AST | AMERICAN SOCIETY OF TRANSPLANTATION

Using Genomics to Guide Immunosuppression Therapy

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CEOT CUTTING EDGE of TRANSPLANTATION

TRANSPLANT SUMMIT 2019

NO SIZE FITS ALL: *Uncovering the Potential of Personalized Transplantation*

FEBRUARY 21–23, 2019 • ARIZONA BILTMORE • PHOENIX, AZ

Disclosure

Consulting: Livanova, Getinge, Abbott, Abiomed
Speaker: Novartis
Research: Astellas, Abbott

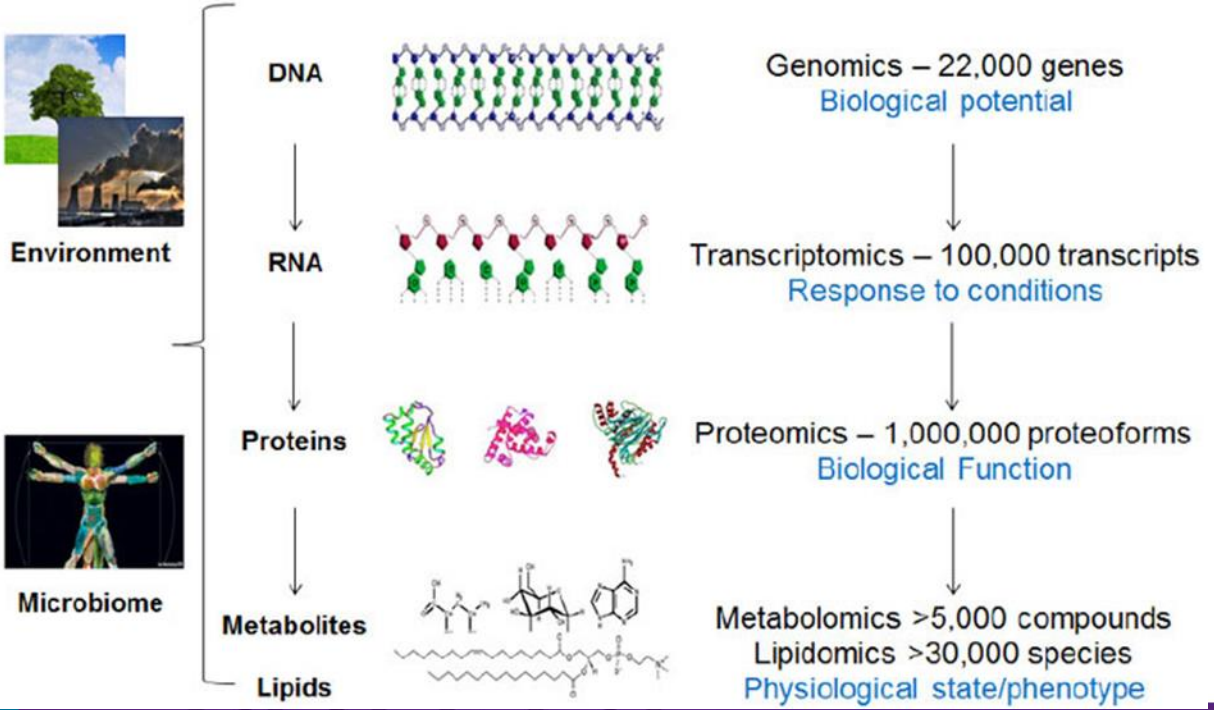
Learning Objectives

Understand the difference between the different –omics approaches
Identify the major areas of research in genomics as they apply to transplantation

Outline

- Guide to the –OMICS
- Genomic work in transplantation
  - Drug metabolism
  - Immunosuppression
  - Assessment of risk of rejection
  - Genomics of active rejection
- Future Directions

Guide to the -OMICS



What is genomic medicine?

- **NHGRI defines genomic medicine as** "*an emerging medical discipline that involves using genomic information about an individual as part of their clinical care (e.g., for diagnostic or therapeutic decision-making) and the health outcomes and policy implications of that clinical use.*"

Proteome Complexity

GENOME

- 4 nucleotides.
- Double helix.
- Same in all cells.

PROTEOME

- 20 amino acids.
- Each protein has unique 3D shape.
- Differs with cell type.

We have got the map (genomic sequence) to find treasure so that we can get treasure chest.

Even if we get the treasure chest (target gene), we can't open it (because we don't understand its function in disease.)

Current genomic researchers have tried pulling out of all nails on the chest.
However, the number of the nails may be infinite…

Key to open the chest
(Post genomic technology)

Getting treasure (new drugs) !!

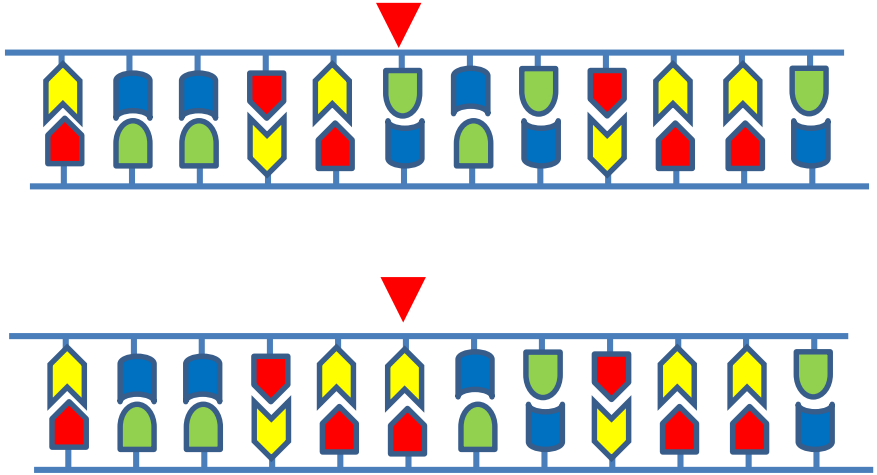
“Genetic Testing”

- Not whole genome sequencing
- We sequence small specific pieces
- “Single Nucleotide Polymorphisms”
- Can screen thousands of SNPs on a SNP-Chip

Polymorphism Markers

- Polymorphism marker: Difference of DNA sequence on the genome
- High polymorphism, but the distribution is less and heterogenious
  - Mini-satellite：Repeat of several to tens of base sequence
  - Micro-satellite：Repeat of 1 to 4 base sequence
  - Base insertion and deletion： Insertion /Deletion of 1-tens of base sequence

- Low polymorphism, but are a lot of distributed on genomic DNA uniformly
- Single nucleotide polymorphism（SNP):
  - 1 /1000 bases
  - 3-10 millions SNP on human genome



