

**AIBT Summer School**  
**Ercolano, 13-14 giugno 2019**

# **BIOMARCATORI NON INVASIVI DI RIGETTO DEL TRAPIANTO**

**Silvia Deaglio, MD, PhD**

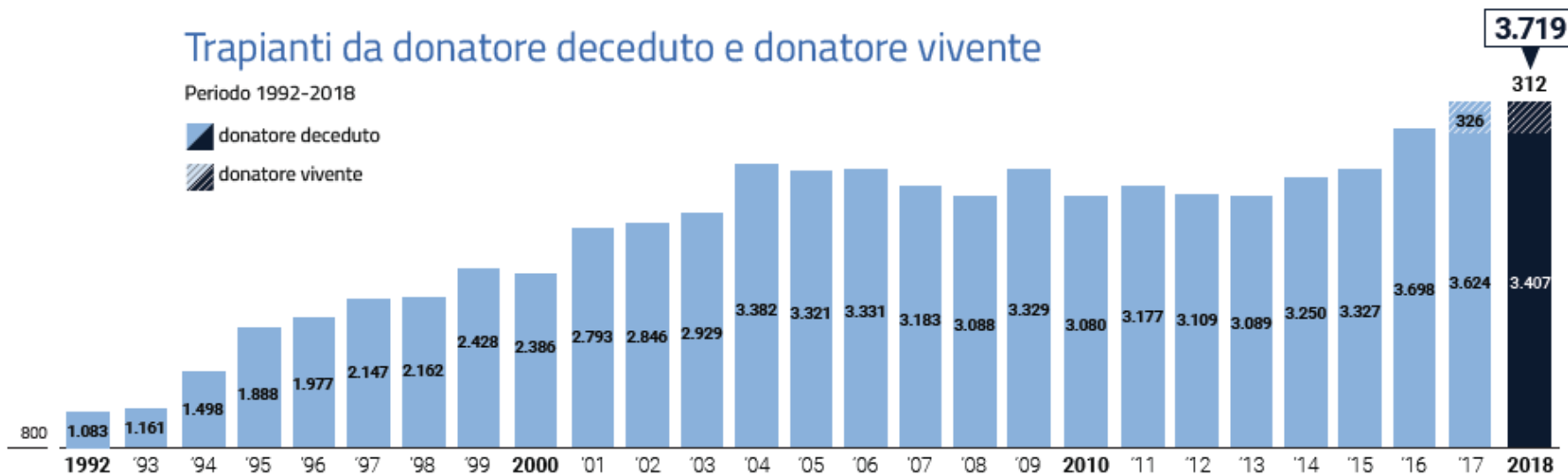


# Solid organ transplantation activity in Italy

## Trapianti da donatore deceduto e donatore vivente

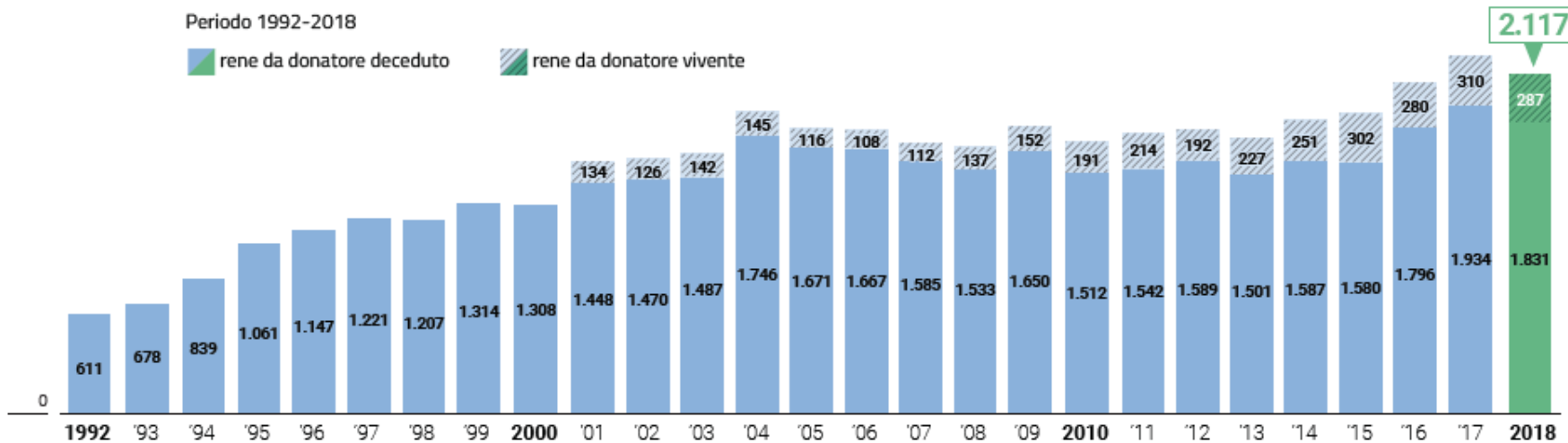
Periodo 1992-2018

■ donatore deceduto  
▨ donatore vivente



Periodo 1992-2018

■ rene da donatore deceduto    ▨ rene da donatore vivente



Source: CNT

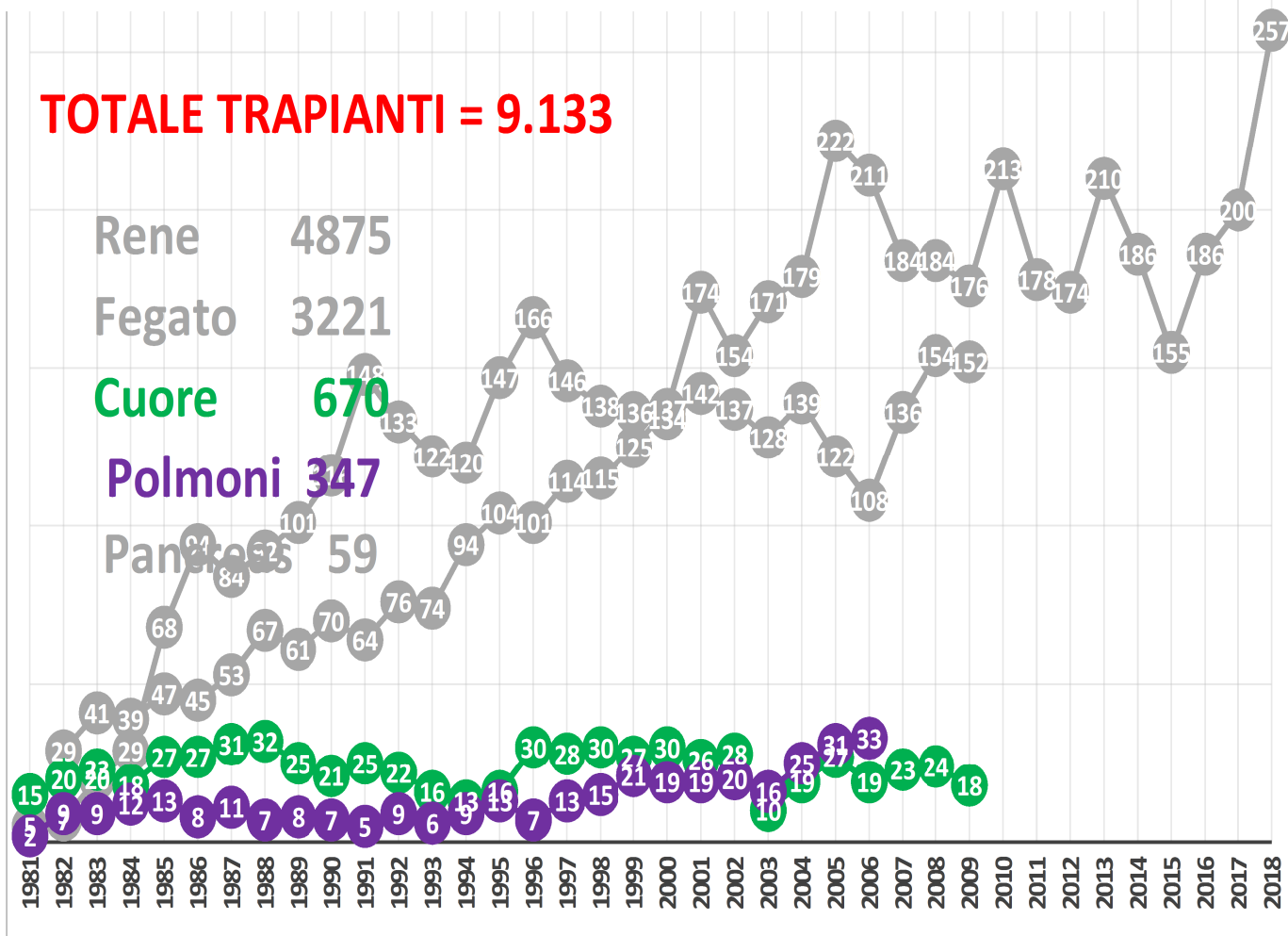
# Solid organ transplantation activity in Piedmont

## Attività di trapianto in Piemonte 2018

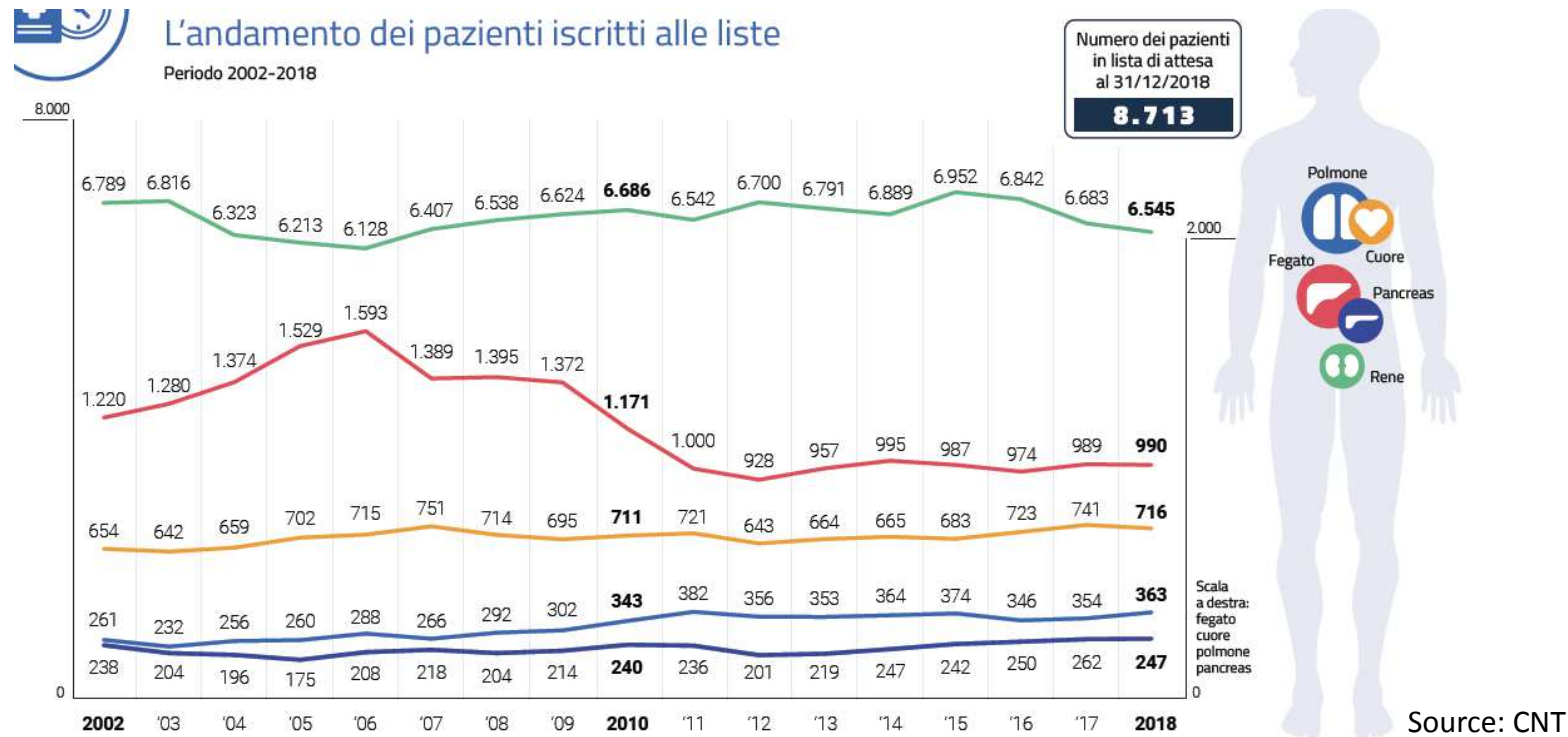
(inclusi i trapianti combinati ed i trapianti da donatore vivente)



**TOTALE TRAPIANTI = 9.133**



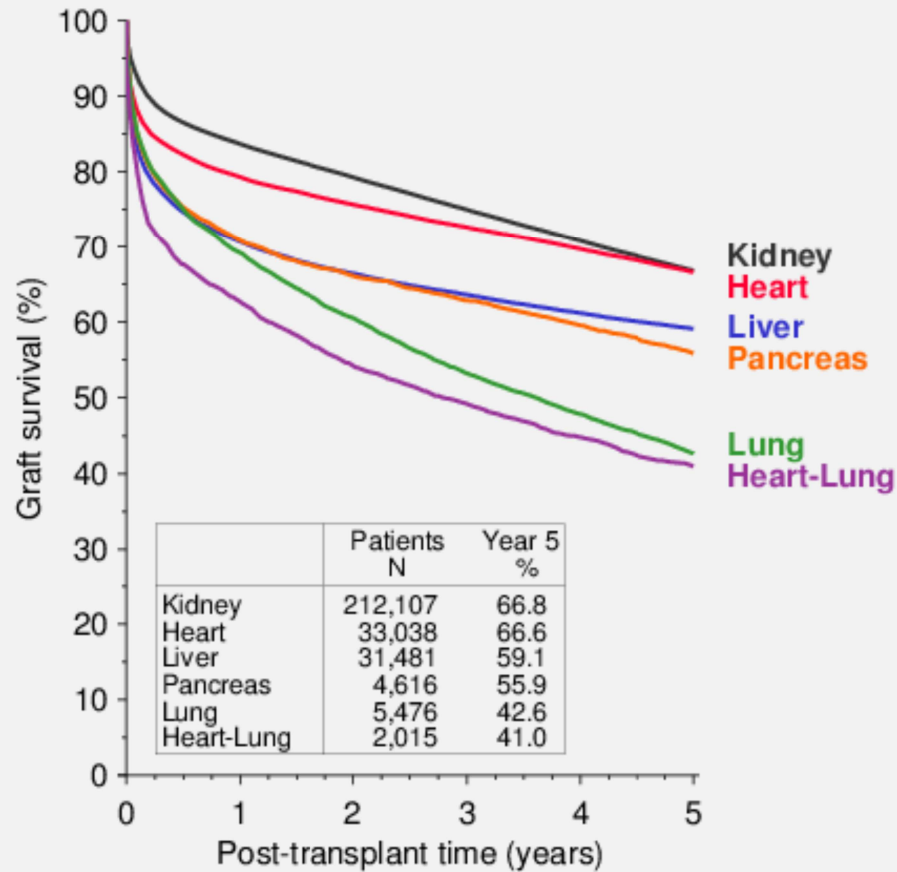
# Open issues in transplantation: the gap between supply and demand



