

X-LINKED GENETIC FACTORS BEHIND GENDER BIAS IN RHEUMATOID ARTHRITIS

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INSERM UMRS1097 “*HLA-DR genes, Autoantibodies, and Microchimerism in Rheumatoid Arthritis and Scleroderma*”
MARSEILLE LUMINY, FRANCE

under the supervision of **Dr. Nathalie C. LAMBERT**

Female predominance in autoimmunity

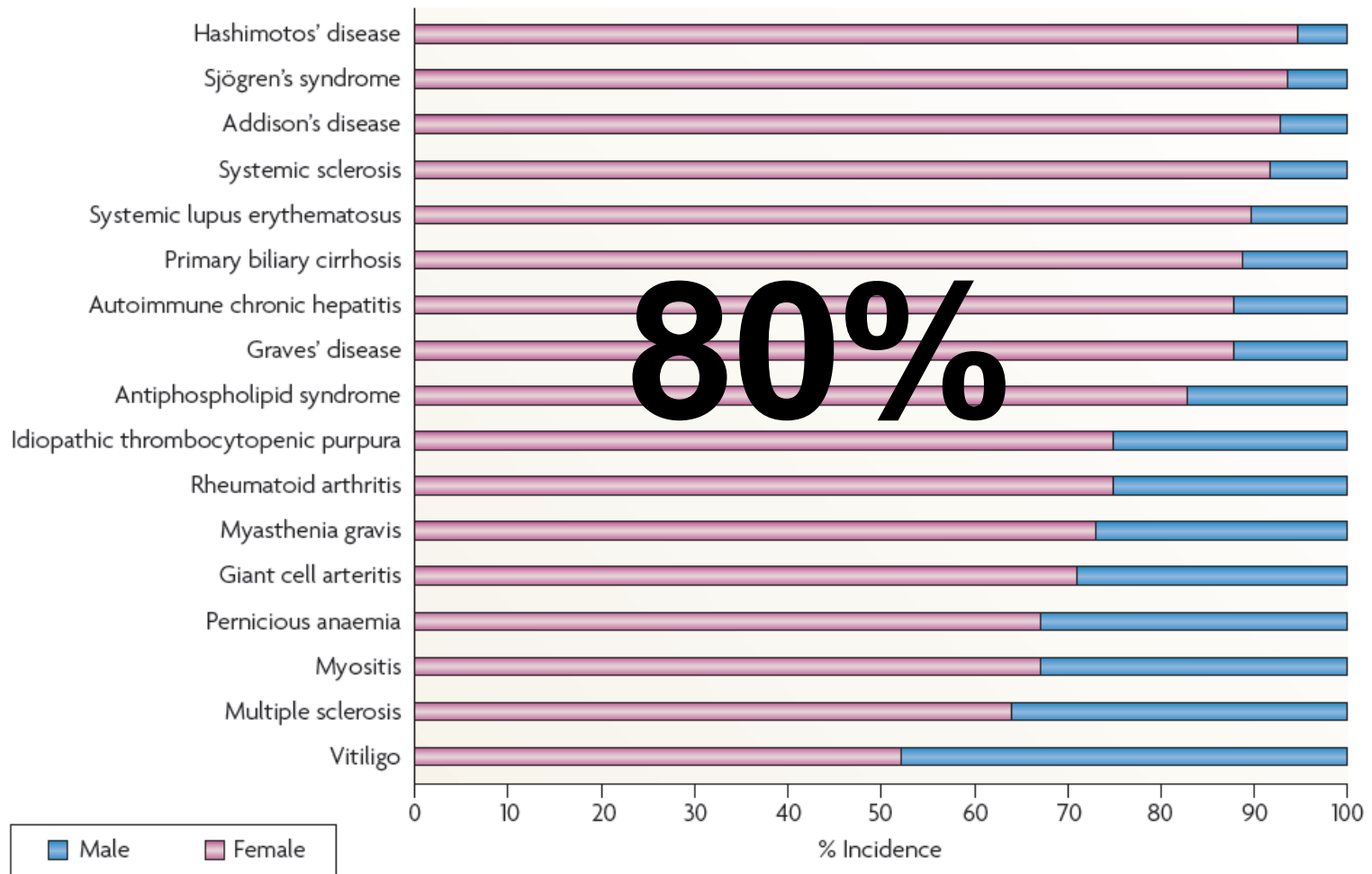
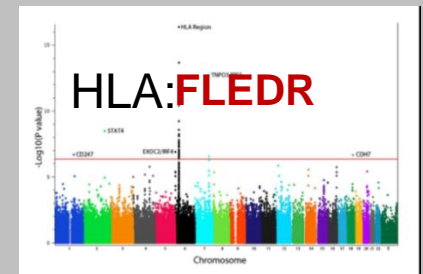
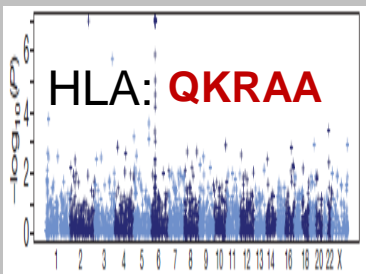


