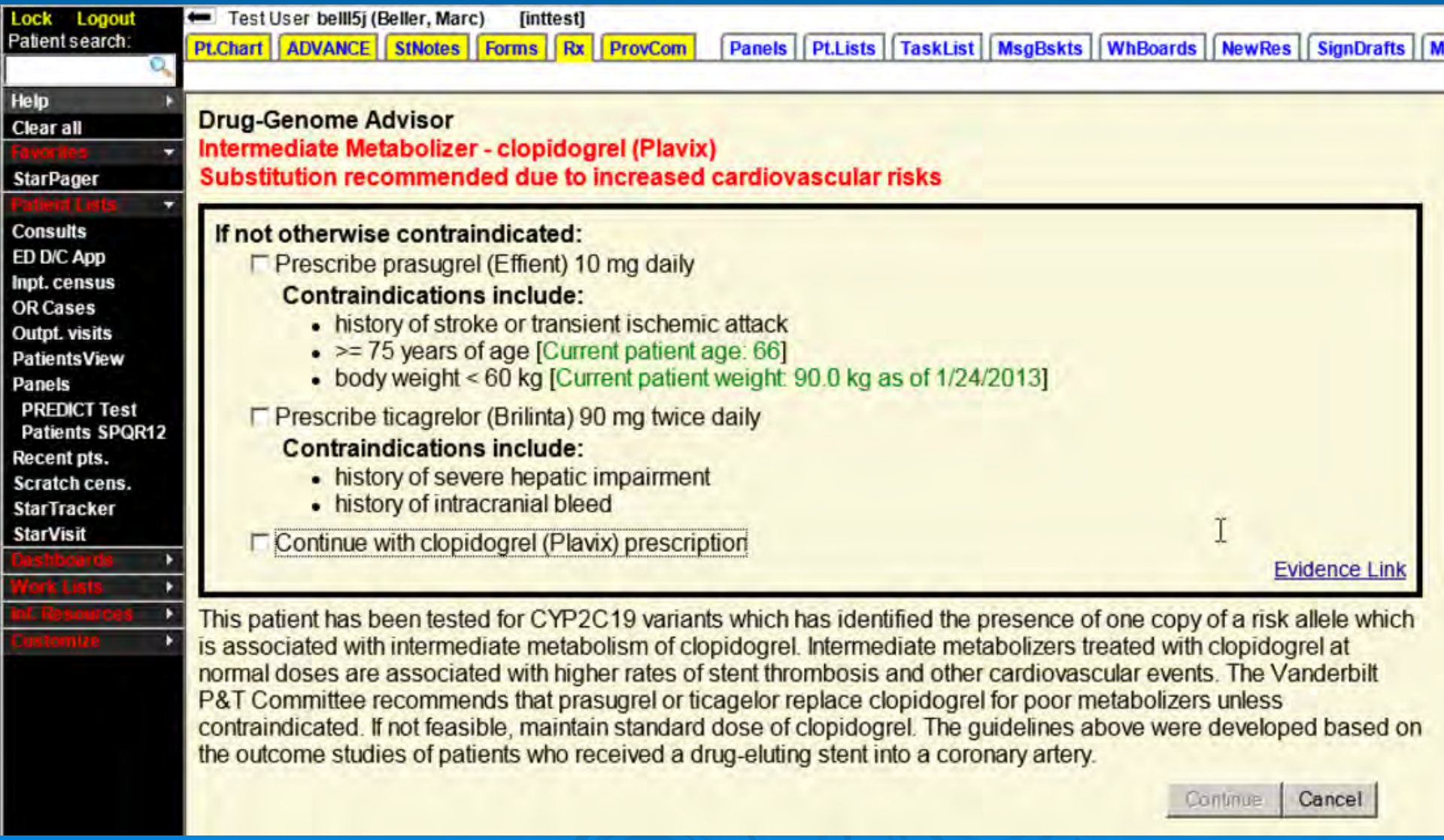
Drug class(es)	Drug	Gene(s)	FDA Label for PGx testing	PharmGKB level of evidence	CPIC level (s)
Anti-Viral	Abacavir	*HLA-B*	Testing required	1A	A
	Atazanavir	*CYP3A5, UGT1A1*		2B, 1A	C, A
	Interferon alfa-2b	*ITPA*		2B	C/D
	Peginterferon alfa-2a	*IFNL4, IFNL3*		1A, 1A	D, A
	Peginterferon alfa-2b	*IFNL4, IFNL3, VDR*	Actionable PGx (IFNL3)	1A, 1A, 2A	D, A, D
	Ribavirin	*VDR, IFNL3*	24096968 (IFNL3)	2A, 1A	D, A
	Tenofovir	*ABCC4*		2B	D
Chemotherapy (skin cancer)	Fluorouracil	*DPYD, UMPS, TYMS, NQO1, GSTP1, MTHFR*	Actionable PGx (DPYD)	1A, 2B, 2A, 2A, 2A, 3	A, D, D, D, C/D, C
Chemotherapy (other)	Anthracycline	*CBR3, SLC28A3, NQO1, HAS3*		2B, 2B, 2A, 2B	D, D, D, D
	Belinostat	*UGT1A1*	Actionable PGx	3	B
	Cisplatin	*TP53, TMEM43, GSTM1, COMT, XPC*		2B, -, 2B, 3, 1B	D, D, D, C/D, D
	Irinotecan	*UGT1A1, SEMA3C, C8orf34*	Actionable PGx (UGT1A1)	2A, 2B, 2B	A, D, D
	Nilotinib	*UGT1A1*	Actionable PGx	3	C
	Sunitinib	*ABCB1*		2B	C/D

The major classes of drugs prescribed to patients post-transplantation are shown along with specific drugs and the genes of known interaction