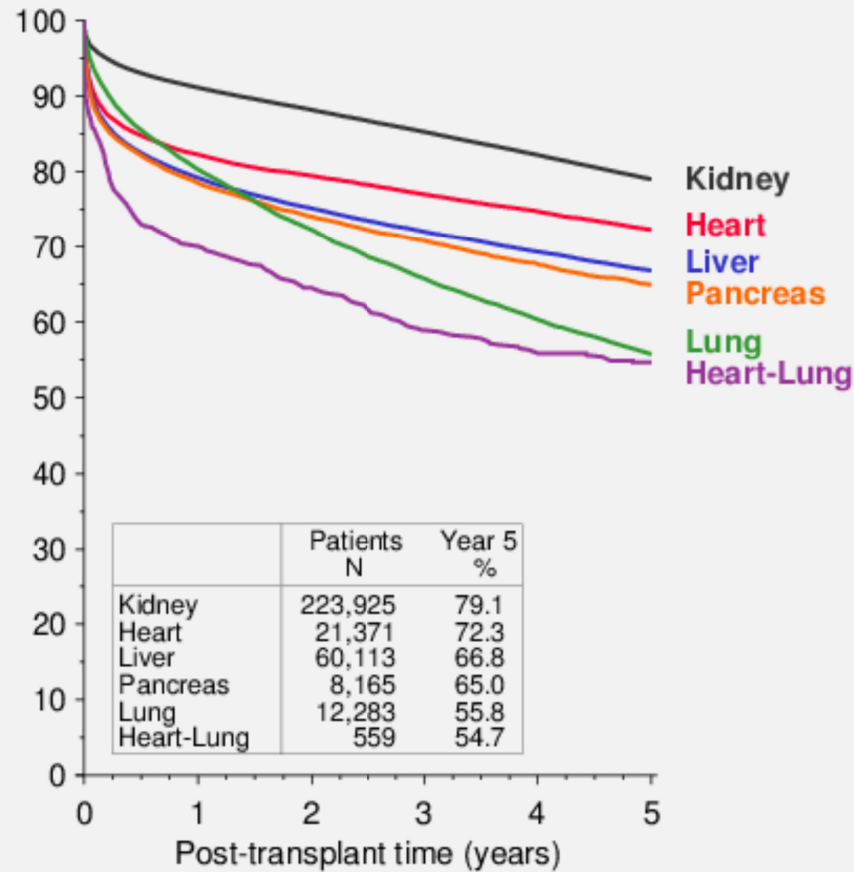
- Three most important issues in solid organ transplantation today are as follows:
  - ✓ need for more organs
  - ✓ longer graft survival rates
  - ✓ reduction of rates of rejection.

# Rejection is the major cause for loss of graft survival

1985–1999

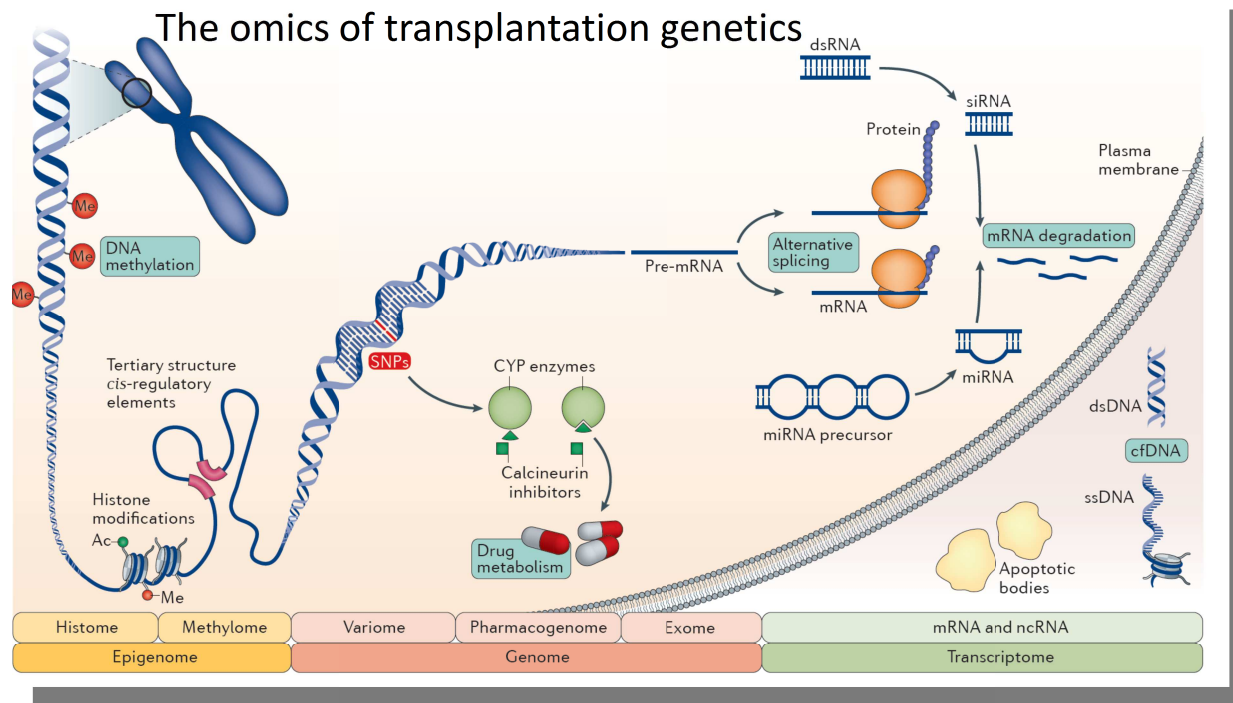


2000–2015



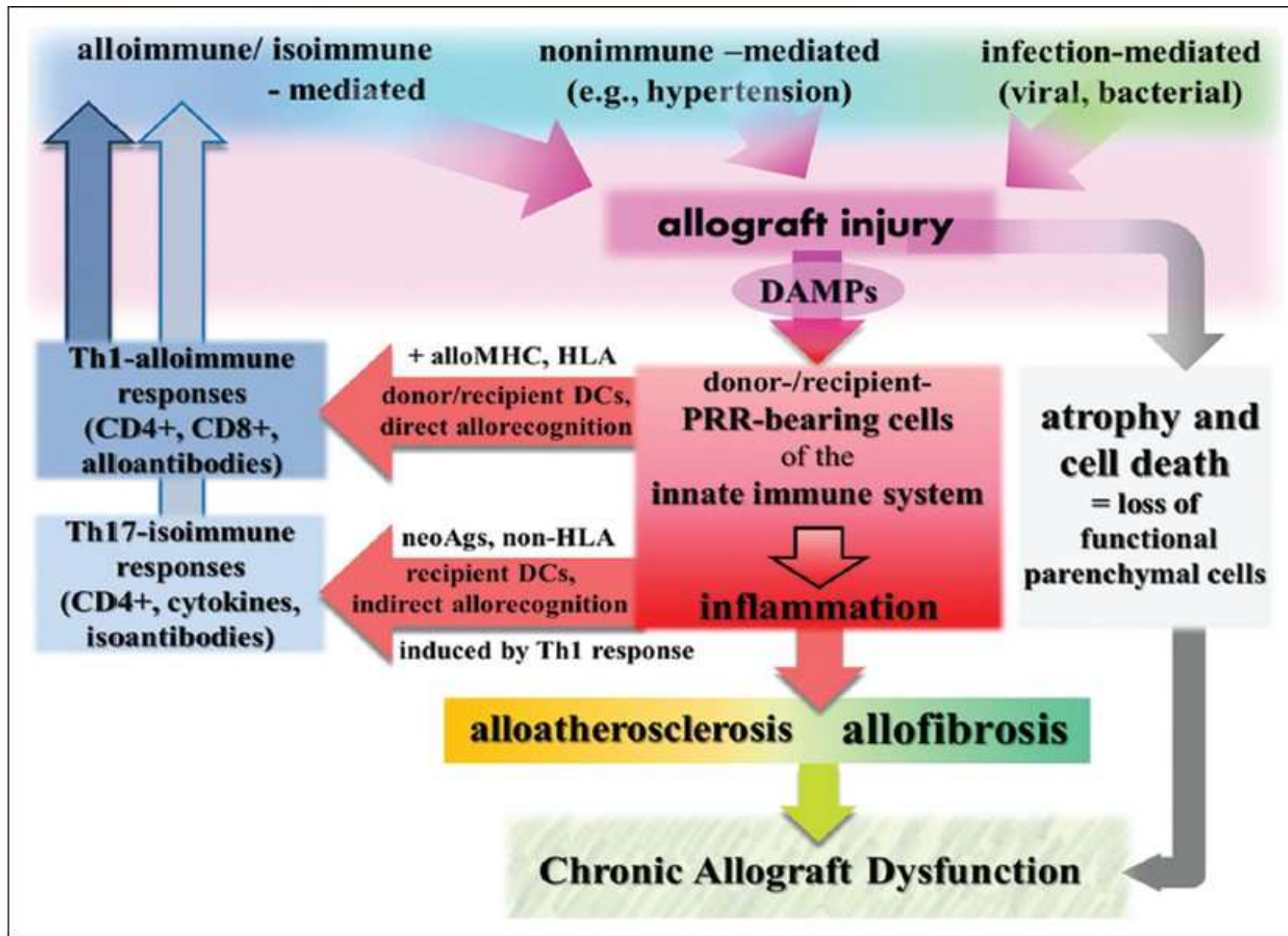
# What causes rejection?

- Despite the 'man-made' nature of organ transplantation, much remains to be understood about the combination of factors that leads to graft dysfunction or tolerance

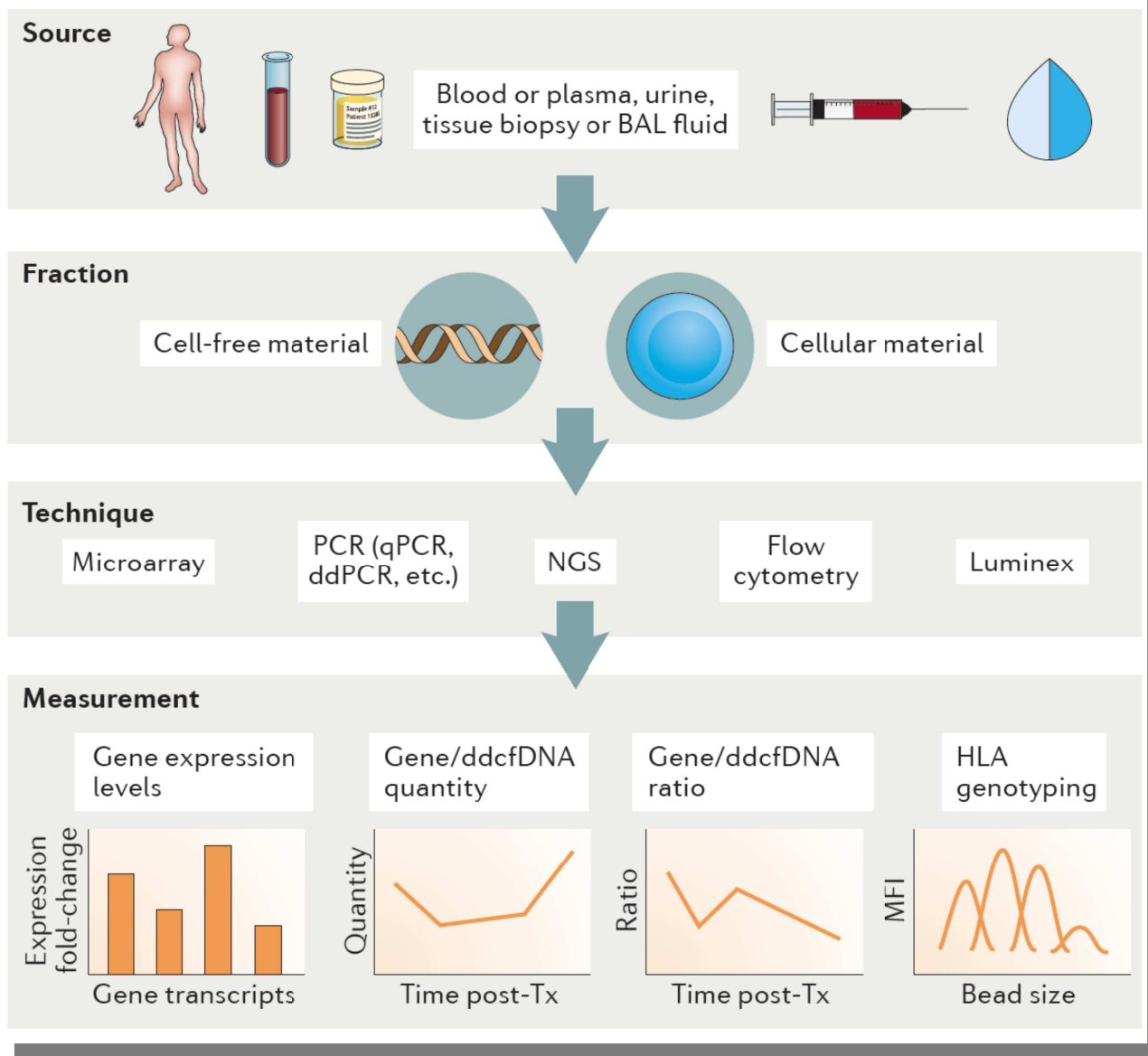


- Both the donor and the recipient genomes contribute to the diversity of the types and modifications of nucleic acids that are relevant to transplantation genetics.