SNPs

- Single Nucleotide Polymorphism
- Responsible for 90 % of all human genetic variation
- A SNP occurs every 100-300 Base pairs
- dbSNP database has more than 112 million validated entries
- Most are not responsible for disease

Pharmacogenetics

American Journal of Transplantation 2017; 17: 1008–1019
Wiley Periodicals Inc.

doi: 10.1111/ajt.14040

Predictive Modeling of Tacrolimus Dose Requirement Based on High-Throughput Genetic Screening

C. Damon[1,*], M. Luck[1,2], L. Toullec[3], I. Etienne[4],
M. Buchler[5], B. Hurault de Ligny[6],
G. Choukroun[7], A. Thierry[8], C. Vigneau[9],
B. Moulin[10], A.-E. Heng[11], J.-F. Subra[12],
C. Legendre[13], A. Monnot[1], A. Yartseva[1],
M. Bateson[1], P. Laurent-Puig[2,3,14],
D. Anglicheau[13], P. Beaune[2,3,14], M. A. Loriot[2,3,14],
E. Thervet[2,15] and N. Pallet[2,3,14,15,*]

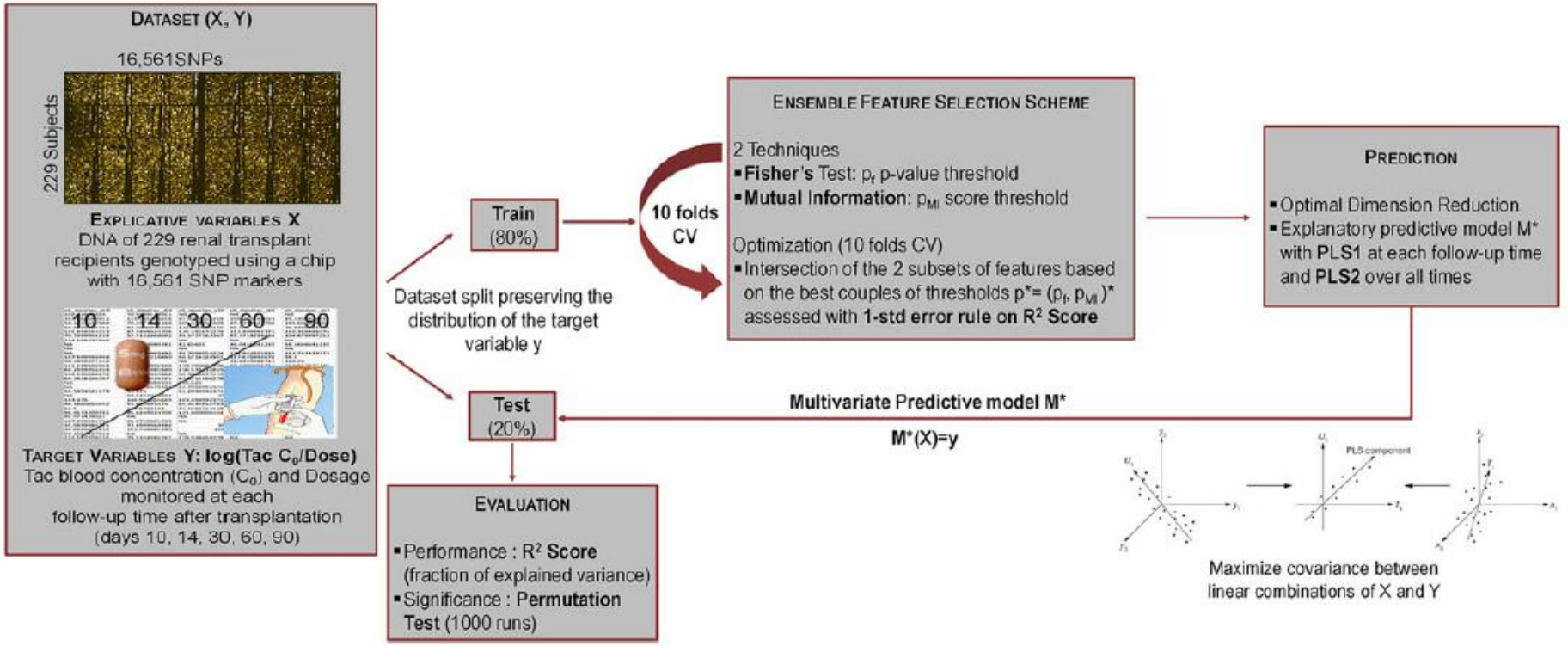


Figure 1: Data-mining methodology. Our predictive approach has two steps. In step 1, an ensemble feature-selection strategy

Table 1: Performance, statistical significance, and complexity of the predictive models at each follow-up time after transplantation (days 10, 14, 30, 60, 90) with PLS1 model and for all times with PLS2 model

	PLS1 models					PLS2 models
Time after transplantation (days)	10	14	30	60	90	10, 14, 30, 60, and 90 days
Performance (R^2 value)	0.30	0.27	0.41	0.7	0.62	0.28
Significance (p-value)	0.001	0.001	0.001	0.001	0.001	0.001
Model complexity (number of SNPs)	5	19	12	44	33	7

PLS, partial least squares; SNP, single-nucleotide polymorphism.

Genetic Component of TAC Metabolism

Using a high-throughput genetic screening approach to predict variability of Tac dose requirement in KTRs, we demonstrated (i) that SNP networks explain 30–70% of the interpatient variability of Tac metabolism, depending on the model generated and the time after transplantation; (ii) that gene interaction networks related to oxidoreductase functions and monooxygenase activity, including *CYP3A4* and *CYP3A5,* have a major impact on Tac metabolism; and (iii) that the multidrug transporter ABCC8 and the nucleoside carrier SLC28A3 appear to be involved in Tac metabolism.