eMERGE Phase III Members



Embedding of CLIA genotyping into EMR's & applying to real-life patients



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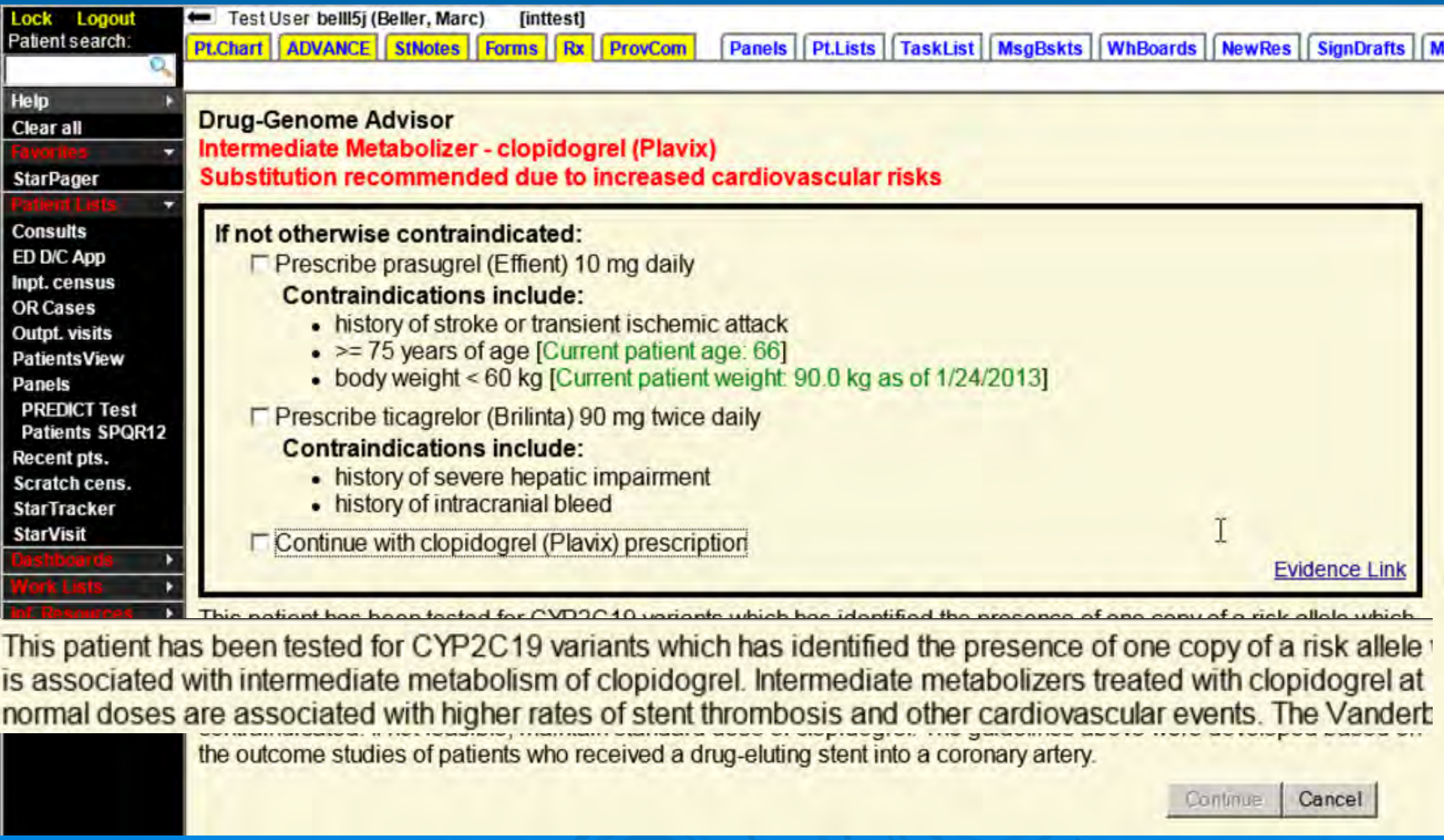

Industry/Academia Biomarker Collaborations

- Genome-wide Studies to discover gene knock-out effect and/or targets for common & rare disease.

- Pharma Whole-exome sequencing studies using Large Biobanks
  - UK BioBank > 500,000 WES underway
  - Geisinger Health System >200,000 WES completed to date
  - Mayo Clinic – recent agreement for > 100,000 WES
  - Penn Biobank/Penn DNAs – 160,000 WES
  - Genomics England – 100,000 Whole Genome Seq
- Rare Disease studies
  - Numerous Pediatric Hospitals
  - NIH Undiagnosed Diseases Program, NIAID, NIDDK

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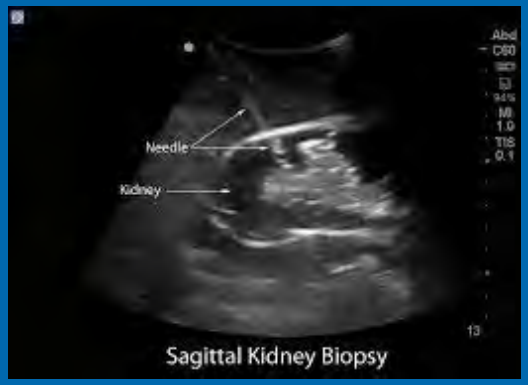
- iGeneTRAiN
  - Over 2,000 Penn Tx samples subjected to WES to date
  - HLA Sequencing of key Class I & II loci in > 700 patients to date
  - Scaling up to 45,000 samples (GWAS, WES & HLA Class I/II regions)

Biomarkers for diagnoses & prognostication of post-transplant outcomes

Urinary mRNA prediction of Acute Rejection in Kidney Tx

- Gold standard for diagnosing rejection in kidney is still needle biopsy



  - Complications: procedural errors, inter-observational variability, bleeding
  - Biopsies are typically ‘for cause’: graft damage may already have occurred

- NIH Clinical Trials in Organ Transplantation (CTOT) 04 study
  - Assessed if mRNA urinary profiles were predictive of acute rejection
  - n=485 kidney tx recipients recruited in multicenter observational clinical study
  - > 10 Urines collected in 1st year for diagnostic prediction of acute rejection