# What causes rejection?



# How can we study rejection?



- By analysing a variety of different biological samples from different sites, both cellular and acellular, of a single individual, many different biomarkers that are associated with graft outcome can be measured.
- These can be measured directly from a biopsy sample of the graft, peripheral blood and plasma, or — in the case of specific transplants — from urine (for kidney transplants) and bronchoalveolar lavage (BAL) fluid (for lung)

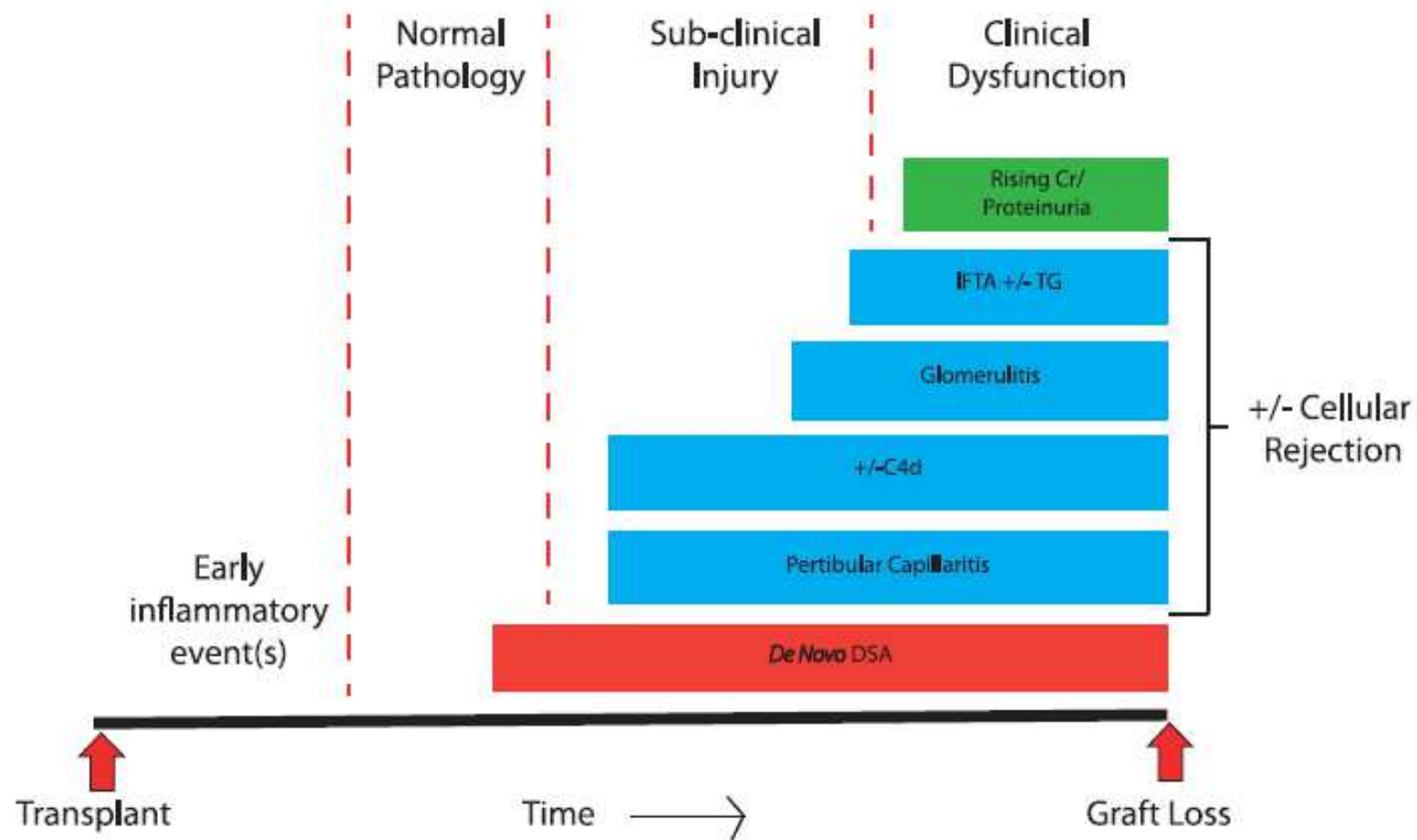
# What is a biomarker?

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A characteristic that is objectively measured and evaluated as an indicator of a normal biological process, pathologic process, or pharmacologic responses to a therapeutic intervention

- ✓ Non invasive
- ✓ Easily obtainable
- ✓ Measurable with standardized assays
- ✓ Fast results
- ✓ Reasonable costs

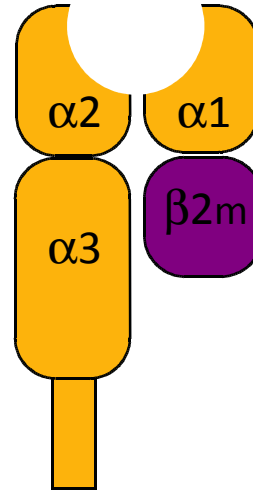
# DSA in transplantation



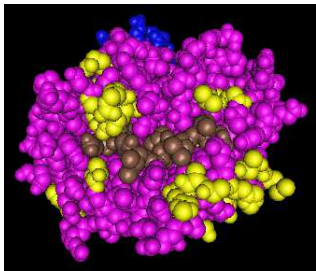
Proposed model for patients developing *de novo* DSA as they evolve from transplantation to graft failure.

# HLA matching in transplantation

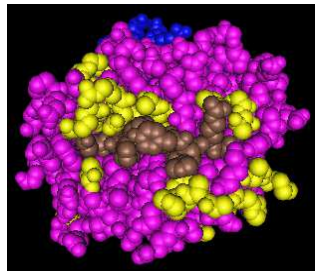
- ✓ Allelic variations in MHC genes underpin histocompatibility in transplantation, and HLA-A, HLA-B and HLA-DR are recognized to have the greatest importance for successful HLA matching



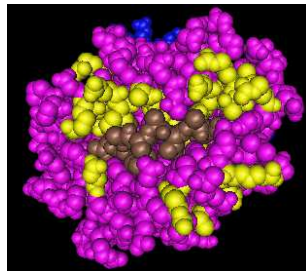
HLA-A2



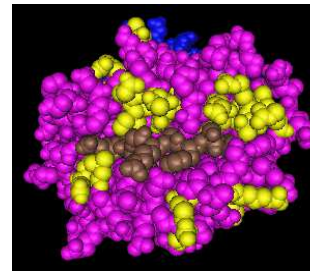
HLA-A68



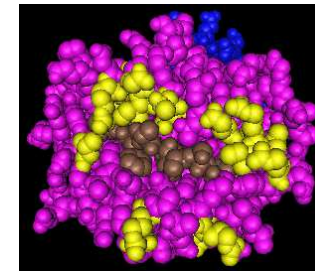
HLA-B27



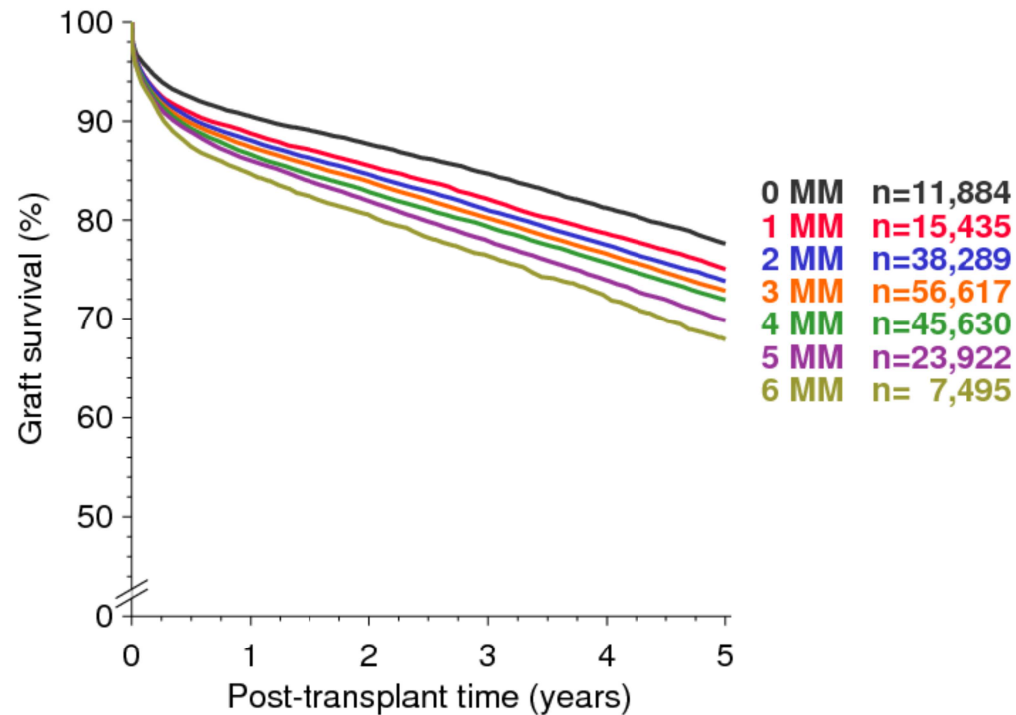
HLA-B44



HLA-B51



# Role of HLA mismatch in kidney transplant rejection



✓ Even patients who receive HLA-identical transplants can undergo acute or chronic rejection, suggesting a role for non-HLA factors in alloimmunity

Graft loss at 10 years among recipients of cadaveric organs is caused by:

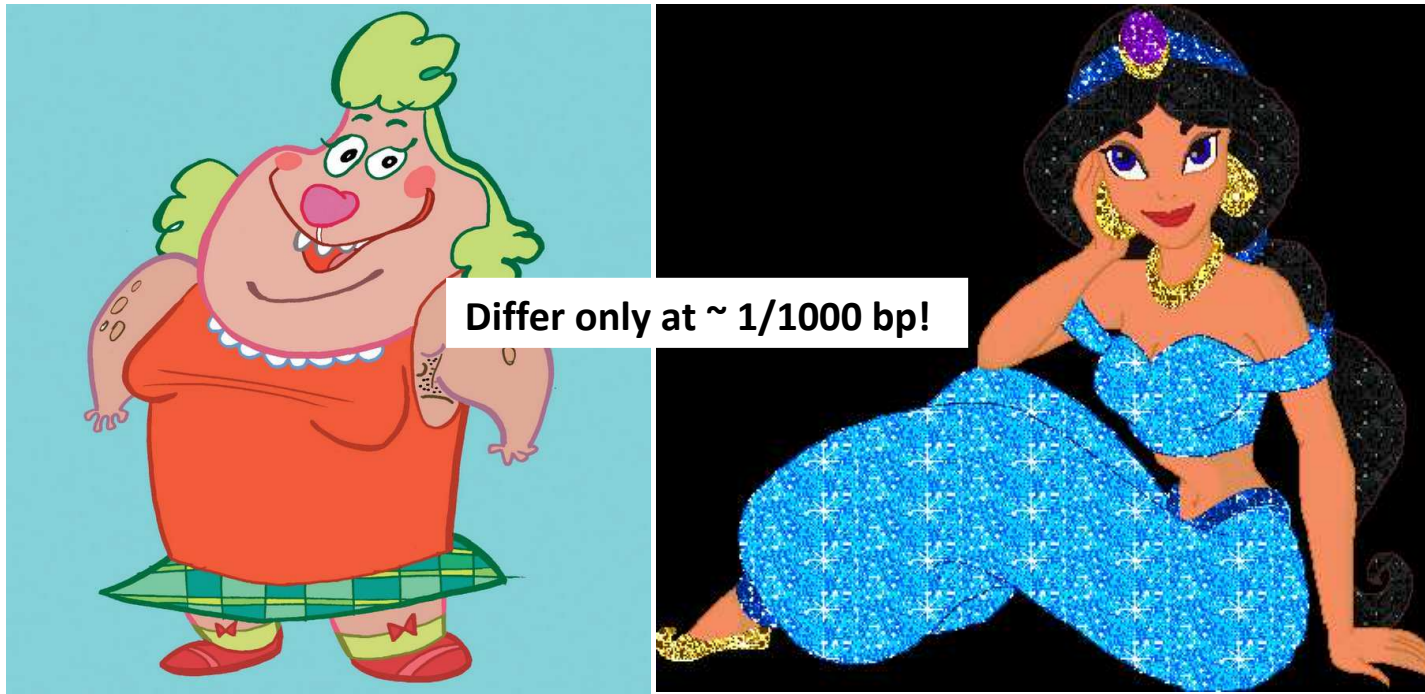
- ✓ HLA-associated factors (18%)
- ✓ **non-HLA factors (38%)**
- ✓ 43% by non-immunological factors

# How do we identify genetic predisposition to rejection beyond HLA genes?

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- ✓ Exploiting genetic variability
- ✓ If there is recognition and rejection there must be a difference between donor and recipient;
- ✓ Humans are 99.6% identical at the sequence level
- ✓ Evolutionary perspective:
  - H. Sapiens is a young species with a small founding population (~10,000)
  - Similarity with our relatives
    - 70-90% identity with mouse
    - 98.5% identity with chimp

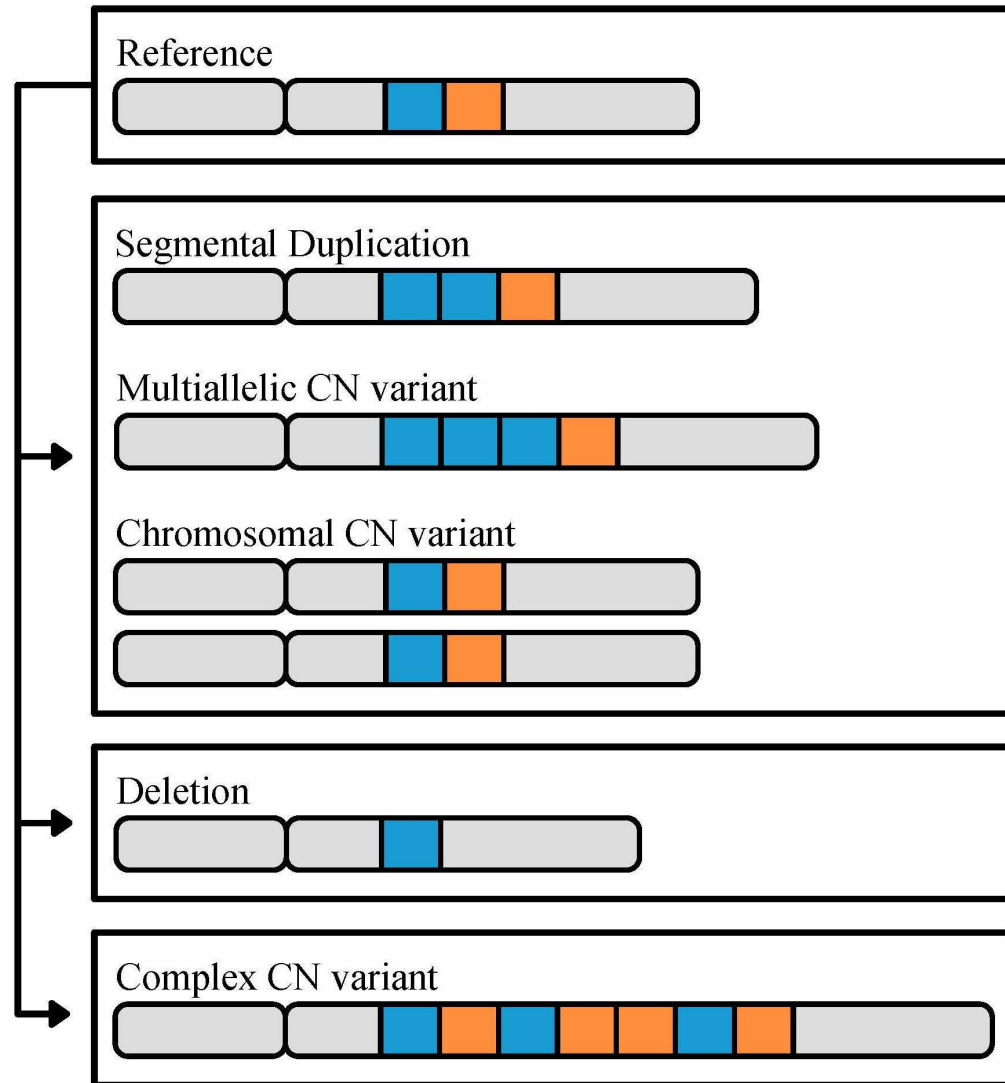
# Where is genetic variability?