ORIGINAL ARTICLE

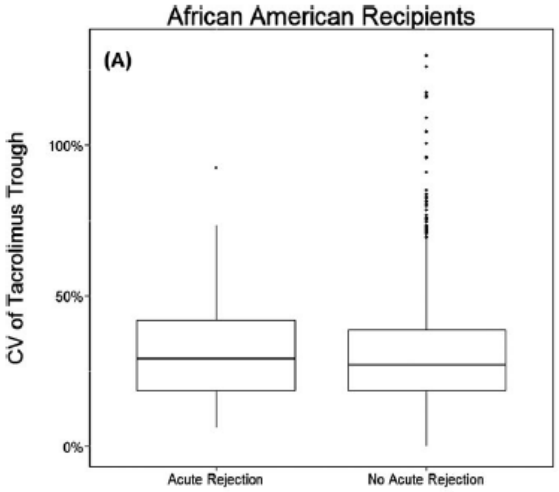
WILEY Clinical TRANSPLANTATION
The Journal of Clinical and Translational Research

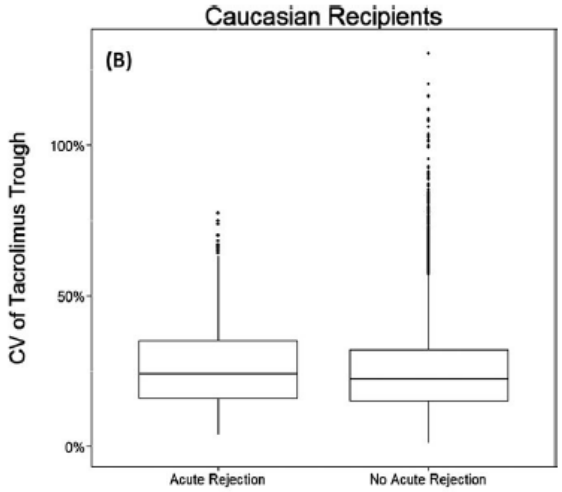
Tacrolimus trough and dose intra-patient variability and CYP3A5 genotype: Effects on acute rejection and graft failure in European American and African American kidney transplant recipients

Stephan R. Seibert[1] | David P. Schladt[2] | Baolin Wu[3] | Weihua Guan[3] | Casey Dorr[4] | Rory P. Remmel[5] | Arthur J. Matas[6] | Roslyn B. Mannon[7] | Ajay K. Israni[8] | William S. Oetting[9] | Pamala A. Jacobson[1]

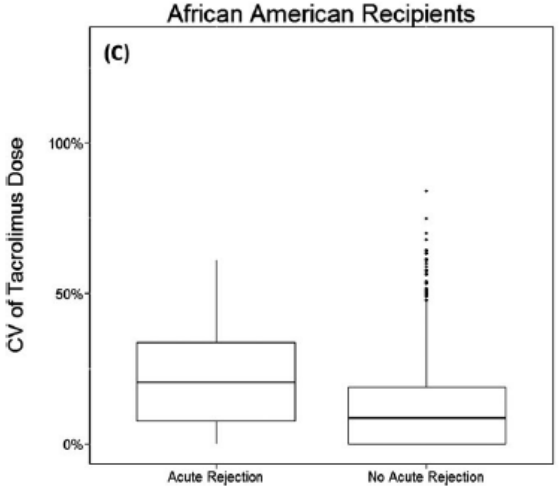
Clinical Transplantation. 2018;32:e13424.

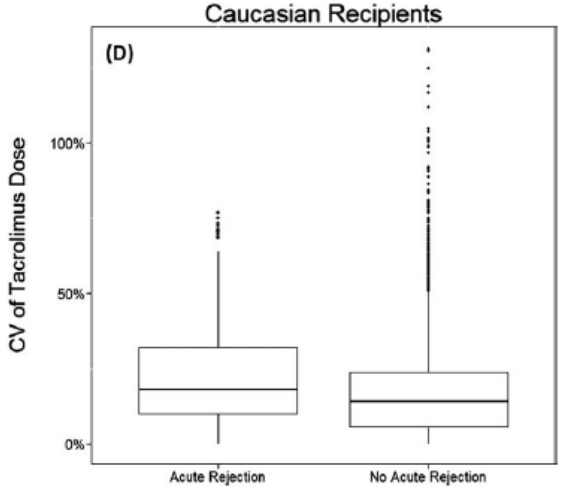
Tac Level





Tac Dose



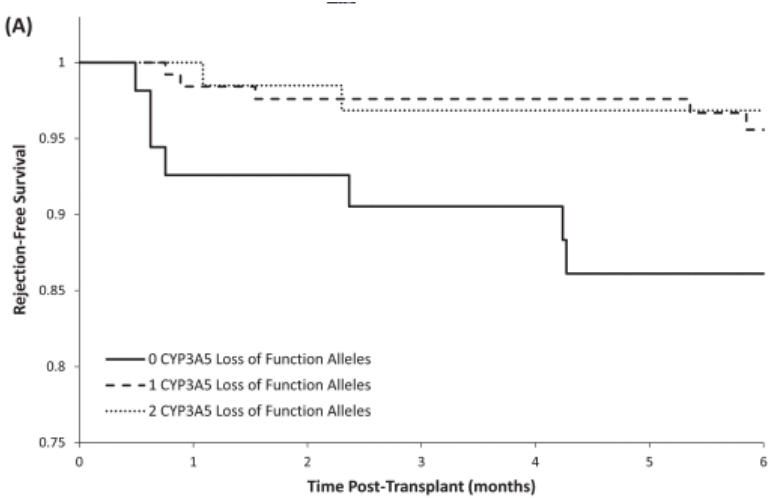


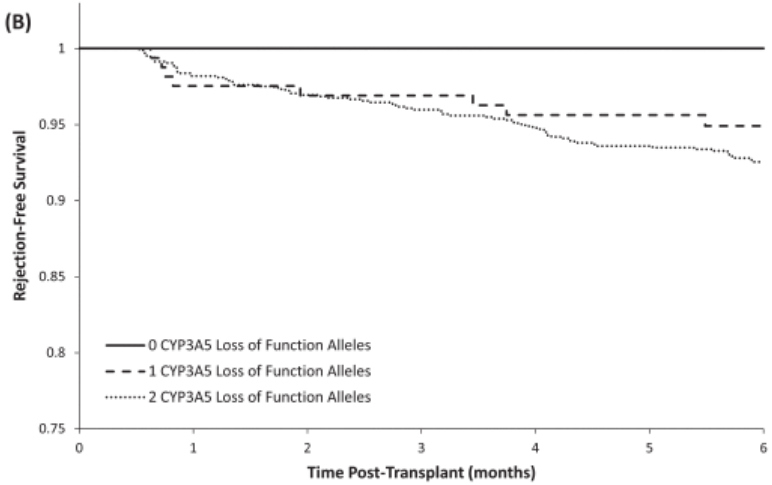
Variable	African American		European American	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
CV of TAC dose (highest quartile)[a]	33.53 (5.54-202.85)	0.0001	1.81 (1.14-2.86)	0.012
Number of CYP3A5 loss-of-function alleles	0.16 (0.05-0.49)	0.0015	1.51 (0.74-3.11)	0.26
No. of HLA mismatches				
1 or 2	0.30 (0.01-6.42)	0.85	3.15 (0.87-11.37)	0.0073
3 or 4	0.37 (0.04-3.71)		3.64 (1.11-11.96)	
5 or 6	0.39 (0.03-4.30)		5.95 (1.82-19.41)	
B- or T-cell crossmatch	3.01 (0.46-19.65)	0.25	2.25 (1.13-4.50)	0.022
Donor age at transplant	1.01 (0.97-1.06)	0.65	1.02 (1.00-1.04)	0.039

CV, Coefficient of Variation; HLA, Human Leukocyte Antigen; TAC, tacrolimus.
[a]Highest CV quartile is >19% for AA and >24% for EA. CV was calculated as described in methods for acute rejection.