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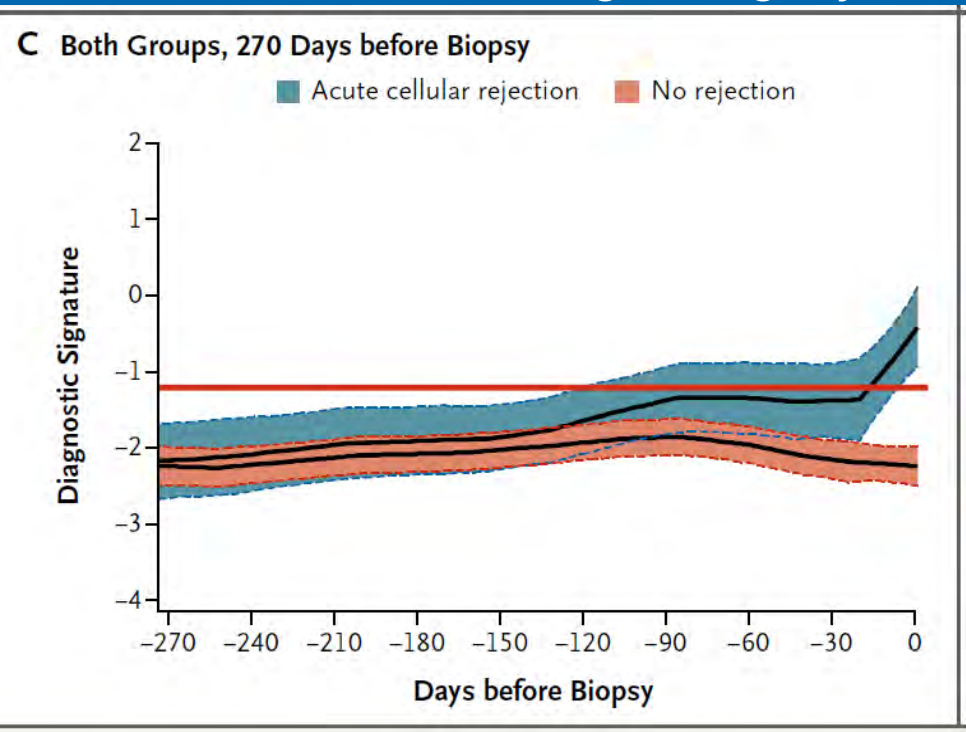
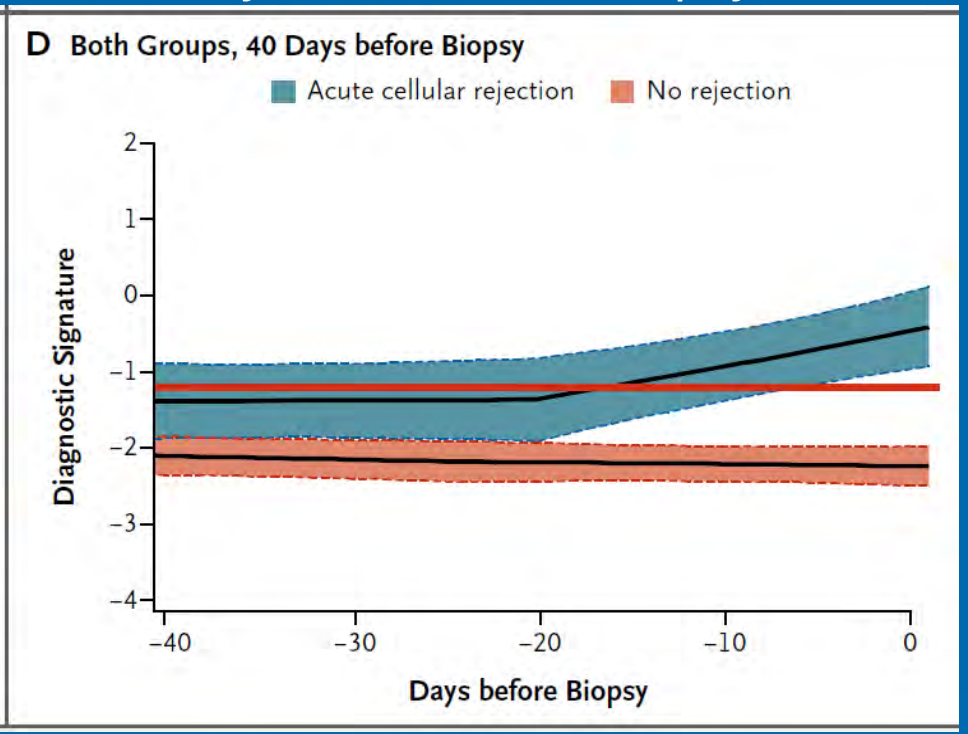




- *CD3ε & CXCL10* mRNAs discriminated rejection vs non-rejection
  - AUC, 0.85; 95% CI: 0.78-0.91; *P*<0.001 by ROC curve analysis

Blue: n= 38 patients with 1st biopsy showing ACR (201 urine samples)

Orange: n=113 patients showing no rejection (833 urine samples)

Bridging Pediatric & Adult Therapeutics: VIRTUUS

- Minimally invasive Transplant Biomarker study: 450 pediatric kidney tx recipients prospectively in 12 sites (recruitment from Summer/Fall 2017)

NIH U.S. National Library of Medicine

ClinicalTrials.gov

Home > Search Results > Study Record Detail

VIRTUUS Children's Study (VIRTUUS)

ClinicalTrials.gov Identifier: NCT03719339

Recruitment Status : Recruiting
First Posted : October 25, 2018
Last Update Posted : October 1, 2019

See Contacts and Locations

Sponsors and Collaborators

Children's Hospital of Philadelphia

Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

Investigators

Principal Investigator: Brendan Keating, DPhil Children's Hospital of Philadelphia and Hospital of The University of Pennsylvania

Bridging Pediatric & Adult Therapeutics: VIRTUUS

Whole Urinary metabolite screening of Kidney Tx Patients

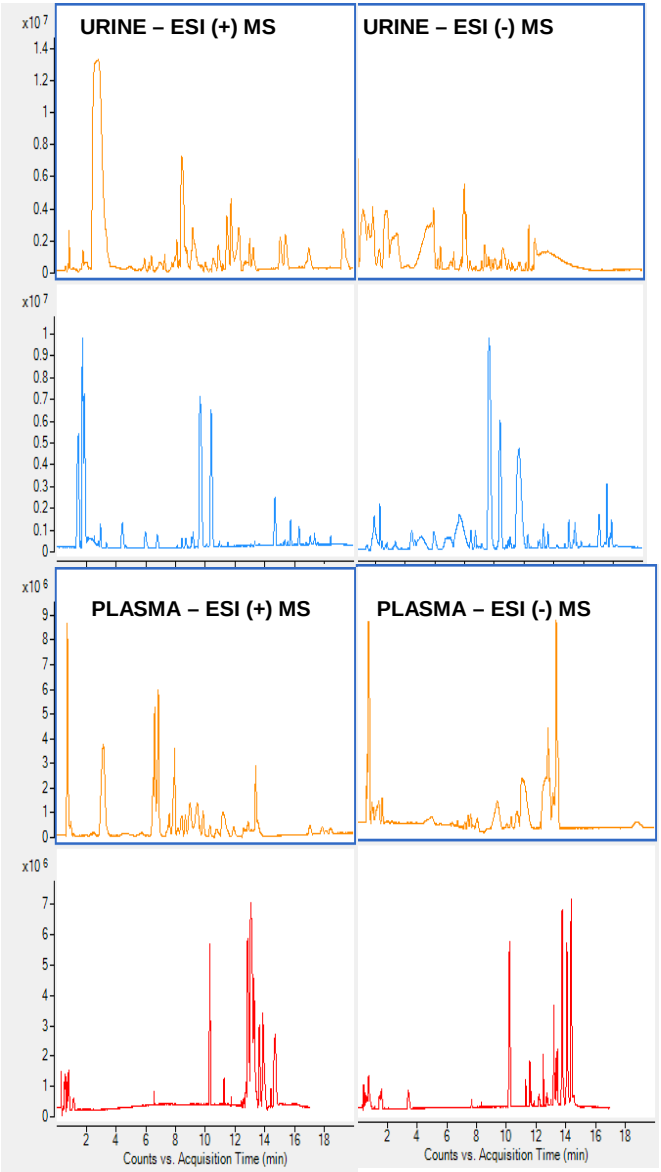
Additional Sites

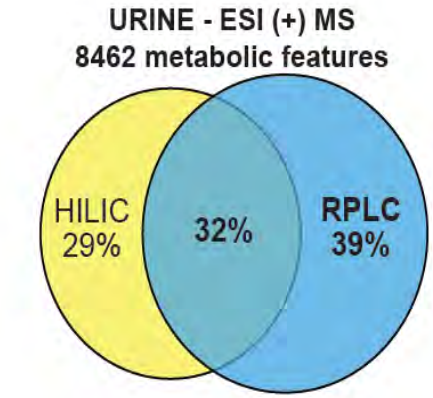
- UPenn, USA
- Samsung, Korea
- BIOMARGIN (EU)*
- HdR, Sao Paulo Brazil
- King Faisal, Riyadh
- Univ Med Vienna,
- DeKAF