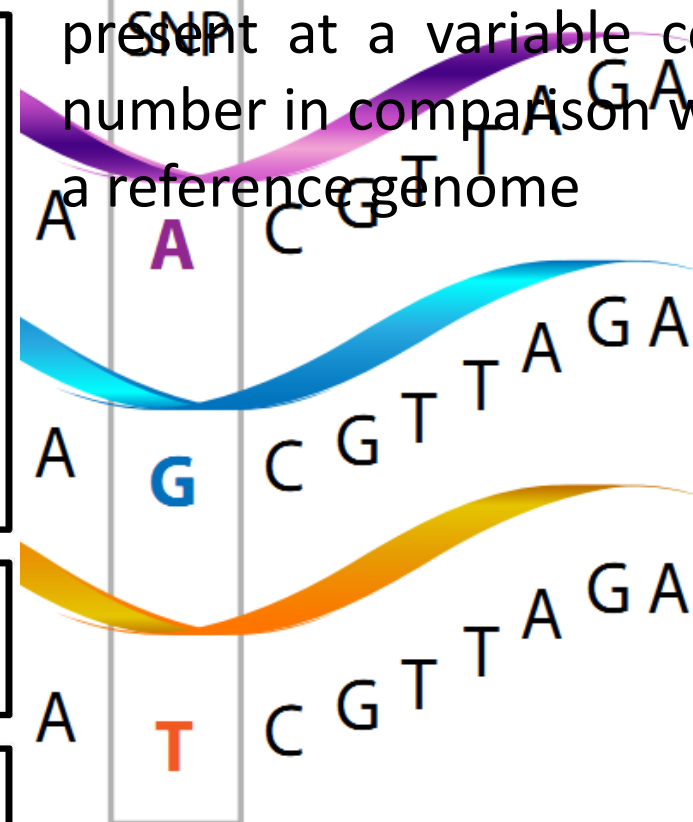
Where is genetic difference?

- Insertions / deletions – «indels» ~ 10%
- Length polymorphism ~ 5%
- Single nucleotide polymorphisms (SNP) ~ 45%
- Copy number variants ~ 40%
- Inversions ?
- RECOMBINATION (reshuffling at each generation)

# How do we identify genetic predisposition to rejection beyond HLA genes?



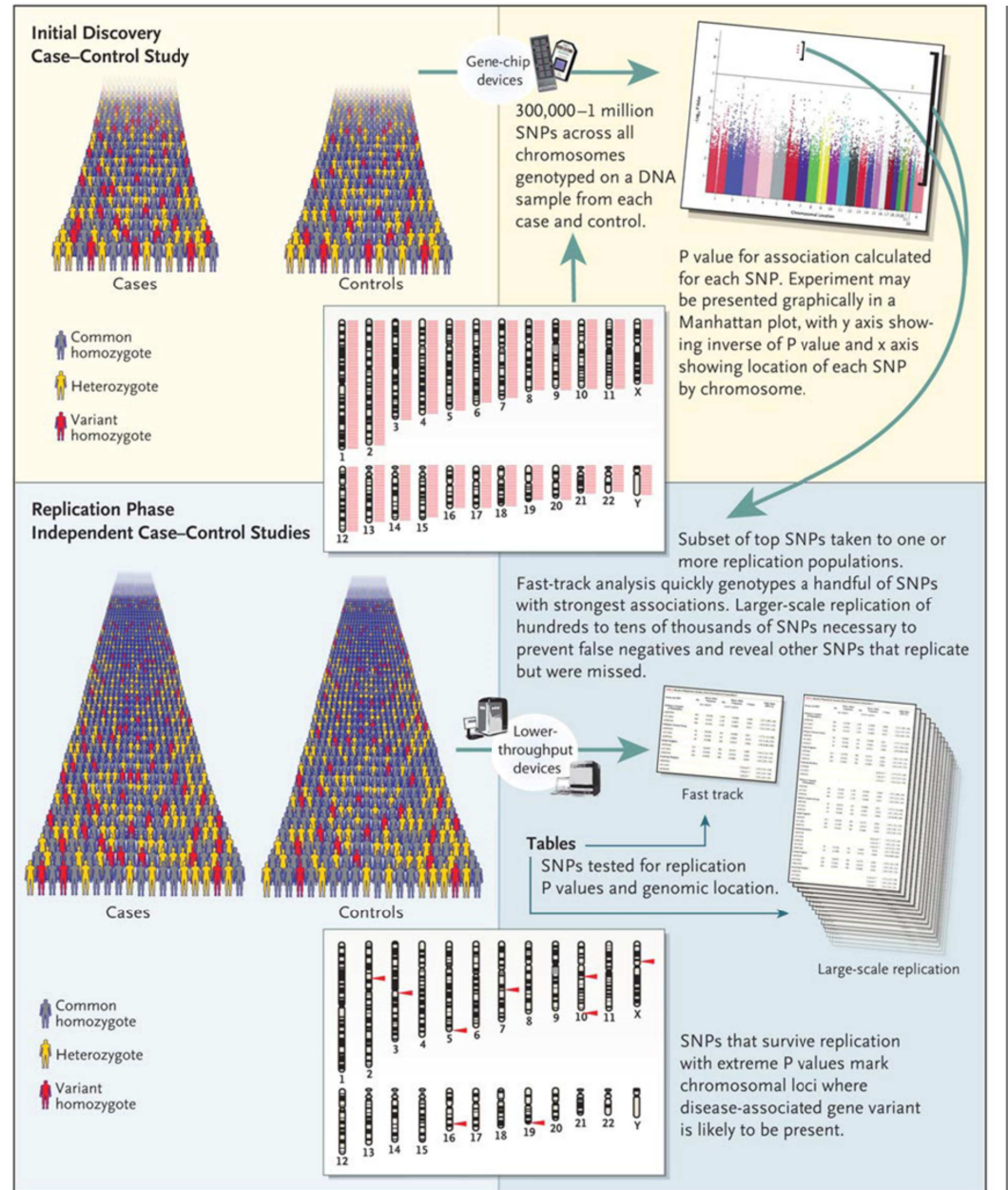
CNV: a segment of DNA that is 1 kb or larger and present at a variable copy number in comparison with a reference genome



approximately every 1,000 bp...

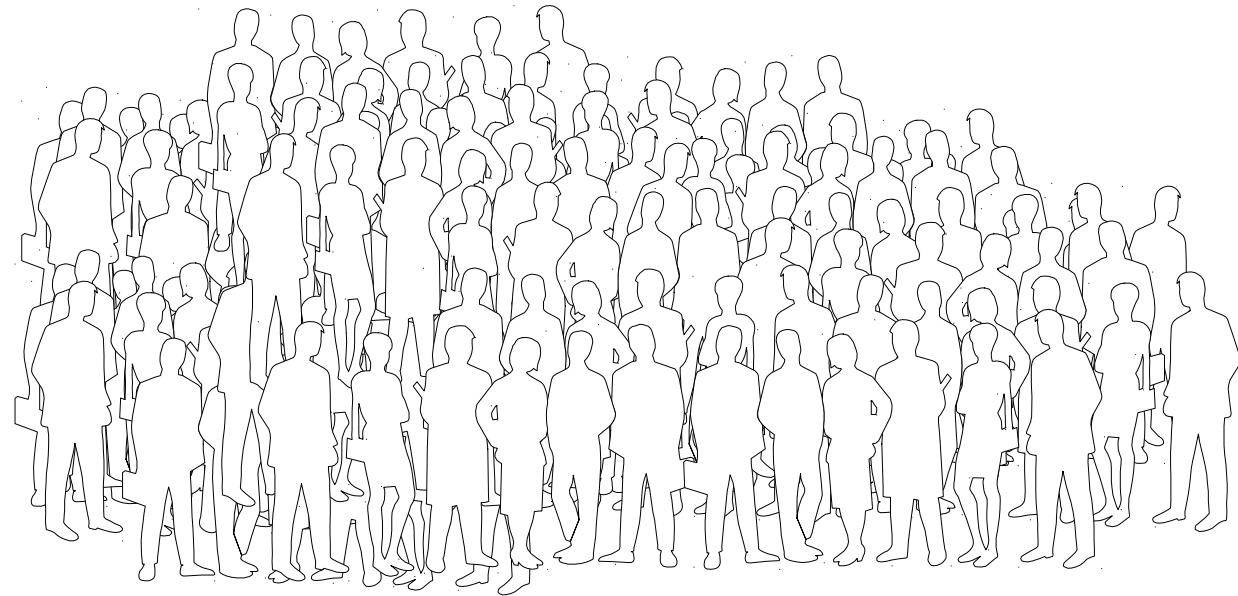
# The principle of GWAS

Genome-wide association studies (GWAS) allow the characterization of hundreds of thousands to several million single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) across the human genome rapidly and inexpensively, so using these advantages we soon might have - in an unbiased manner - new reliable discoveries related to **non-HLA polymorphisms** that influence the graft outcome.



# Genome-wide association studies

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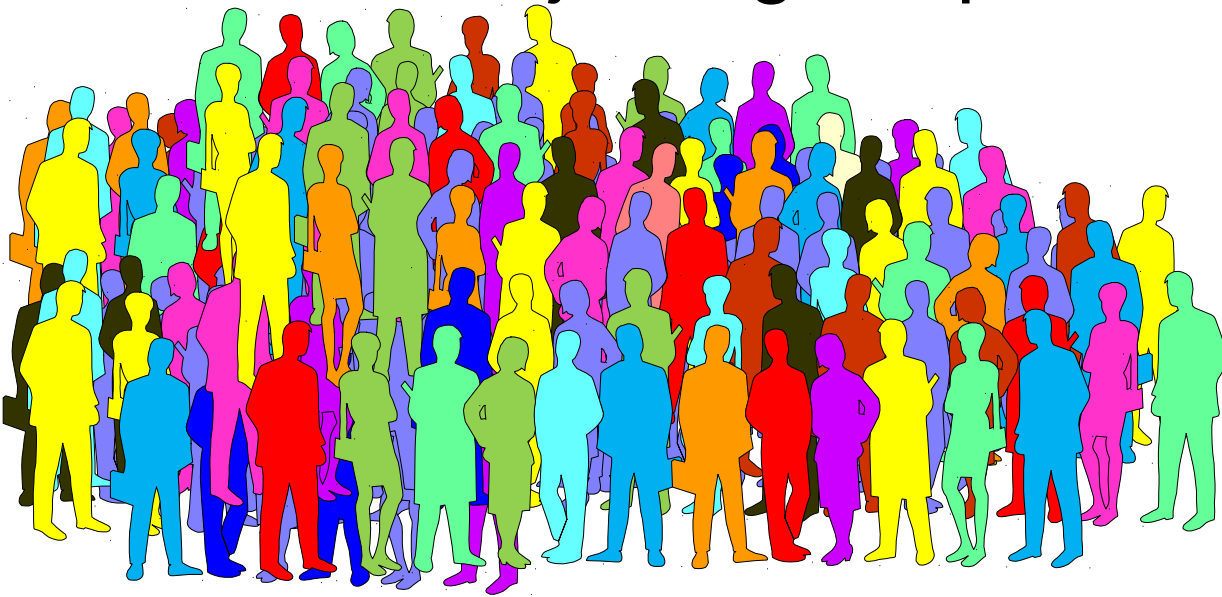


**Non rejecting**

**Rejecting recipients**

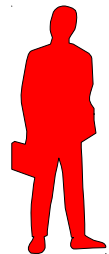


# Non rejecting recipients



5/100

# Rejecting recipients



90/100



# Determining relative risk

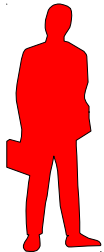
Non rejecting



5/100

Relative risk (RR) is calculated by dividing the frequency of the SNP or CNV in the patient population by the frequency in the general population

$$\text{RR or OR} = \frac{(\text{Ag+}/\text{Ag-}) \text{ disease}}{(\text{Ag+}/\text{Ag-}) \text{ control}} = \frac{(90/10)}{(5/95)} = 171$$



90/100

Rejecting

# GWAS in transplantation

## A brief history of genome-wide association studies in the transplantation field

GWAS	Sample size and cohort type	Transplant type	Transplant-associated outcome or aspect of interest	Ethnicity of recipients	Associated genes or regions
O'Brien <i>et al.</i> (2013)	326 discovery	Kidney	Long-term graft function	Caucasian	TRA <sup>‡</sup> and ZNF516 <sup>*</sup>
McCaughan <i>et al.</i> (2014)	256 discovery and 441 replication	Kidney	NODAT	Caucasian	ATP5F1P6 <sup>‡</sup>
Sanders <i>et al.</i> (2015)	388 discovery	Heart and kidney	Post-transplant cancer	Caucasian	LINC00882 <sup>‡</sup> , CACNA1D <sup>‡</sup> and CSMD1 <sup>‡</sup>
Oetting <i>et al.</i> (2016)	197 discovery and 160 replication	Kidney	Pharmacokinetics (tacrolimus)	African American	CYP3A5 and ZSCAN25
Ghisdal <i>et al.</i> (2017)	778 discovery and 844 replication	Kidney	Acute rejection	Caucasian	PTPRO and CCDC67
Sato-Otsubo <i>et al.</i> (2015)	1,589 discovery	HSC	Acute GVHD	Japanese	HLA-DPB1 and KRAS
Bari <i>et al.</i> (2015)	68 discovery and 100 validation	HSC	Acute GVHD	Mostly Caucasian	SUFU <sup>‡</sup>

# The study

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Working hypothesis: donor-recipient SNP or CNP **incompatibilities** represent an unrecognized risk for the allograft acute rejection in solid organ

Identification of such incompatibilities may lead to the discovery of novel “minor” histocompatibility antigens, outside of the HLA region, that can influence the outcome of the transplantation

# Study design

**3,266 CNVs**

based on CNP data generated with 2.1-million NimbleGen CGH arrays.

**180 CNPs MAF >10%**

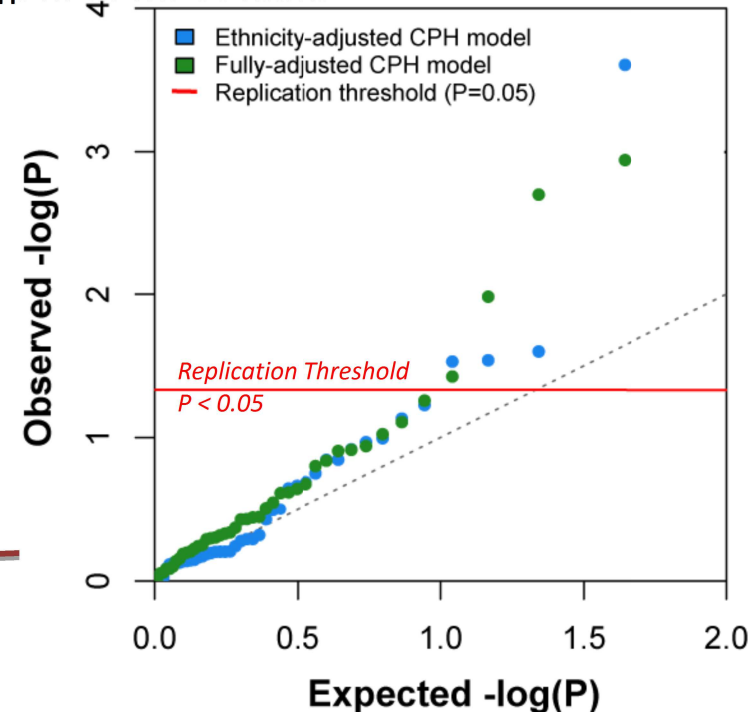
overlapped with a coding segment of the genome

**87 CNPs (48%) were deletions**

Based on the HapMap3 data, a perfect tag SNP ( $r^2 > 0.8$ ) for **54** of the identified deletions was found.