African-American Better metabolizers had more rejx

Caucasian





Tac Dosing / Levels

- Seemingly simple
- Actually complex and outcomes are worse for minority of patients who require high doses of tac and may have more variability in achieved levels

Transplant International

REVIEW

Applying genomics in heart transplantation

Brendan J. Keating[1,2], Alexandre C. Pereira[3], Michael Snyder[4] & Brian D. Piening[4]

Transplant International 2018; 31: 278–290

Genetic Variation

- HLA Class I and 2: Chromosome 6
  - Most polymorphic regions of human genome
- HLA-G
- KIR – Family of 13 genes on chromosome 19
  - Educating / regulating NK cells to sense and respond to HLA Class I surface molecules
  - Involved in immune related diseases

Heart Transplant Matching

- HLA Matching is impractical with hearts given constraints on time
- Immunosuppresion has leveled playing field
- Anti-HLA antibodies are bigger issue
- Cross-Reactivities against HLA groups
- Surprising that outcomes are good despite complexity and universal mismatches

GWAS: Genome Wide Association Study

- Any study of genetic variation across the entire human genome that is designed to identify genetic associations with observable traits (such as blood pressure or weight), or the presence or absence of a disease (such as cancer) or condition

Potential of GWAS

- Hope to personalize medicine
- Compare whole genome with outcome(s) of interest and find SNPs which correlate with desired outcomes
- Find SNPs which are particularly deleterious

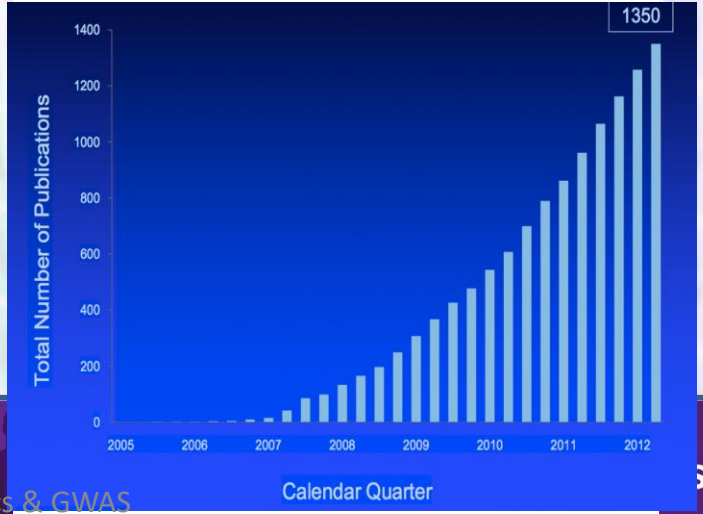
The genomics revolution

- **Sequencing technology**
  - 1977 – Sanger
  - 1995 – 1st bacterial genomes
    - < 10,000

    bases per day per machine
  - 2003 – 1st human genome
    - > 10,000,000,000,000

    bases per day per machine

- **GWAS publications**
  - 2005 – 1st GWAS
    - Age-related macular degeneration
  - 2014 – 1,991 publications
    - 14,342 associations

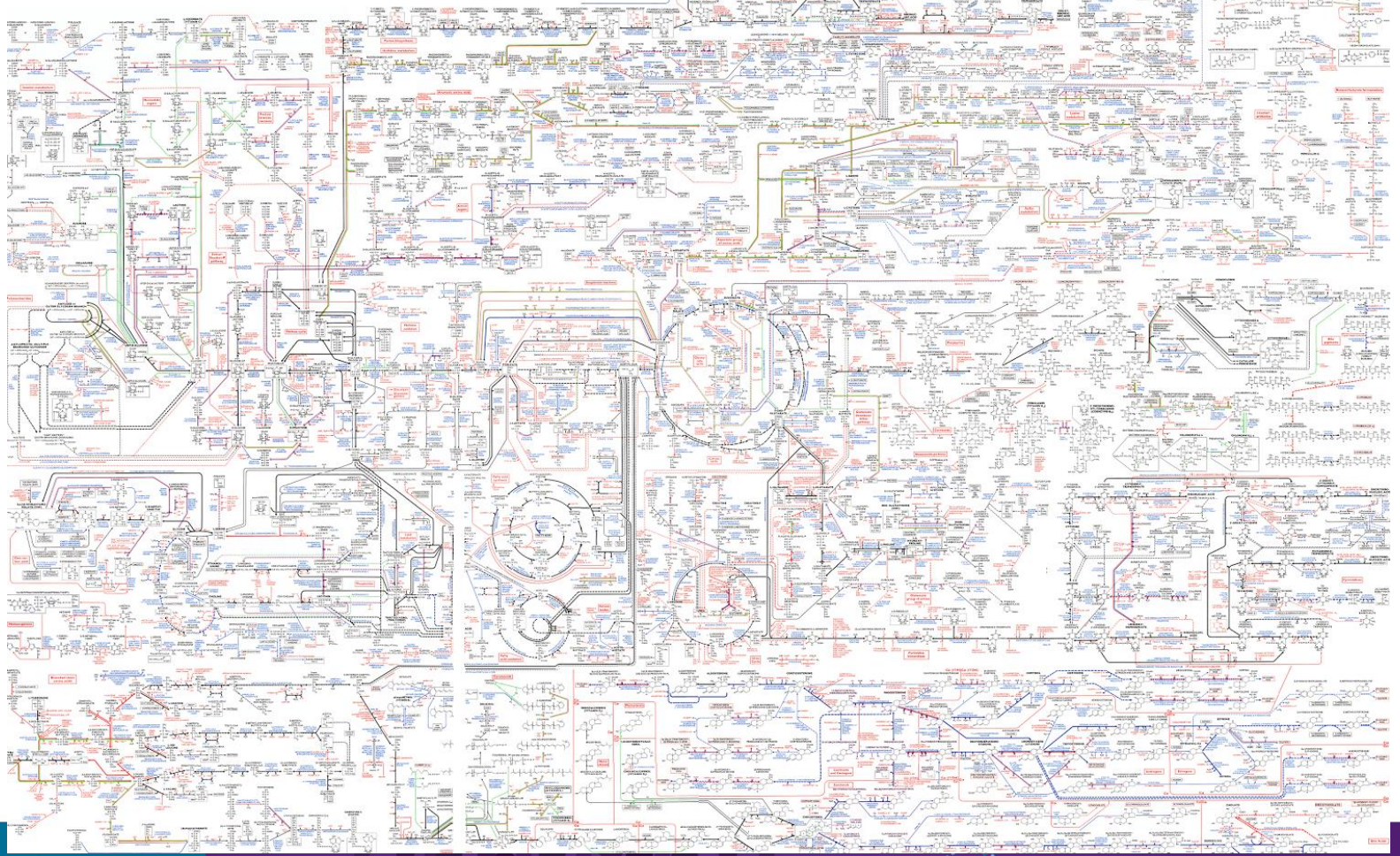


A few GWAS discoveries…


The case of the missing heritability

Why?