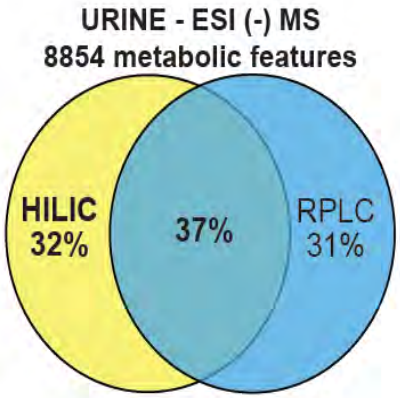
Pending Sites

- GOSH, London
- Temple St. Dublin,

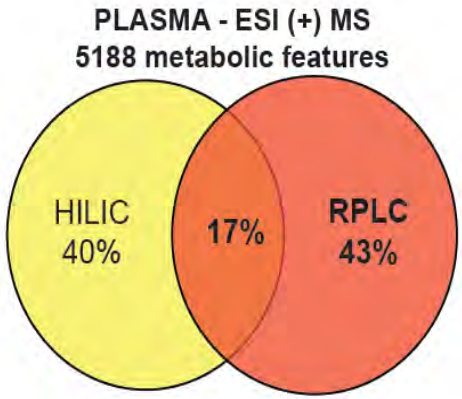
Using Mass Spec to detect metabolites in blood & urine