These 54 deletions disrupt a total of 110 genes, 30% of which are robustly expressed in the adult kidney (based on the HuGE Index and the EMBL Gene Expression Atlas).

These 50 SNPs were genotyped in the cohort of 705 CUMC kidney transplant recipients



\* 50 SNPs, recessive model, fully-adjusted model accounts for ethnicity, age, sex, HLA mismatch, PRA titers, and sensitization risk factors (transfusions, pregnancies, and prior transplants).

Kiryluk K, NEJM., 2019

# Pilot Discovery Study on more than 1,000 renal allograft recipients transplanted between 1998-2014

**3,266 CNVs**

based on CNP data generated with 2.1-million NimbleGen CGH arrays.

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overlapped with a coding segment of the genome

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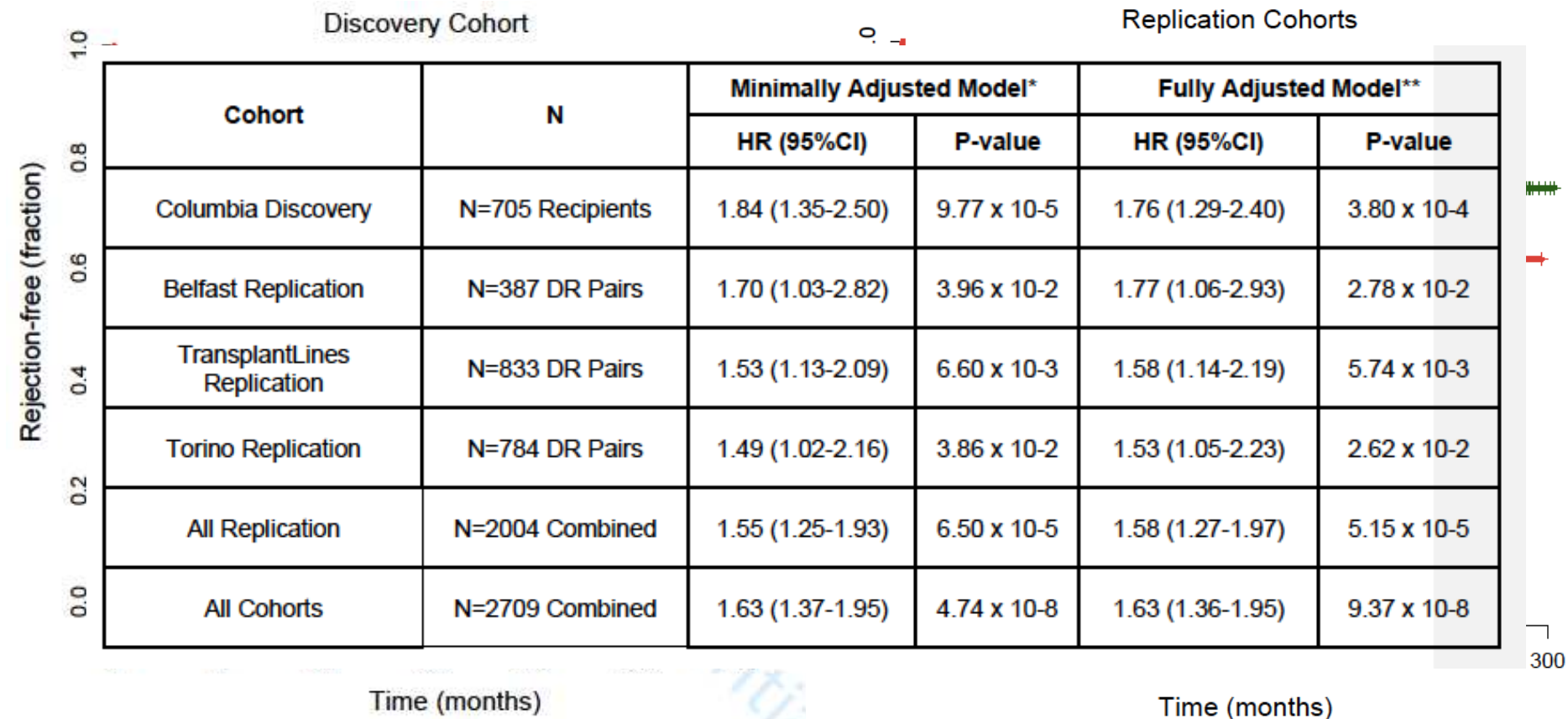
Of these 50 SNPs, 5 came up as correlated with acute rejection in a recessive model.

The top one is **SNP rs893403** (in the promoter) and CNV **CNVR915.1** (downstream) of ***LIMS1*** gene



# Pilot cohort

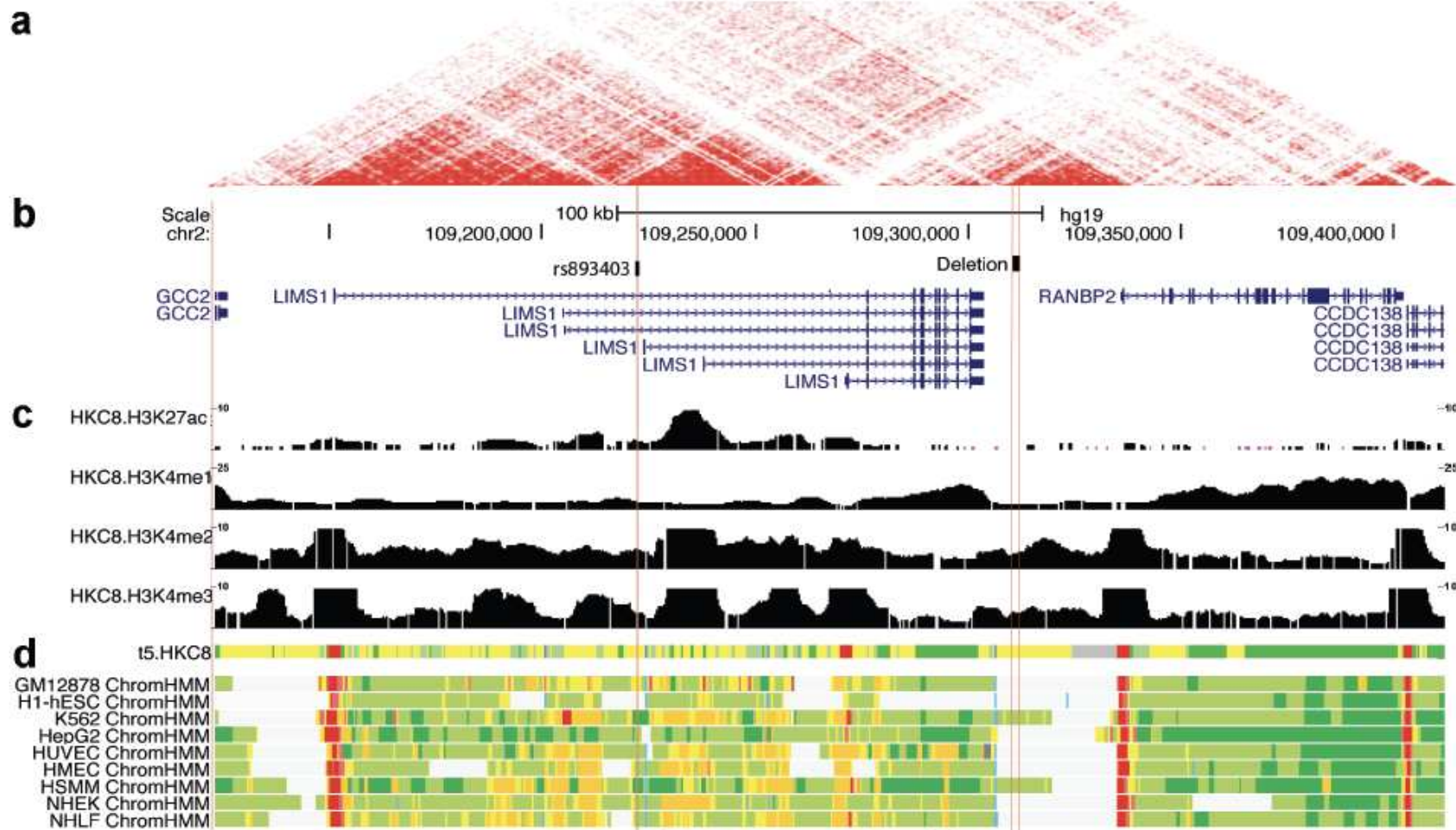
# Validation cohort



Kiryluk K, NEJM., 2019

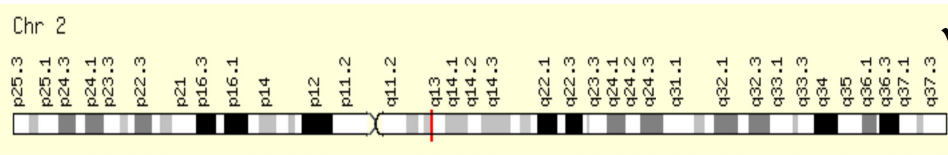
Kidney transplant recipients homozygous for the deletion-tagging allele had 79% higher risk of acute rejection in the ethnicity-adjusted analysis (HR=1.79, p=2x10<sup>-4</sup>)

## Genomic region surrounding rs893403



Kiryluk K, NEJM., 2019

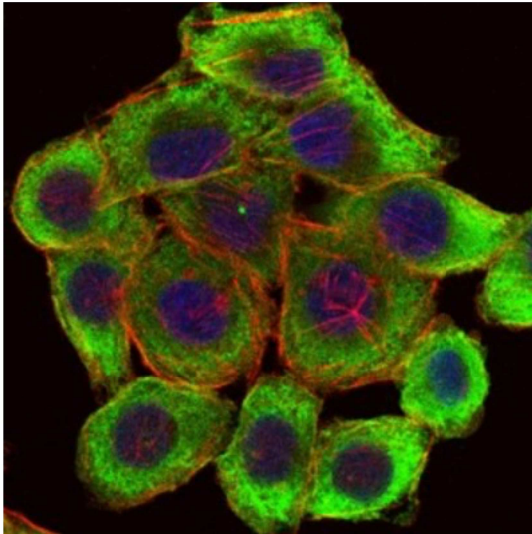
# LIMS1 protein



✓ *LIMS1 is a protein coding gene, located on chromosome 2q12.3.*



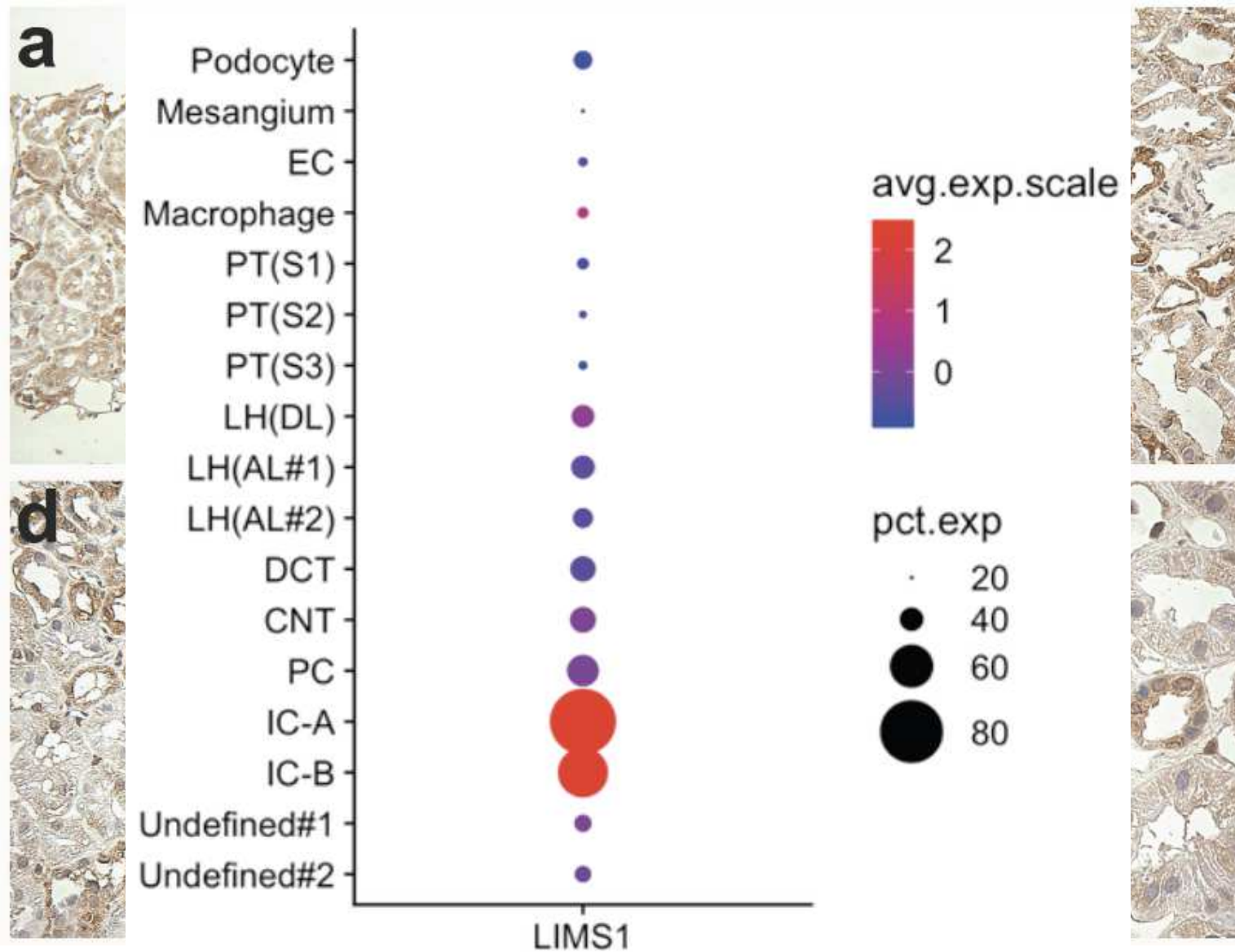
✓ *The protein encoded by this gene is an adaptor protein which contains five LIM domains, or double zinc fingers.*



✓ *The protein is likely involved in integrin signalling.*

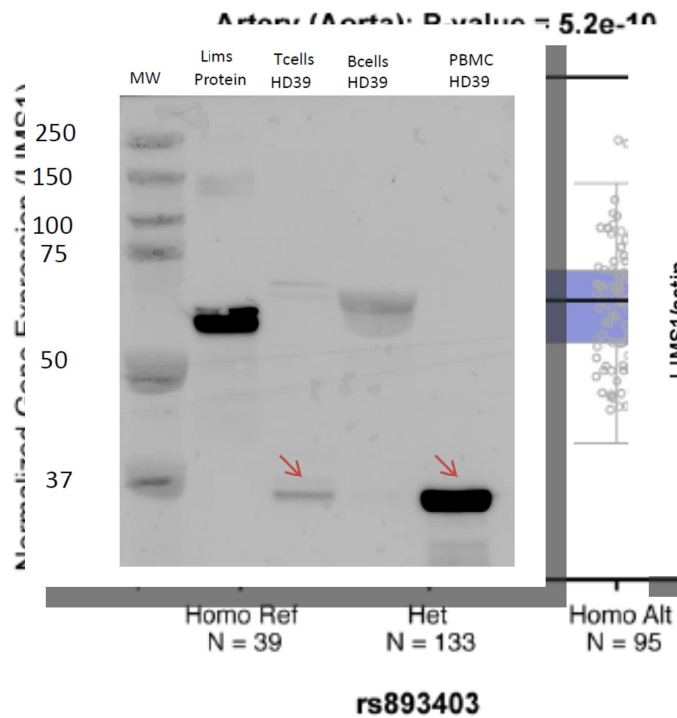
✓ *LIMS1 protein has been connected with regulation of renal glomerular podocyte adhesion and renal tubular epithelial-to-mesenchymal transition.*