- Environment, Gene-Environment interactions
- Complex traits, small effects, rare variants
- Gene expression levels
- GWAS methodology?



Multivariate methods

Penalized Regression

LASSO penalized regression

The elastic net

Ridge regression

Neural Networks

Genetic programming optimized neural networks

Parametric decreasing method

Logic Trees

Logic feature selection

Monte Carlo Logic Regression

Logic regression

Modified Logic Regression-Gene Expression Programming

Bayesian Approaches

Bayesian partitioning

Bayesian Logistic Regression with Stochastic Search Variable Selection

Bayesian Epistasis Association Mapping

Genetic Programming for Association Studies

Set association approach

Factorial Methods

Multi-factor dimensionality reduction method

Sparse-PCA

Supervised-PCA

DAPC-based FS (snpzip)

Non-parametric Methods

Random forests

Restricted partitioning method

Combinatorial partitioning method

Odds-ratio-based MDR

Testing for Association


European Bioinformatics Institute [GB] | https://www.ebi.ac.uk/gwas/search?query=heart%20transplant
Error
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Diagram
Download
Documentation
About
EMBL-EBI
NIH
National Human Genome Research Institute
GWAS Catalog
The NHGRI-EBI Catalog of published genome-wide association studies
heart transplant
Examples: breast carcinoma, rs7329174, Yao, 2q37.1, HBS1L, 6:16000000-25000000
Feedback
GWAS / Search / heart transplant
Refine search results
Publications 1
Catalog stats
Last data release on 2019-01-11
3730 publications
70611 SNPs
89897 unique SNP-trait associations
Genome assembly GRCh38.p12
dbSNP Build 151
Ensembl Build 93
Search results for heart transplant
Genome Wide Association Study Reveals Novel Genetic Loci Associated With Change in Renal Function in Heart Transplant Recipients.
Asleh R et al. 2018 Clin Transplant PMID:30160337
Associations 5 Studies 1

GWAS in Heart Transplant

Received: 29 June 2018 | Revised: 13 August 2018 | Accepted: 23 August 2018

DOI: 10.1111/ctr.13395

ORIGINAL ARTICLE

WILEY Clinical TRANSPLANTATION
The Journal of Clinical and Translational Research

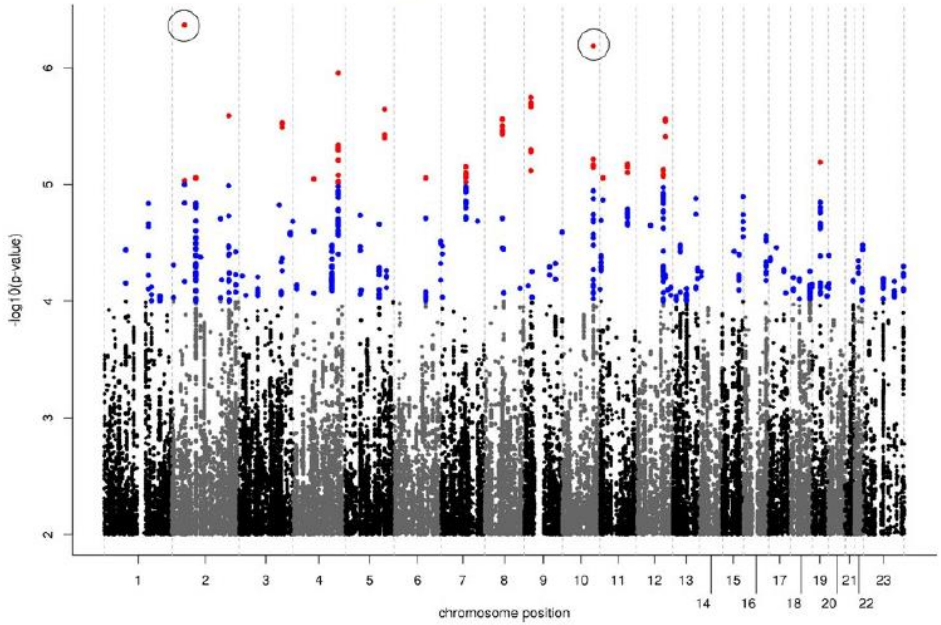
Genomewide association study reveals novel genetic loci associated with change in renal function in heart transplant recipients

Rabea Asleh[1] | David Snipelisky[2] | Matthew Hathcock[3] | Walter Kremers[3] | Duan Liu[4] | Anthony Batzler[3] | Gregory Jenkins[3] | Sudhir Kushwaha[1] | Naveen L. Pereira[1,4]

Clinical Transplantation. 2018;32:e13395.

Details

- 251 Heart transplant patients
- Genotyped for 314,903 SNPs
- Primary endpoint was change in GFR at 1 yr post transplant
- Found 3 significant variants
- 2 in long non-coding RNA gene LINC01121
- One in pseudogene BTBD7P2


-log10(p-value)
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chromosome position

Many SNP Associations

TABLE 3 Significant genetic polymorphisms associated with the change in renal function