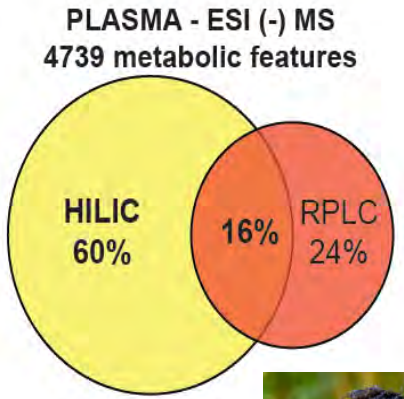




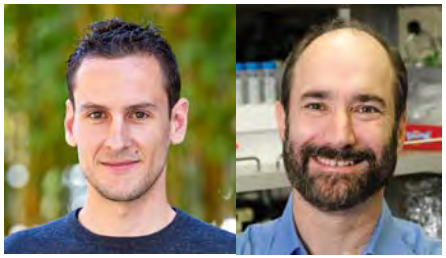


URINE
11,332 unique features in both ESI modes



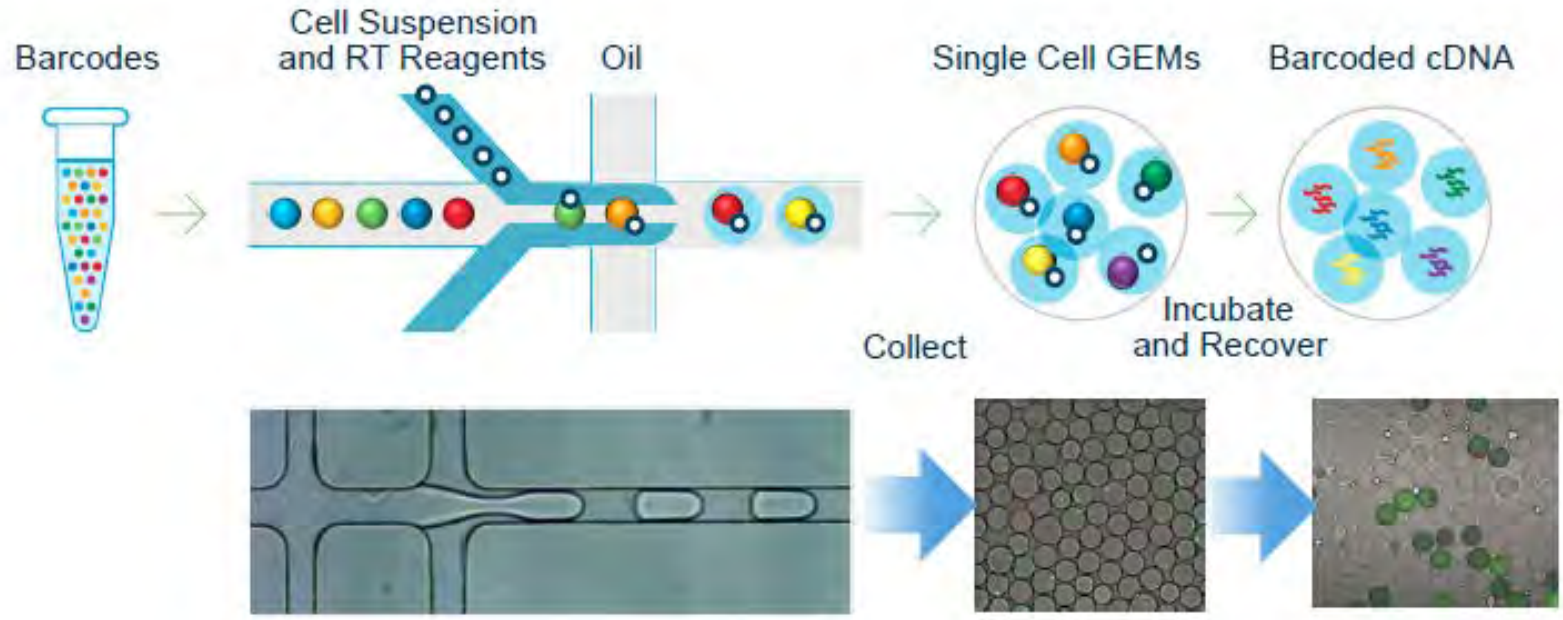


PLASMA
8,287 unique features in both ESI modes



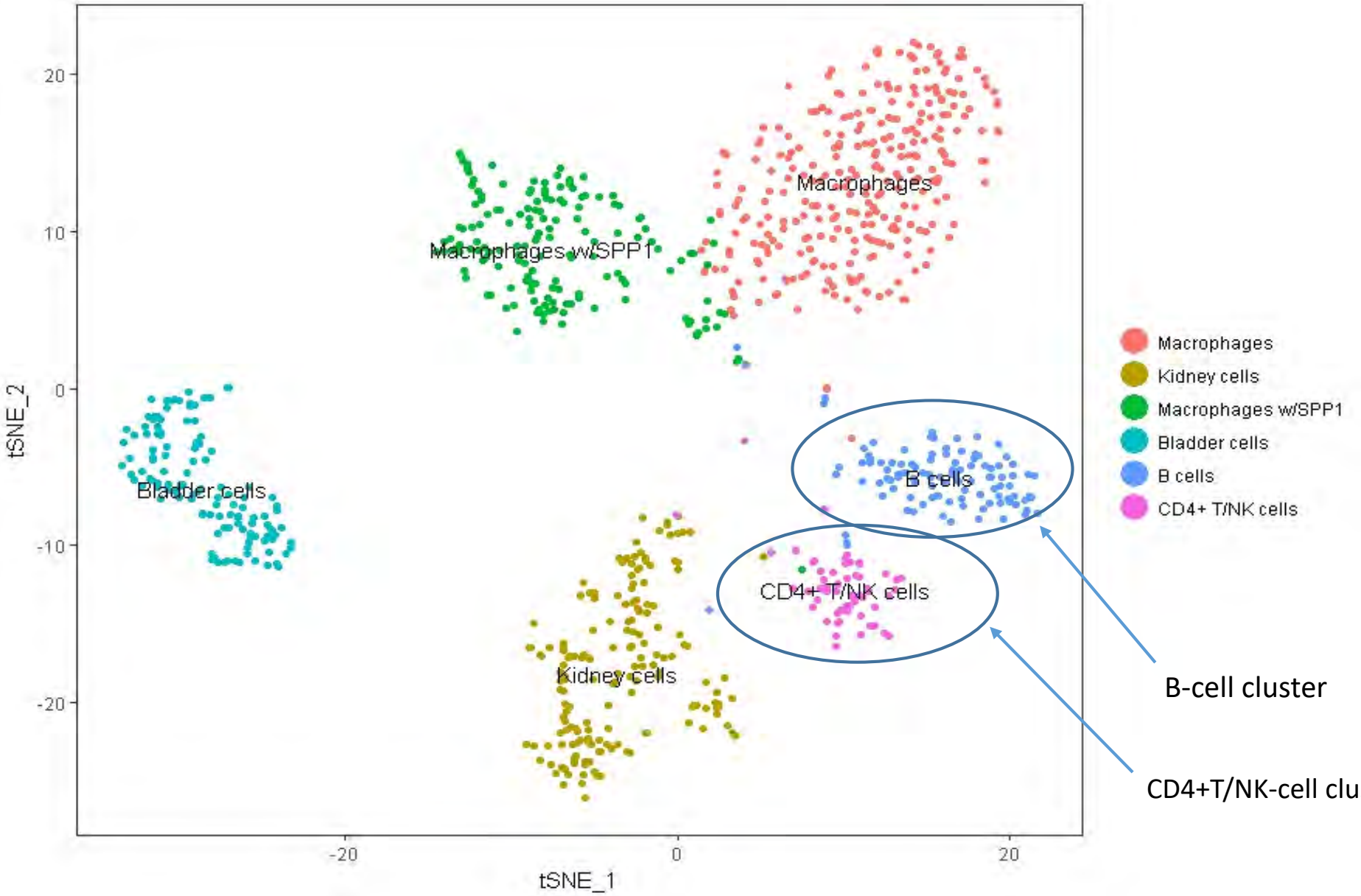
Contrepois K, Jiang L, Snyder M. Mol & Cell Proteomics 2015

Single cell RNA-sequencing



- High GEM fill ratio (~90% of droplets contain beads)
- Poisson loading of cells into GEMs
- Beads dissolve for efficient, liquid phase biochemistry

Urinary cells in patient presenting 40 days prior to acute rejection diagnosis



Liver Transplant: cf-miRNA for detection of Acute Rejection & reducing immuo-suppressants

Can we use minimally invasive biomarkers to diagnoses subclinical liver transplant rejection?

Can we personalize Immunosuppression dosing to minimize side-effects while preserving suppression of immune system?

NIH prospective study samples available

1. NIH CTOT-03 Trial: (NCT-00531921)

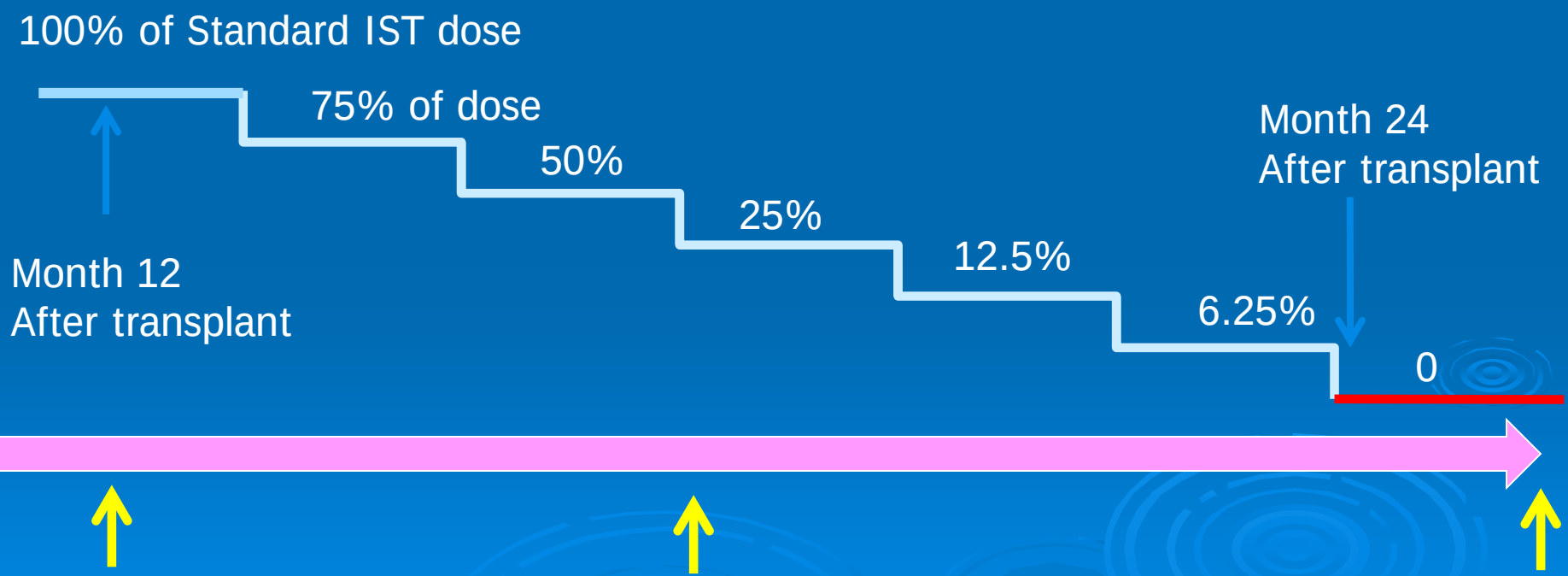
   Prospective trial profiling pro-inflammatory and rejection biomarkers