# LIMS1 protein expression



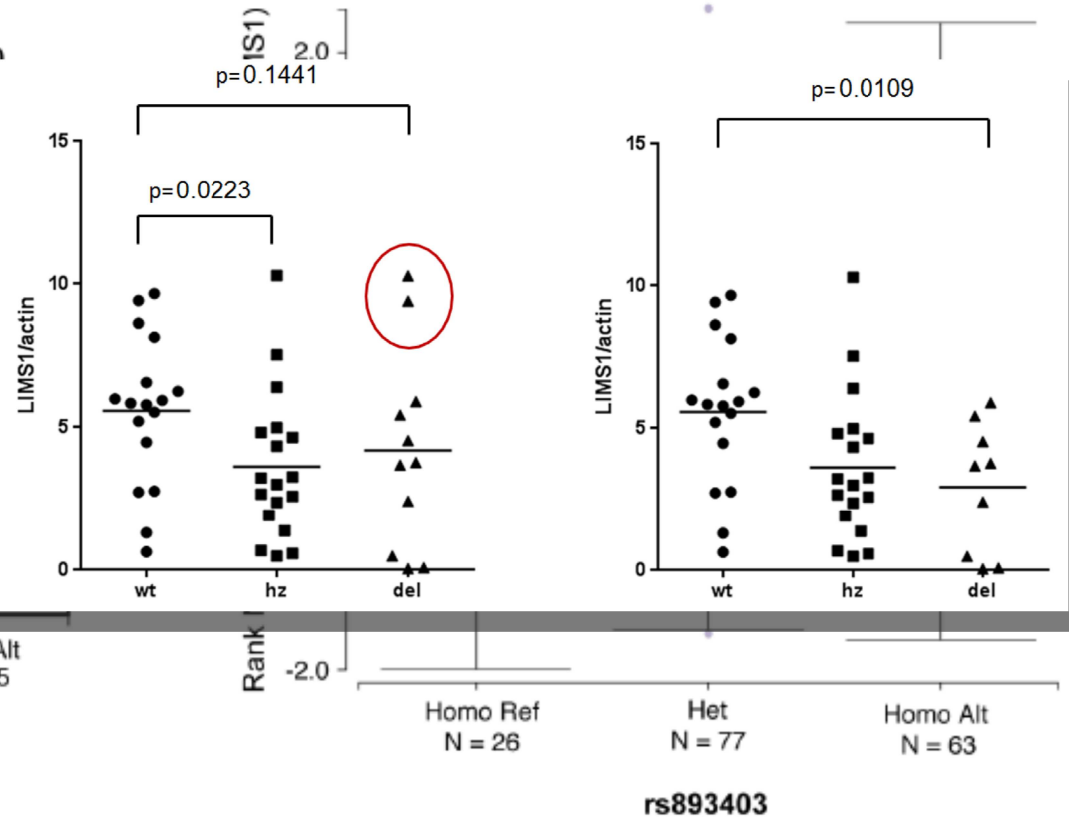
# LIMS1 protein expression

**b**

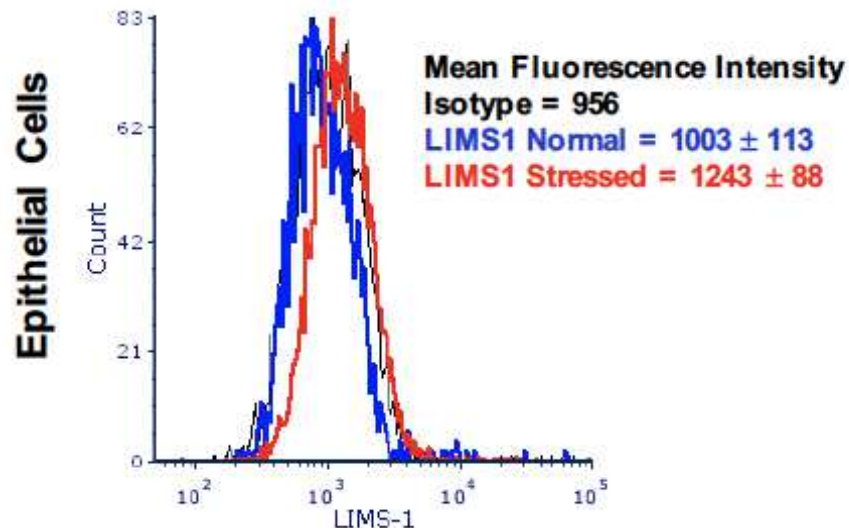
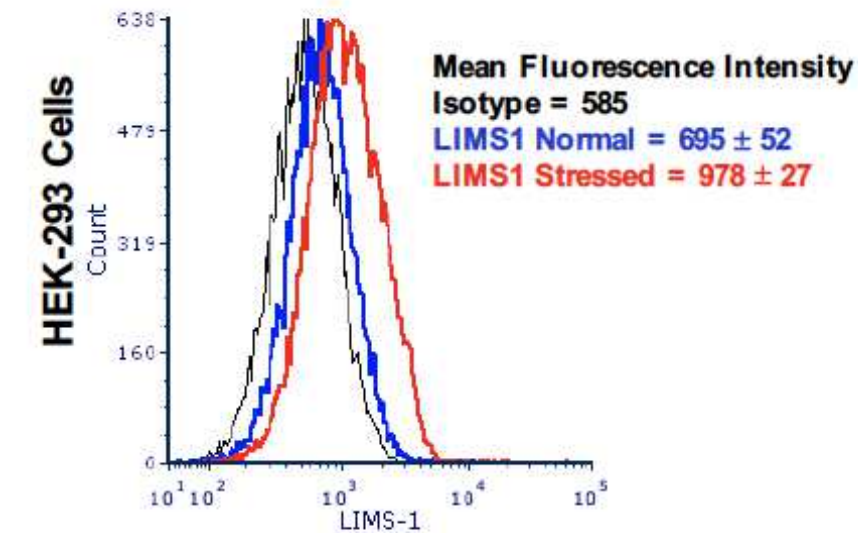