Rs number	Chr.	Base pairs	MA	CA	MAF	β Co.	SE	P-value	t	dosR2	Gene	Gene ID	Variant location
rs17033285	2	45489633	T	A	0.09	17.28	3.33	4.30e-07	I	0.81	UNQ6975	400952	5′upstream
rs76427116	2	45477781	T	C	0.05	17.38	3.84	9.28e-06	I	0.90	UNQ6975	400952	Intron
rs4917601	10	113074200	T	A	0.15	11.64	2.28	6.46e-07	I	0.97	LOC100420392	100420392	5′upstream
rs4617520	10	113062340	C	T	0.12	11.95	2.58	6.07e-06	O		LOC100420392	100420392	5′upstream
rs7095911	10	113066263	G	A	0.13	11.01	2.39	6.75e-06	I	0.99	LOC100420392	100420392	5′upstream
rs11195513	10	113066650	C	T	0.13	11.01	2.39	6.77e-06	I	0.99	LOC100420392	100420392	5′upstream
rs4465313	10	113072148	G	A	0.13	10.92	2.37	6.79e-06	I	0.99	LOC100420392	100420392	5′upstream
rs7923594	10	113067280	G	A	0.13	10.99	2.39	6.95e-06	I	0.99	LOC100420392	100420392	5′upstream
rs4918638	10	113065820	C	G	0.14	10.67	2.33	7.13e-06	I	0.96	LOC100420392	100420392	5′upstream
rs9762450	4	165022251	C	A	0.24	8.70	1.74	1.11e-06	I	0.97	MARCH1	55016	Intron
rs77044648	4	165029280	A	C	0.27	8.00	1.71	4.60e-06	I	0.97	MARCH1	55016	Intron
rs11735194	4	165034085	A	G	0.27	7.96	1.70	4.85e-06	I	0.97	MARCH1	55016	Intron
rs17475702	4	165034536	A	G	0.27	7.96	1.70	4.86e-06	I	0.97	MARCH1	55016	Intron
rs17579154	4	165035111	T	A	0.27	7.95	1.70	4.91e-06	I	0.98	MARCH1	55016	Intron
rs10517799	4	165041073	C	T	0.27	7.92	1.70	5.10e-06	I	0.98	MARCH1	55016	Intron
rs4691111	4	165045284	C	G	0.27	7.82	1.69	6.19e-06	I	0.99	MARCH1	55016	intron
rs34291409	4	165034367	T	A	0.24	7.96	1.75	8.35e-06	I	0.97	MARCH1	55016	Intron
rs13146038	4	165030102	G	T	0.24	7.92	1.75	9.35e-06	I	0.97	MARCH1	55016	Intron
rs13135028	4	165046631	C	G	0.25	7.85	1.74	9.66e-06	I	0.99	MARCH1	55016	Intron
rs12057071	9	23759368	C	A	0.11	13.14	2.68	1.79e-06	I	0.93	ELAVL2	1993	Intron
rs13294337	9	23761695	G	A	0.11	13.04	2.68	2.00e-06	I	0.94	ELAVL2	1993	Intron
rs1431304	9	23768971	T	A	0.11	12.93	2.66	2.07e-06	I	0.95	ELAVL2	1993	Intron
rs2891188	9	23756299	C	T	0.11	13.17	2.71	2.16e-06	I	0.91	ELAVL2	1993	Intron
rs7024224	9	23779696	T	C	0.11	12.25	2.63	5.06e-06	I	0.97	ELAVL2	1993	Intron
rs10966079	9	23781824	C	T	0.11	12.20	2.62	5.27e-06	I	0.97	ELAVL2	1993	Intron
rs10966081	9	23783743	G	A	0.11	12.07	2.64	7.58e-06	I	0.97	ELAVL2	1993	Intron
rs918378	5	143201705	G	A	0.08	14.18	2.93	2.27e-06	O		HMHB1	57824	3′downstream
rs10463361	5	143207881	G	A	0.09	13.63	2.88	3.75e-06	I	0.99	HMHB1	57824	3′downstream
rs72795604	5	143205918	C	T	0.09	13.60	2.88	3.88e-06	I	0.99	HMHB1	57824	3′downstream
rs11167832	5	143204776	C	T	0.09	13.58	2.88	4.00e-06	I	0.99	HMHB1	57824	3′downstream

Chr, chromosome; MA, mutant allele; CA, control allele; β Co., β-coefficient; SE, standard error; I, imputed; O, observed; DosR2, represents the quality of the imputation performed with values ≥0.80 represent high quality.