2. NIH/ITN A-WISH study (NCT-00135694)

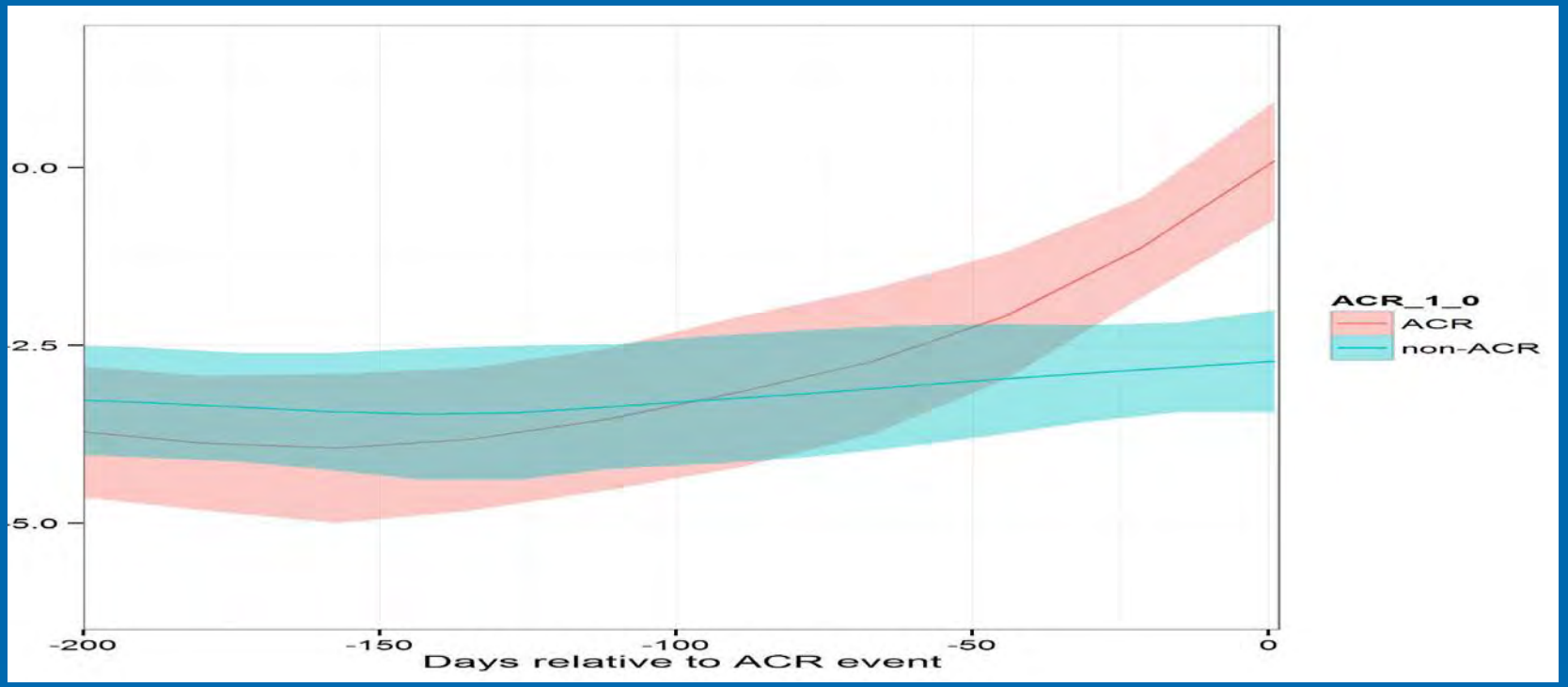
- Phase 2 interventional Trial (initial recruitment of 250 liver tx recipients)

A-WISH: Post-Transplant Immunosuppression reduction

- 1 year post-transplant:
  - 50% of patients were kept on standard immunosuppression (IST)
  - other 50% had immunosuppression reduced by 25% every 10 weeks
- Liver biopsies at 12, 24 & 36 months & blood collected every 3 months



Longitudinal trajectory of 2-miRNA ACR signature as a function of time prior to rejection



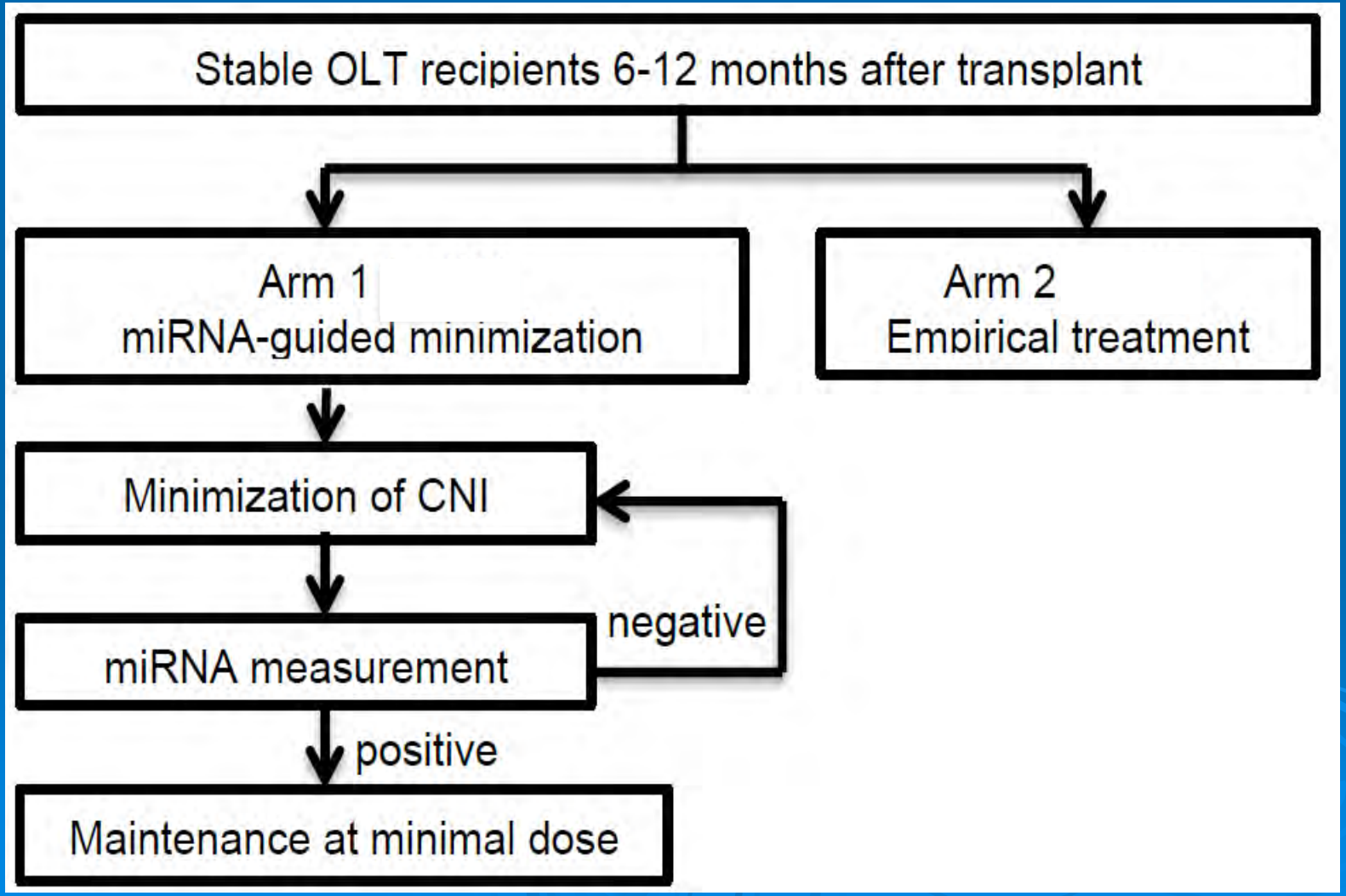
- Combined discovery & replication sets (n= 37 ACR & 93 non-ACR)
  - 752 miRNAs assessed per sera sample (Exiqon)
  - 31 miRNAs associated with ACR after replication (FDR p < 0.005)