**c**

**Kidney Tubulointerstitium: P-value = 0.0144**

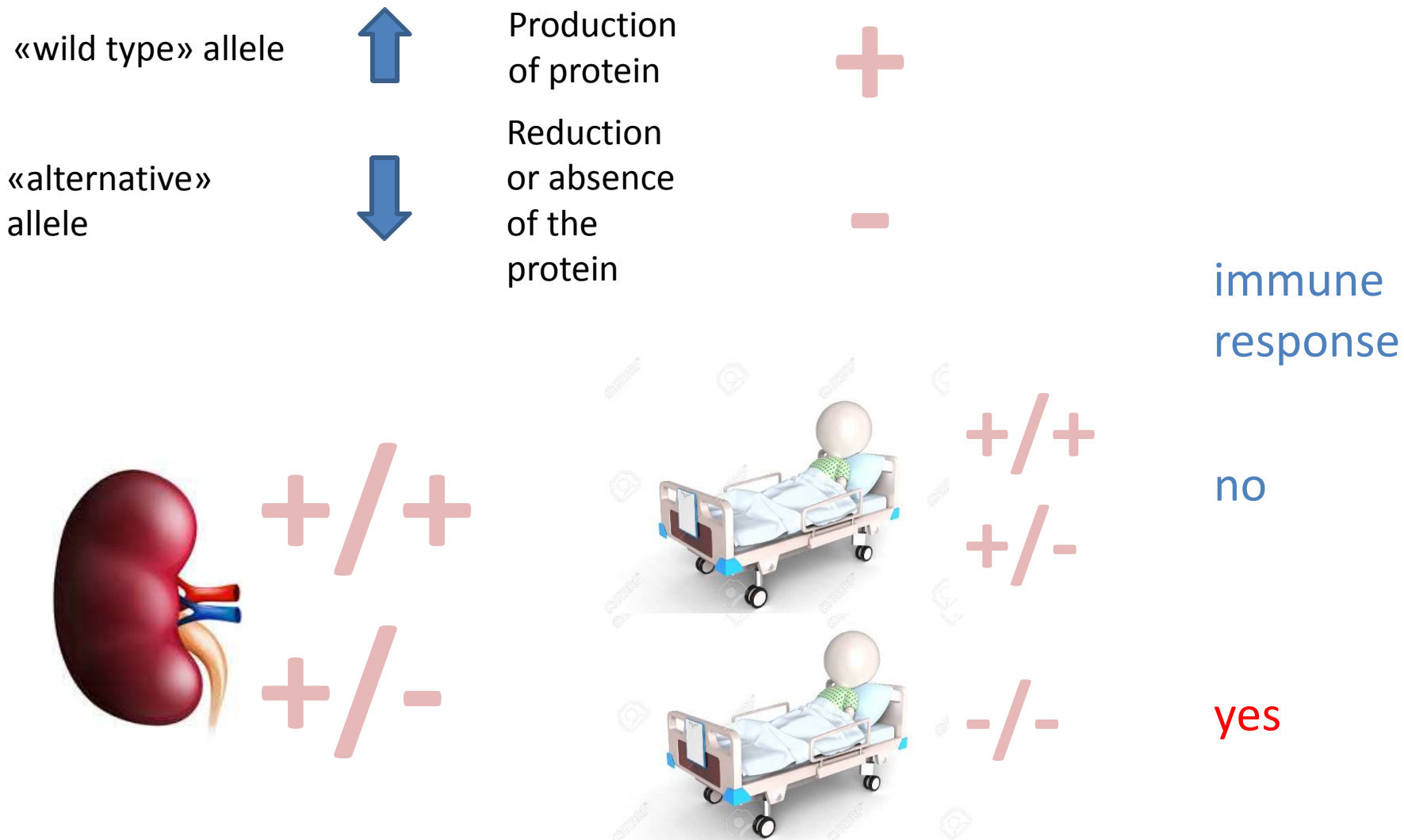


# LIMS1 protein expression under stress

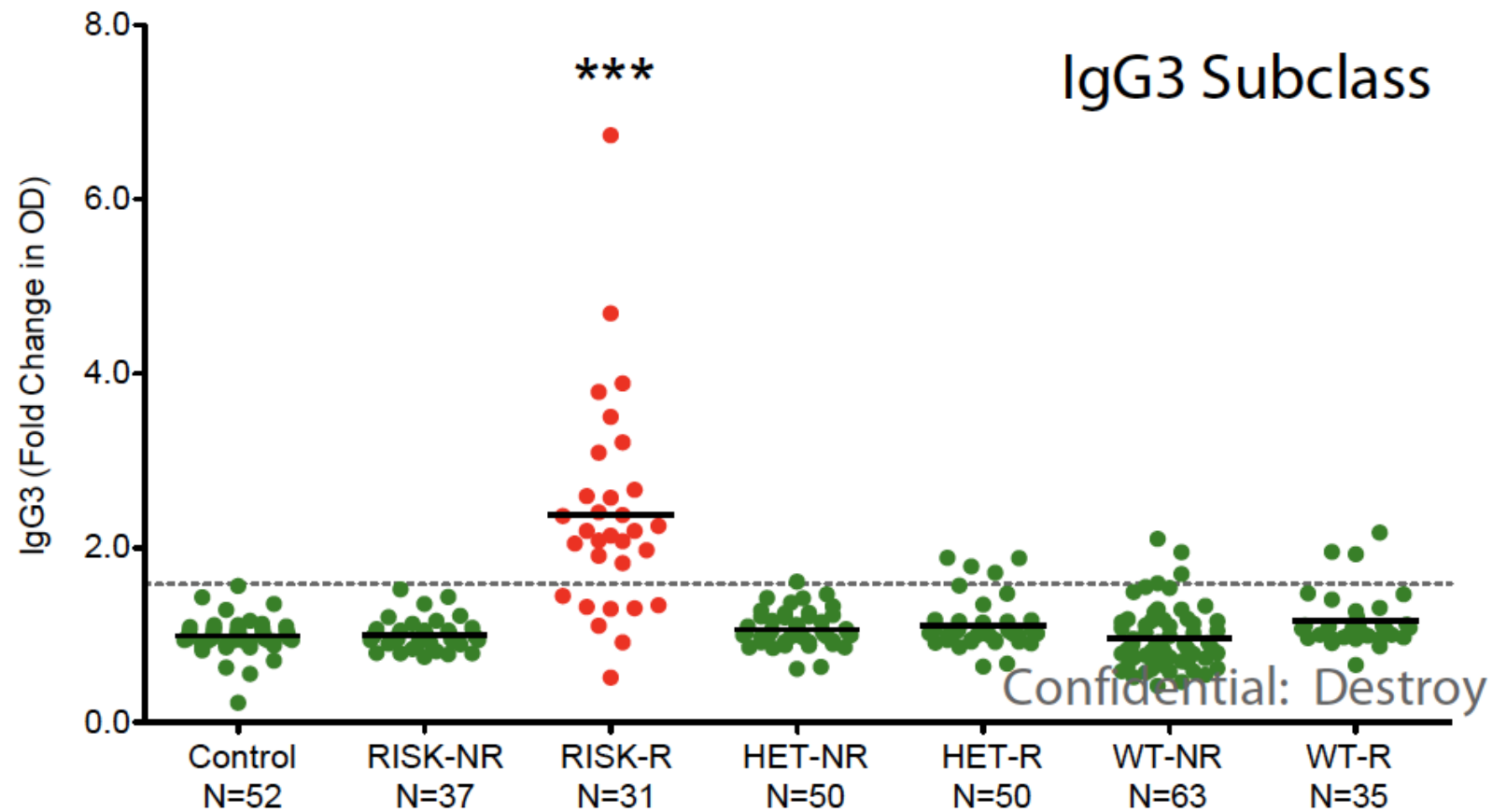


Kiryluk K, NEJM., 2019

# Working hypothesis in the transplant setting

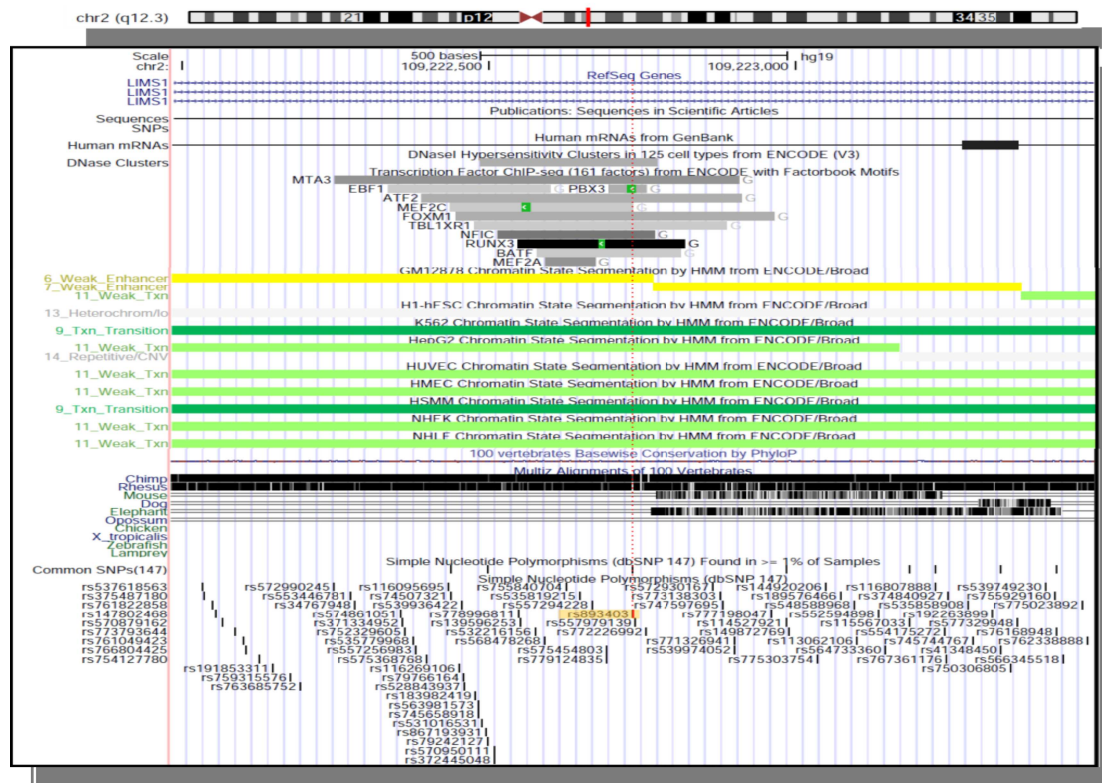


# Detecting anti-LIMS1 antibodies



# Other possible functional implications?

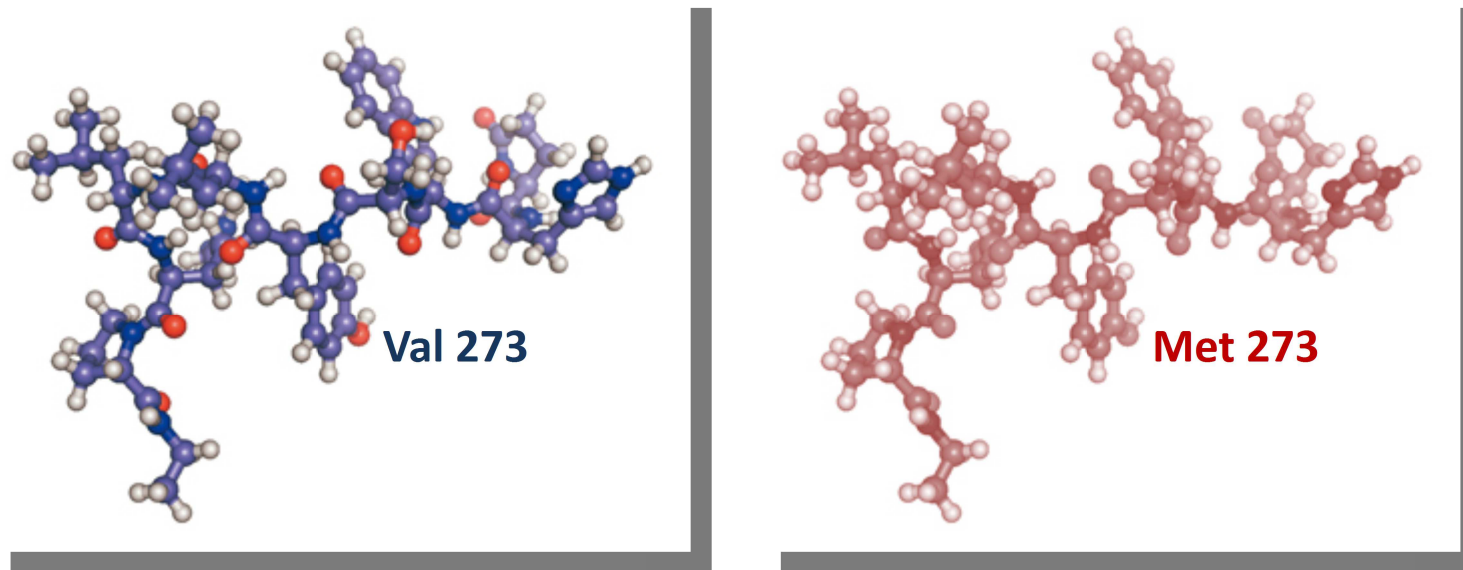
To explore a possible functional impact of rs893403 and CNVR915.1, we exploit ENCODE (Encyclopaedia of DNA Elements) data available at the [University of California, Santa Cruz](#) (UCSC) Genome Browser.



- SNP rs893403 is located inside a weak enhancer region and a transcriptional-transition region.
- CNVR915.1: the deletion seems to affect a heterochromatin region without a regulatory function.
- Possible involvement of other genes of the region and their polymorphisms with LIMS1 and effects of our candidate SNP and deletion are still to be investigated.

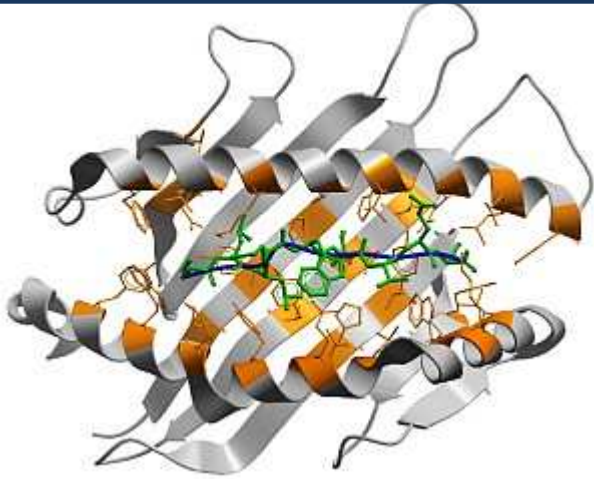
# There are other SNPs in the LIMS1 gene!

- Rs150889011 modifies the structure of the LIMS1 protein (V273M) and variant 273M is in strong LD with CNVR915.1 deletion
- The expected genotype frequencies are: Val/Val: 55%; Val/Met: 30% and Met/Met: 15%



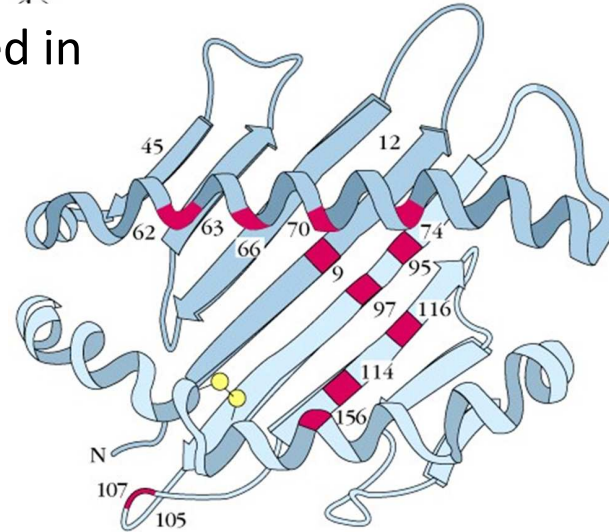
- Peptides derived from LIMS1 might be immunogenic in mismatched combination, e.g.: Don Val 273+ in recipient Val 273-negative

# Could this SNP generate an immunogenic peptide?

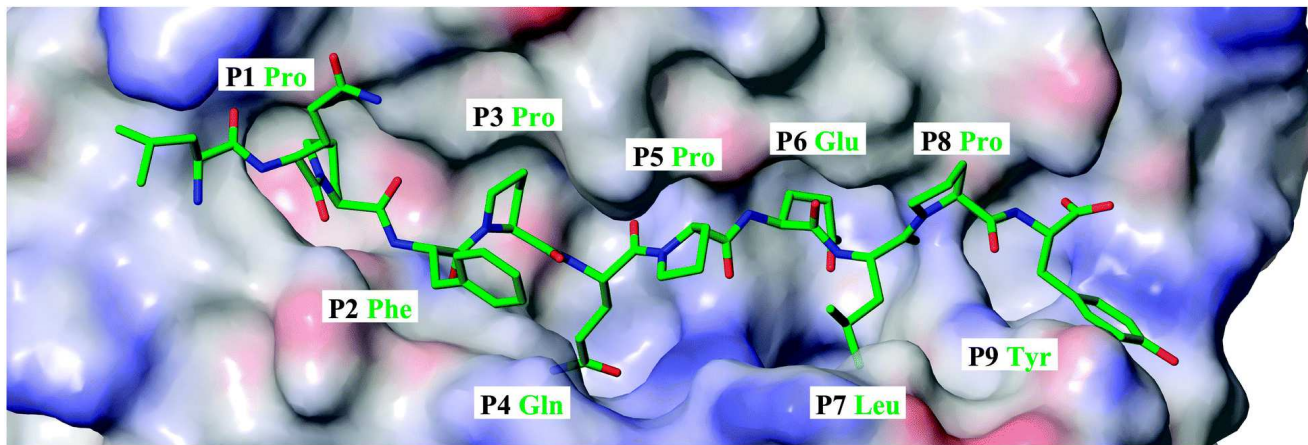


Peptides are presented in the HLA pocket

HLA molecules are highly polymorphic



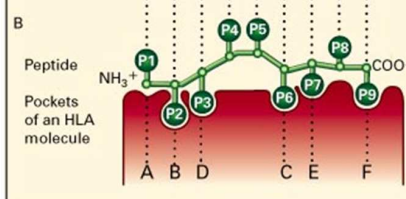
Depending from individual HLA polymorphisms, the repertoire of peptides is different, explaining diversity in the immune response from an individual to another



**A**

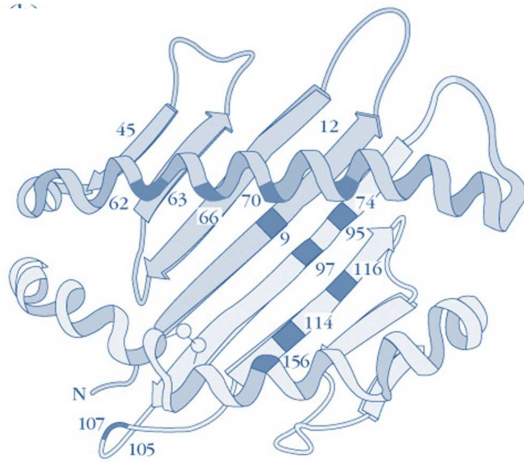
	Peptides								
	P1	P2	P3	P4	P5	P6	P7	P8	P9
HLA-A*0201	W	L	S	L	L	V	P	F	V
	L	L	F	G	V	P	V	Y	V
	I	L	K	E	P	V	H	G	Y
HLA-A3	R	L	R	P	G	G	K	K	K
	I	L	R	G	S	V	A	H	K
	R	L	R	A	E	A	G	V	K
HLA-A*6801	K	T	G	G	P	I	Y	K	R
	E	V	A	P	P	E	Y	H	R
	A	V	A	A	V	A	A	R	R
HLA-B7	G	P	G	P	Q	P	G	P	L
	I	P	Q	C	R	L	T	P	L
	P	P	P	I	F	I	R	R	L
HLA-B27	R	R	V	K	E	V	V	K	K
	G	R	I	D	K	P	I	L	K
	R	R	I	K	E	I	V	K	K

**B**

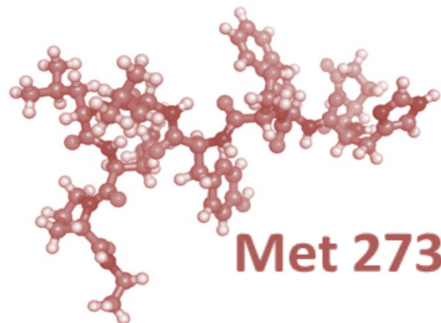
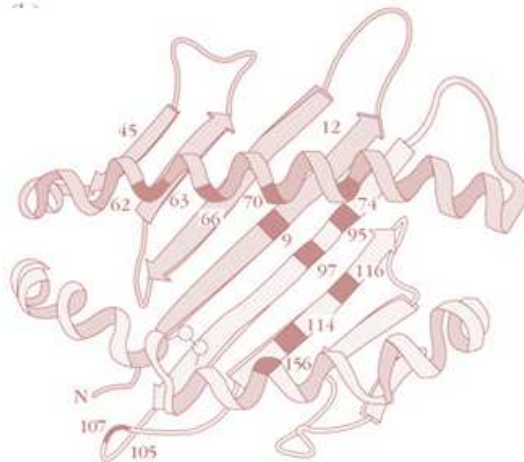


**A**

# Some HLA alleles might present Val273 peptides at higher affinity



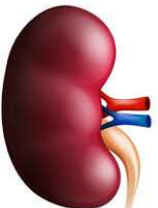

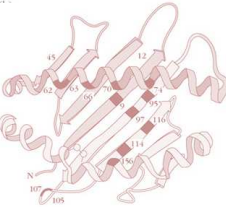
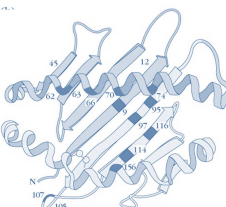

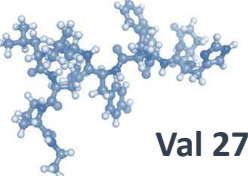
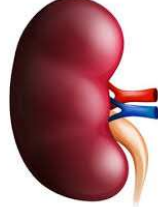


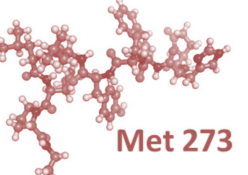
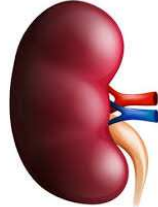


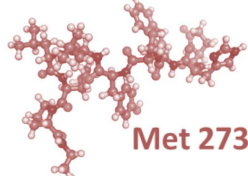
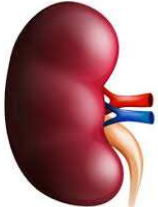
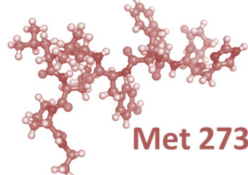

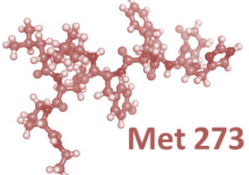
Val 273



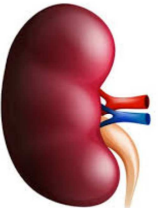


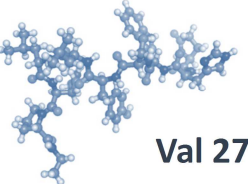
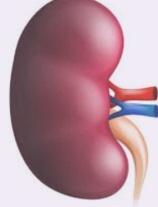