Only Kidney Tx GWAS

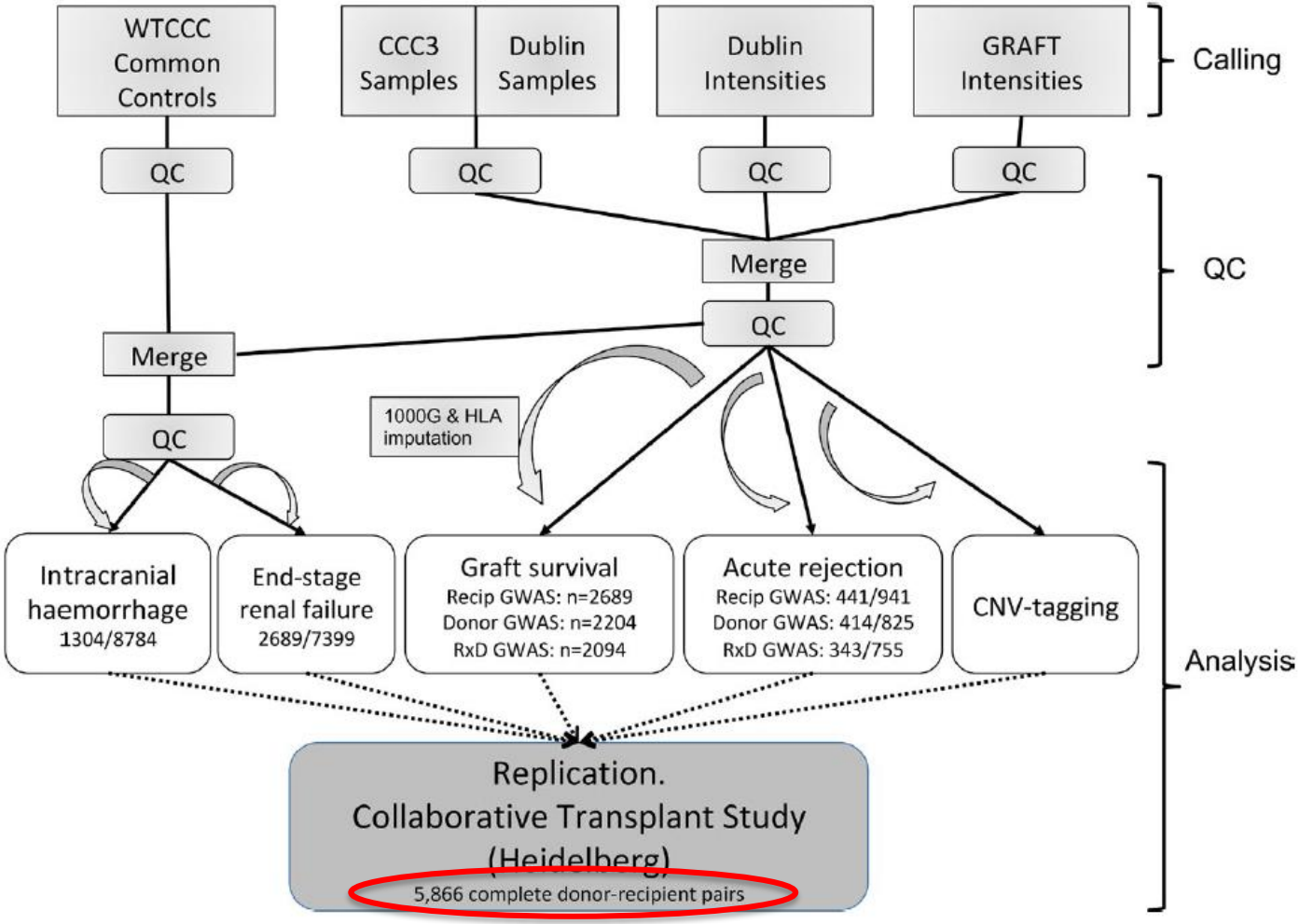
ORIGINAL ARTICLE

AJT

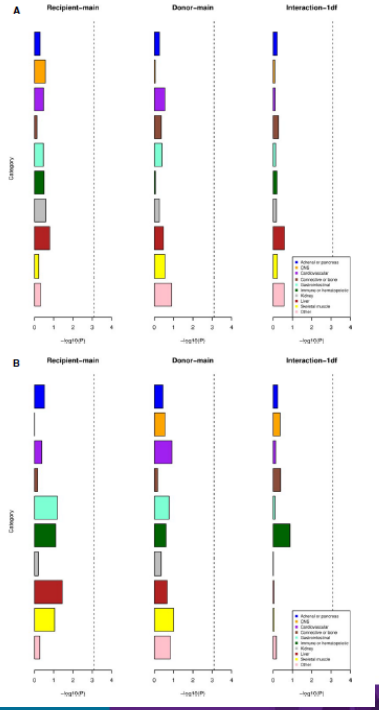
Long- and short-term outcomes in renal allografts with deceased donors: A large recipient and donor genome-wide association study

Maria P. Hernandez-Fuentes[1] | Christopher Franklin[2] | Irene Rebollo-Mesa[1] |

Am J Transplant. 2018;18:1370–1379.

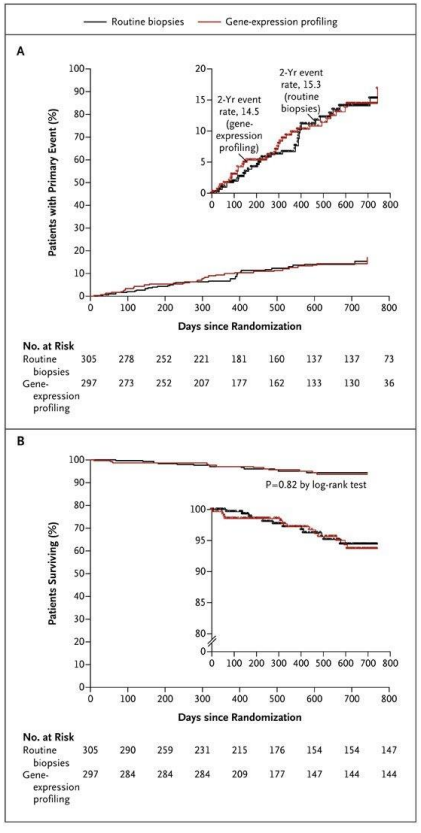

WTCCC Common Controls
CCC3 Samples
Dublin Samples
Dublin Intensities
GRAFT Intensities
Calling
QC
QC
QC
QC
QC
Merge
QC
Merge
QC
1000G & HLA imputation
Intracranial haemorrhage
1304/8784
End-stage renal failure
2689/7399
Graft survival
Recip GWAS: n=2689
Donor GWAS: n=2204
RxD GWAS: n=2094
Acute rejection
Recip GWAS: 441/941
Donor GWAS: 414/825
RxD GWAS: 343/755
CNV-tagging
Analysis
Replication.
Collaborative Transplant Study
(Heidelberg)
5,866 complete donor-recipient pairs

Results



- GWAS failed to show heritable component to explain rejection or graft survival
- Number of patients too small and outcomes are multifactorial

Genomics to Detect Rejection: IMAGE



- 602 pts
- 297 with GEP, 305 with Biopsy surveillance
- Allomap assay uses expression of 11 genes
- GEP non-inferior to Bx

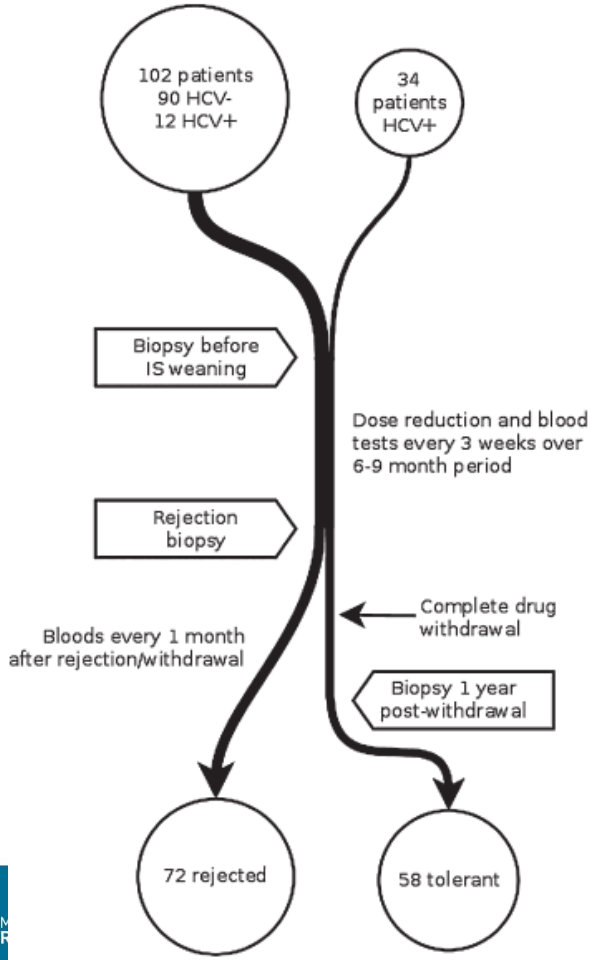
Pham MX et al N Engl J Med. 2010 May 20;362(20):1890-900

Molecular Characterization of Acute Cellular Rejection Occurring During Intentional Immunosuppression Withdrawal in Liver Transplantation

E. Bonaccorsi-Riani[1,†], A. Pennycuick[1,†], M.-C. Londoño[2], J.-J. Lozano[3], C. Benítez[2], B. Sawitzki[4], M. Martínez-Picola[2], F. Bohne[5], M. Martínez-Llordella[1], R. Miquel[1], A. Rimola[2] and A. Sánchez-Fueyo[1,2,*]

but not HCV-positive, patients. Changes were detectable 1–2 mo before rejection was diagnosed. Our results provide insight into the molecular processes underlying acute cellular rejection in liver transplantation and help clarify the potential utility and limitations of transcriptional biomarkers in this setting.