Regression -> 2 miRNAs for ACR signature (hsa-miR-483-3p & hsa-miR-885-5p)

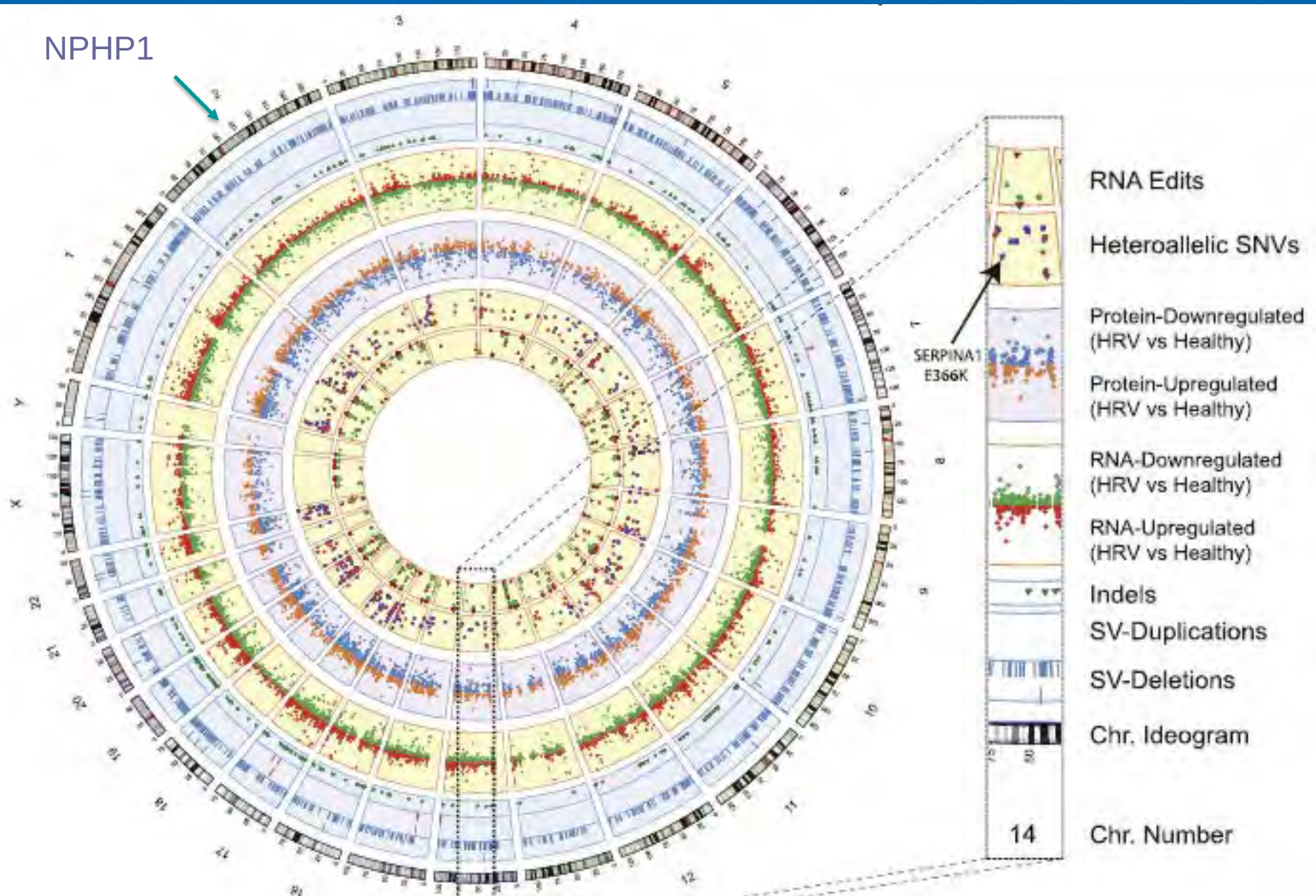
- Ability to differentiate ACR vs nonACR: AUC=0.895 (95%CI=0.84-0.95)
- 92.6% sensitivity & 84.2% specificity (*p*=0.0001) PPV 0.72; NPV 0.93

(Hepatology 2017 PMID: 27533743)

miRNA guided detection of rejection & immunosuppression therapy minimization vs standard of care in Liver Tx



Integrative personal omics profiling (iPOP)