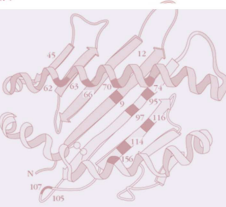

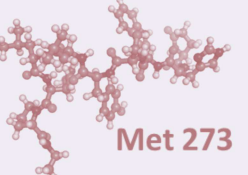
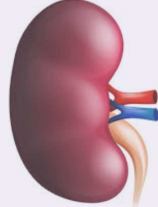

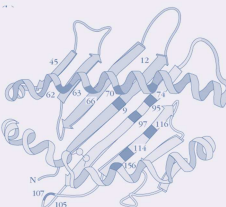

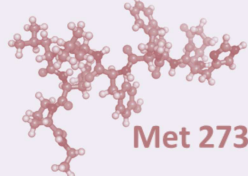
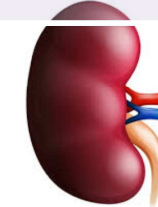
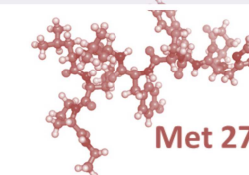

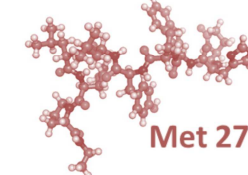
Met 273

Recipients  
Met273/Met273  
homozygotes, when  
receiving organs from  
Donors Val273+, might  
recognize the Val273  
peptide as immunogenic,  
**especially when HLA of  
the donor (or of the  
recipient) are able to bind  
it at high affinity**

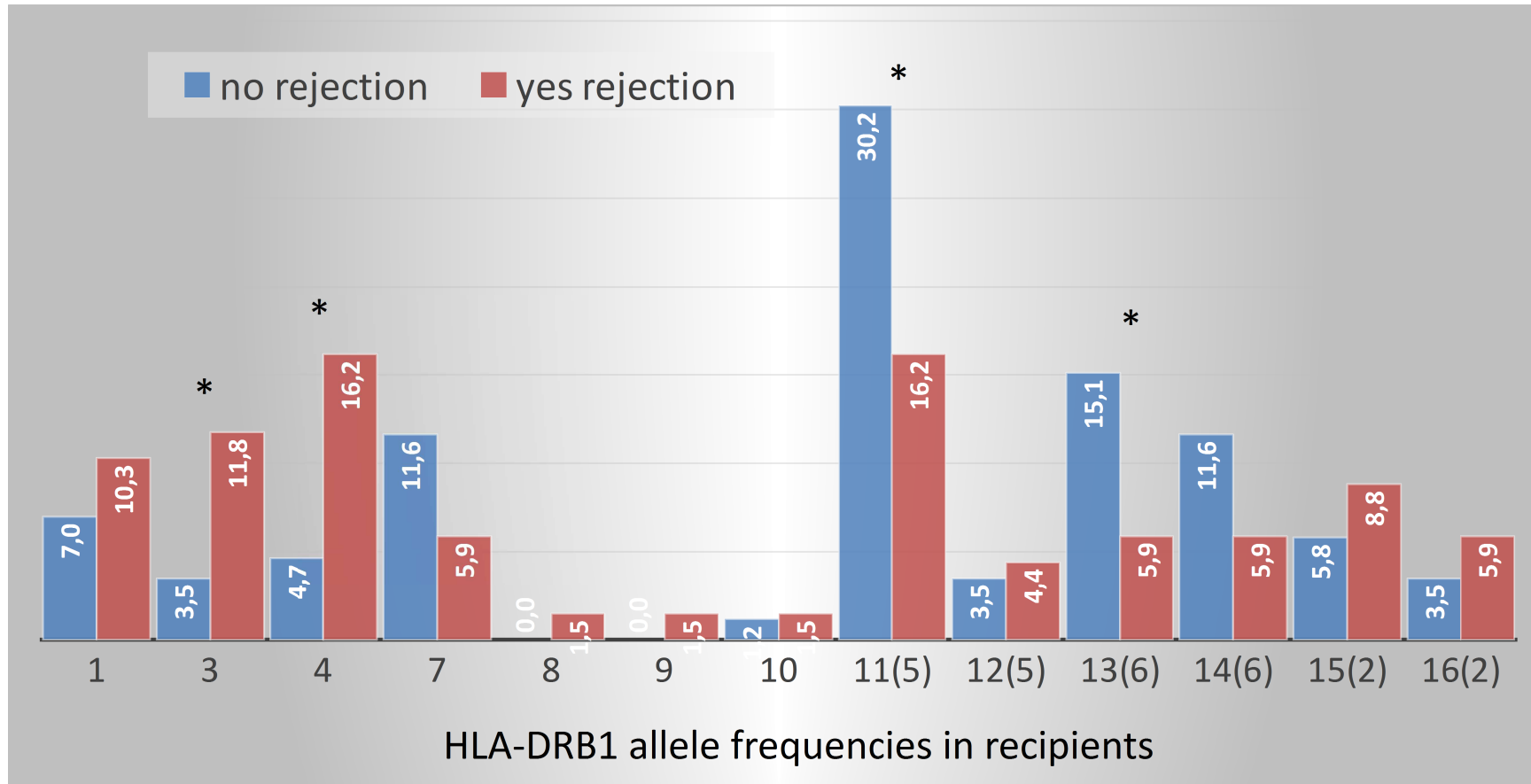
# Is there HLA restriction?

DONOR	LIMS1	HLA Donor or recipient	RECIPIENT	LIMS1	immune response
	 Val 273	 		 Val 273	no
	 Val 273			 Met 273	no
	 Val 273			 Met 273	yes
	 Met 273			 Met 273	no

# Is there HLA restriction?

DONOR	LIMS1	HLA Donor or recipient	RECIPIENT	LIMS1	immune response
	 Val 273			 Val 273	no
	 Val 273			 Met 273	no
	 Val 273			 Met 273	yes
	 Met 273			 Met 273	no

# HLA-DRB1 Frequencies of the recipients of CNVR15.1 mismatched grafts, divided according to rejection (n=77)



\* DRB1\*03  $p=0.05$  and DRB1\*04  $p=0.01$   
DRB1\*11  $p=0.06$  and DRB1\*13  $p=0.02$ ;

# Possible influence of LIMS1 (V273M) mutated form on outcome of mismatched group of patients

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- rs150889011 modifies the structure of the LIMS1 protein (V273M)
- it might generate an immunogenic peptide (Val273) in recipient lacking it, that could be presented
  - at higher affinity by HLA-DRB1\*03 and DRB1\*04 molecules
  - at lower affinity by HLA-DRB1\*11 and DRB1\*13
- It might be responsible of alloreactivity and higher risk of rejection when Val273-positive grafts were transplanted in Val273-negative recipients, in combination of HLA molecules which are able to present it

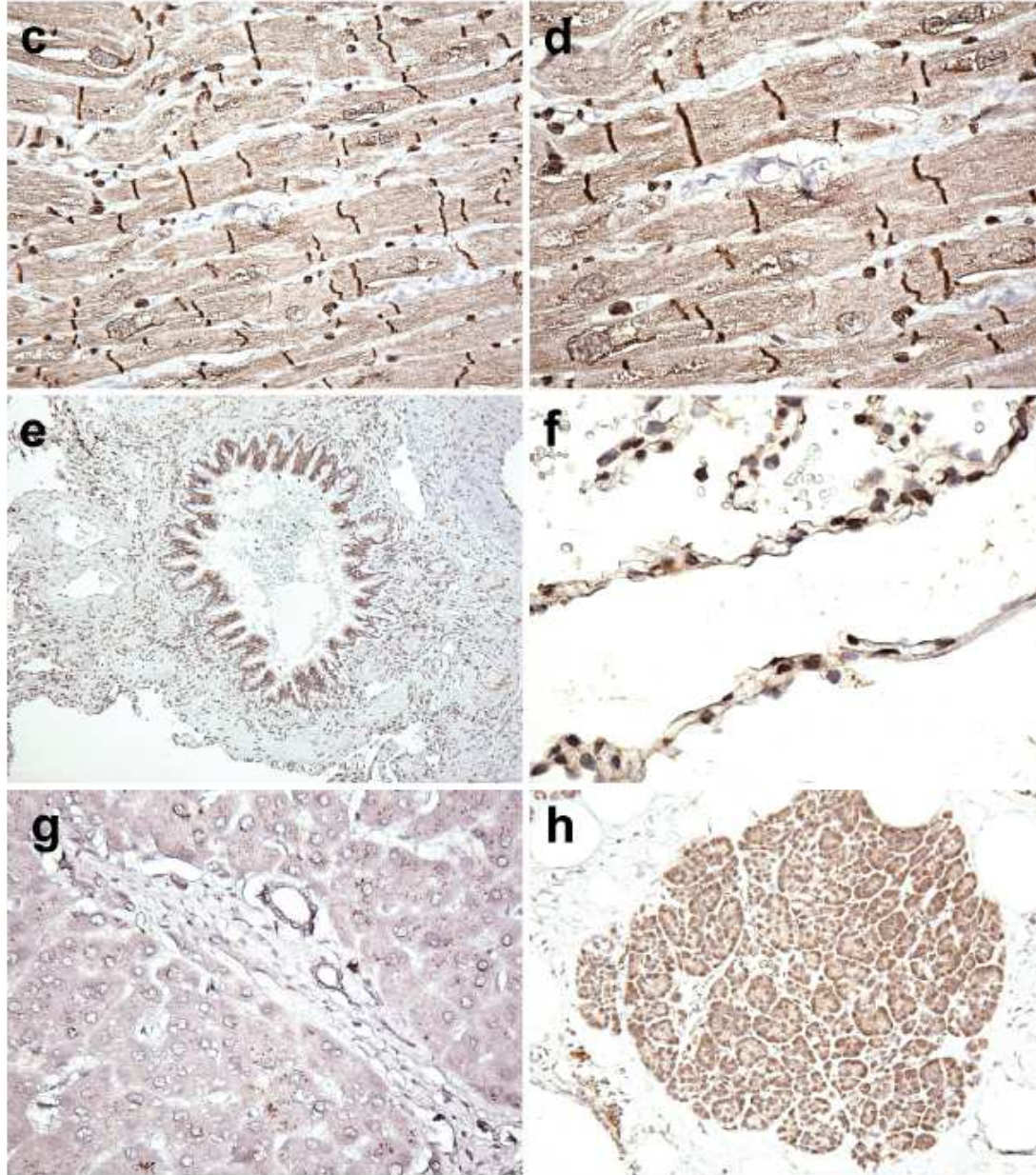
- Evaluate distribution of HLA-DRB1 frequencies, in the other cohorts,
- Genotype cohort of the kidney transplant pairs for rs150889011

# Conclusions

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- ✓ Candidate-gene approaches and GWAS studies have enabled a deeper understanding of the complex interplay of the donor–recipient interactions that lead to transplant tolerance or rejection.
- ✓ LIMS1 is a novel minor histocompatibility antigen.
- ✓ Genomic “collision” at this locus is associated with rejection and production of anti-LIMS1 IgG2 and IgG3 antibodies in mismatched donor-recipient pairs.
- ✓ The risk of rejection may be modifiable by genetic organ matching based on the rs893403 genotype.

# Beyond the kidney



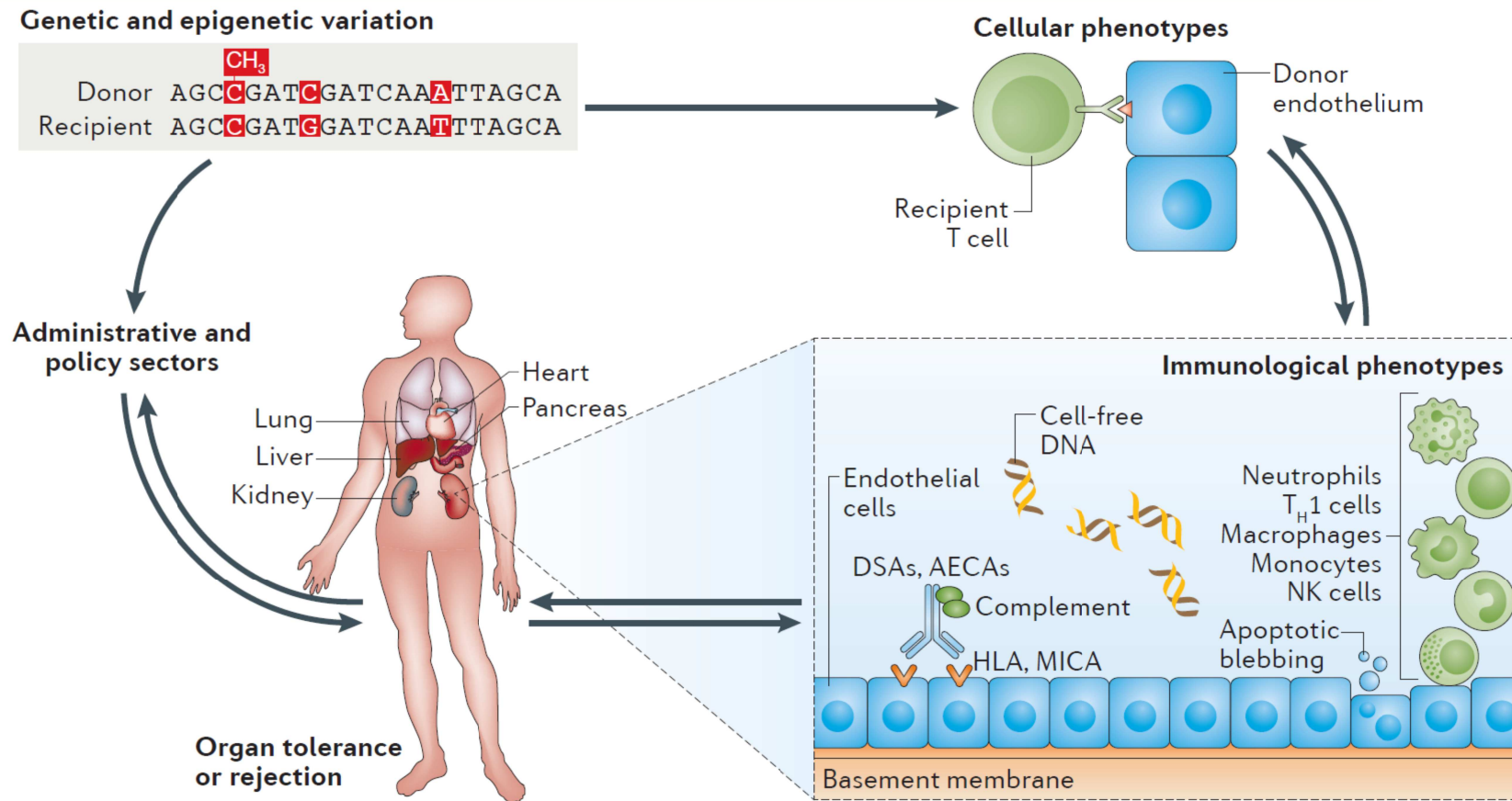
**Heart**

**Lung**

**Liver**

**Pancreas**

# Towards «transplantomics»



Epigenetic or genetic differences (top left) between a donor and recipient can influence cellular phenotypes, such as mRNA transcription and degradation, alternative splicing and protein–protein interactions (top right).

In turn, these processes can affect immunological processes such as cell–cell interactions and donor–recipient antibody binding (bottom right)

# Acknowledgements

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Trasversali di Eccellenza nelle Scienze mediche Esplorando le Omiche