American Journal of Transplantation 2016; 16: 484–496



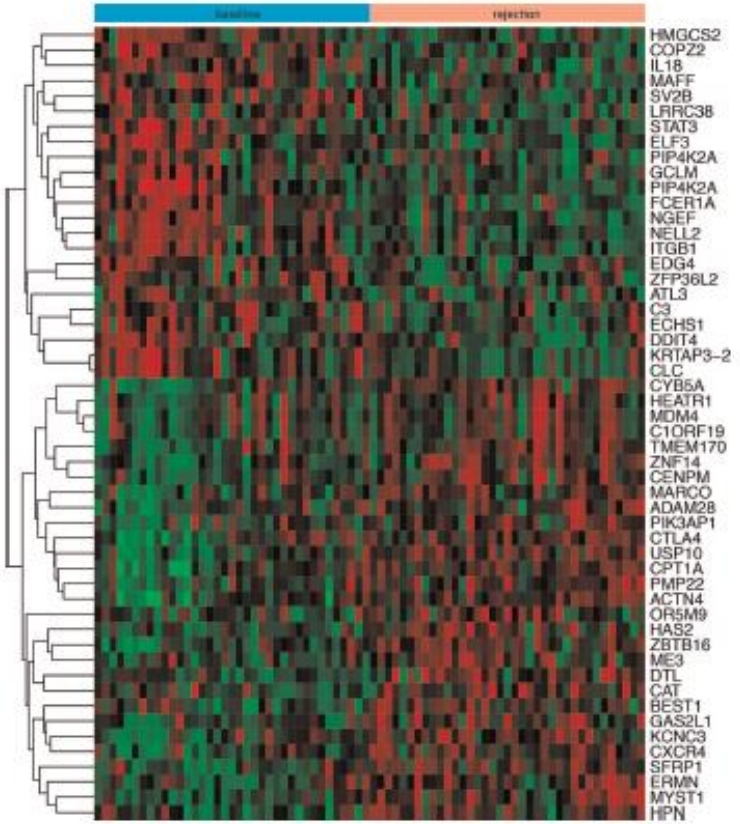
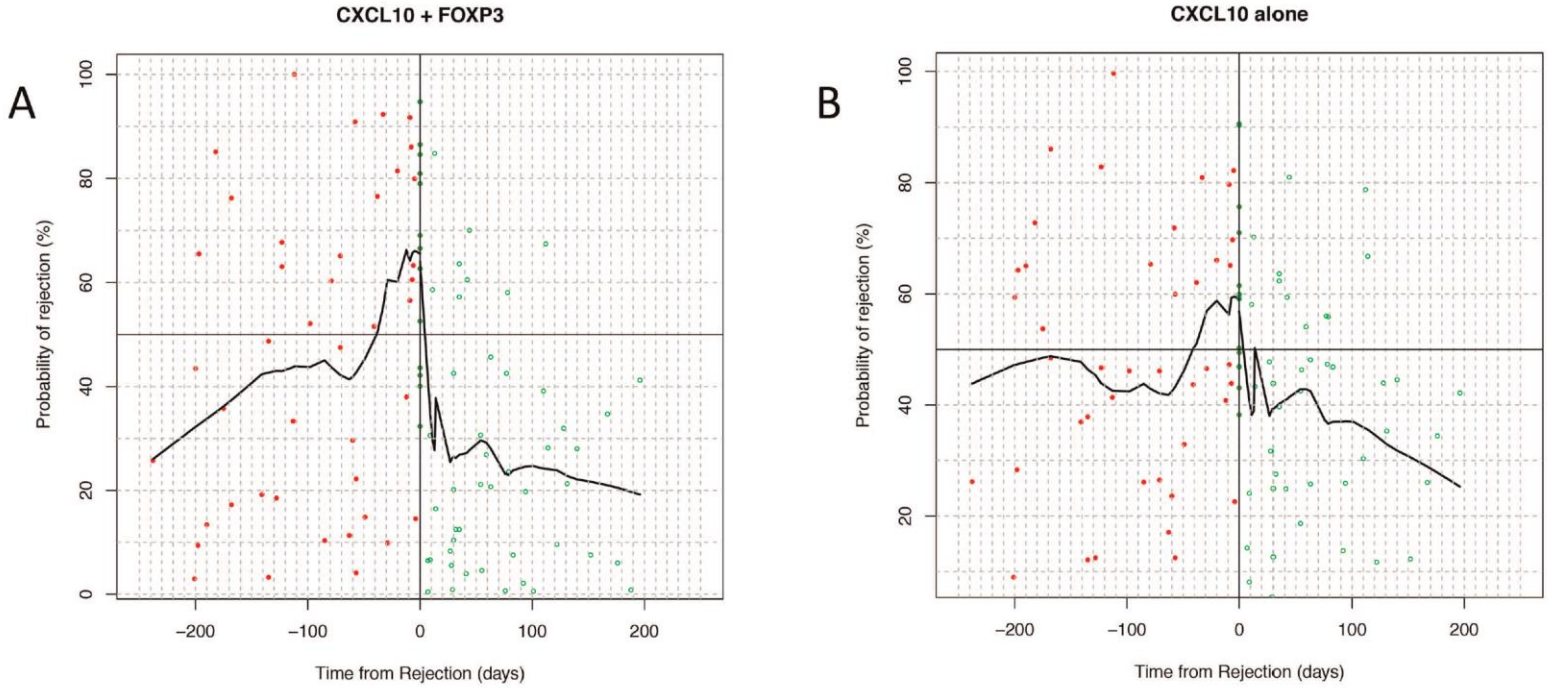


Figure 4: Differentially expressed genes in whole blood. Heat map of the top 50 genes differentially expressed in whole blood based on t-statistic comparing paired baseline (preweaning) and rejection samples. All patients were negative for hepatitis C virus.

Genetic Markers Precede Rejx (Liver)



Conclusion

- Genomics has progressed tremendously
- Improved understanding of problems such as drug metabolism
- Few approaches like Allomap have been successful
- Genomics unlikely to replace other methods of organ surveillance long term