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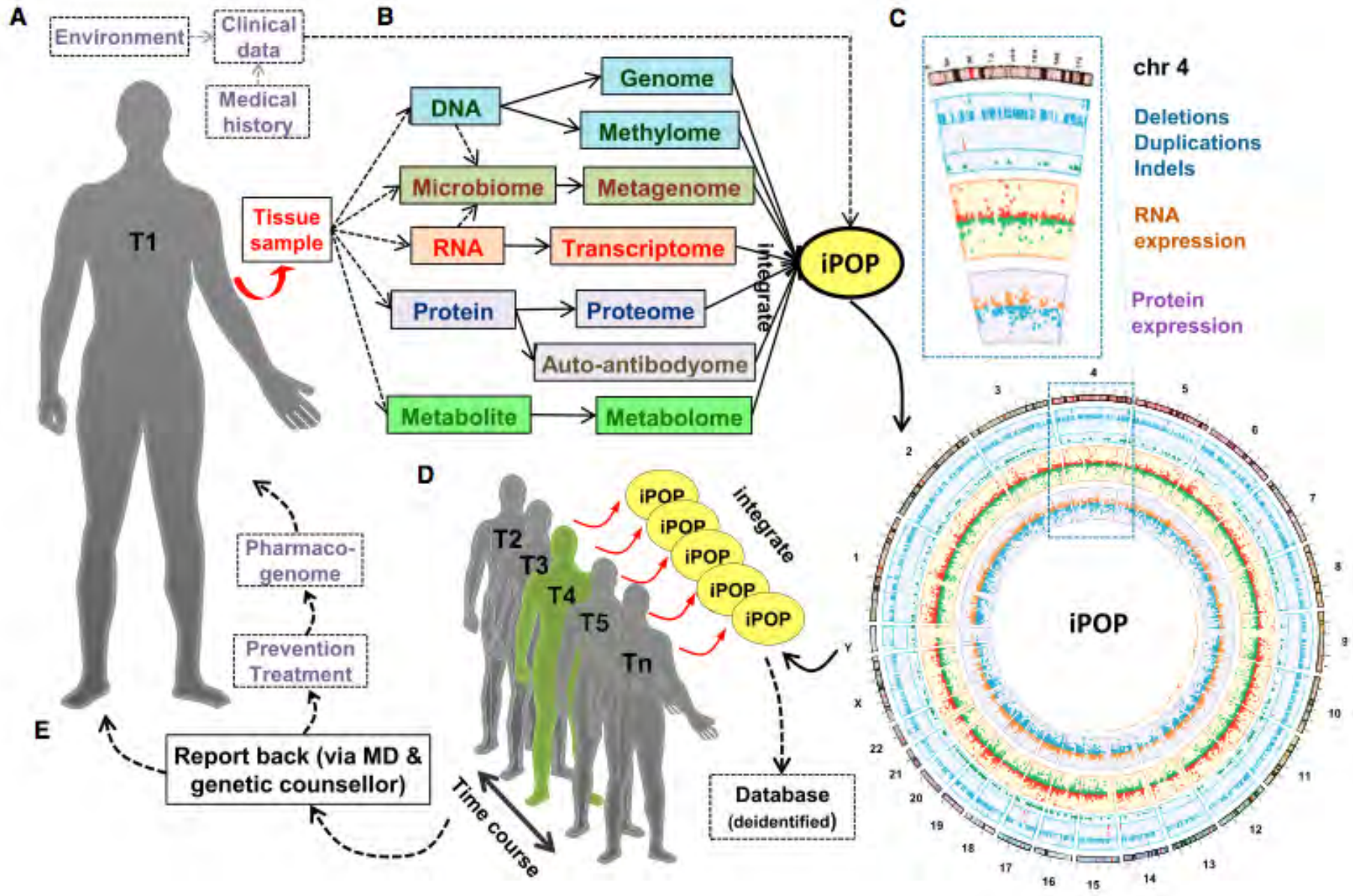


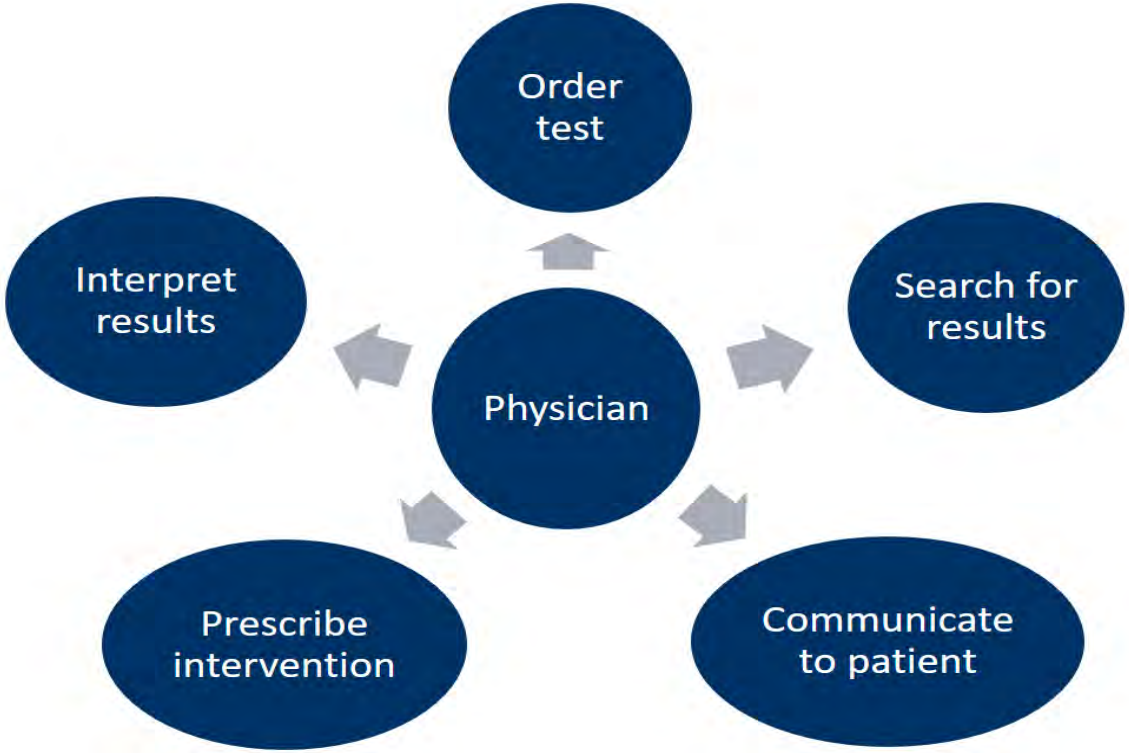
Chen *et al* Cell 2013 PMID: 22424236 and Li-Pook-Than et al., 2013

Barriers to Adoption

- Relieve burden of PGx CDS & implementation from physician
  - Compelling evidence exists for TPMT clinical utility
    - Assays, GI guidelines & billing codes in place BUT only 20% of assays are actually performed.

Concerns:

- **Lack of bandwidth**
- **Lack of PGx familiarity**
- **Liability**



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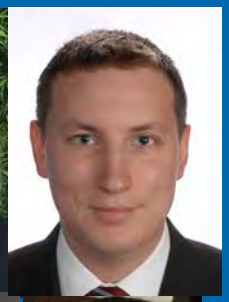
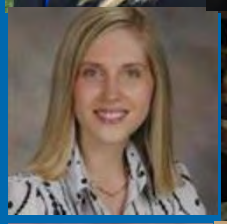

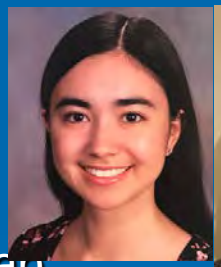


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