Figure 2 | **Sex distribution of the most important autoimmune diseases.** The depicted diseases are the ones which usually show female predominance^{21,102}.

Rheumatoid Arthritis (RA)

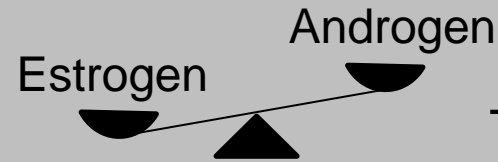
Scleroderma (SSc)

Etiology	Unknown	Unknown
Women:Men	3:1	3:1 → 14:1
Prevalence	0.5% to 1%	Rare: 3-24 per 100,000
Features	Joint destruction disability, pain	Extensive fibrosis, Excessive collagen synthesis vascular alterations...
Genetic Predisposition	HLA: QKRAA	HLA: FLEDR
Auto-antibodies	<ul style="list-style-type: none"> • ACPA 	<ul style="list-style-type: none"> • Limited cutaneous → Anti-centromere (80%) • Diffuse cutaneous → Anti-topoisomerase (30%)



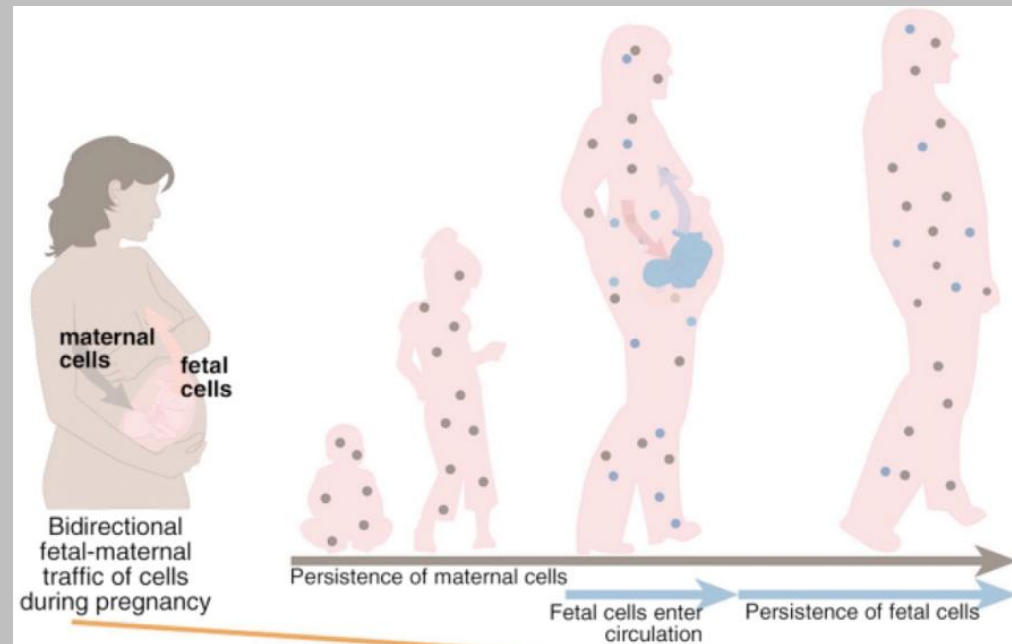
Sexual discrepancy explained by

Hormonal factors



→ Autoimmunity
RA, Scleroderma, Lupus,
Multiple sclerosis...

Microchimerism



Autoimmune thyroid diseases

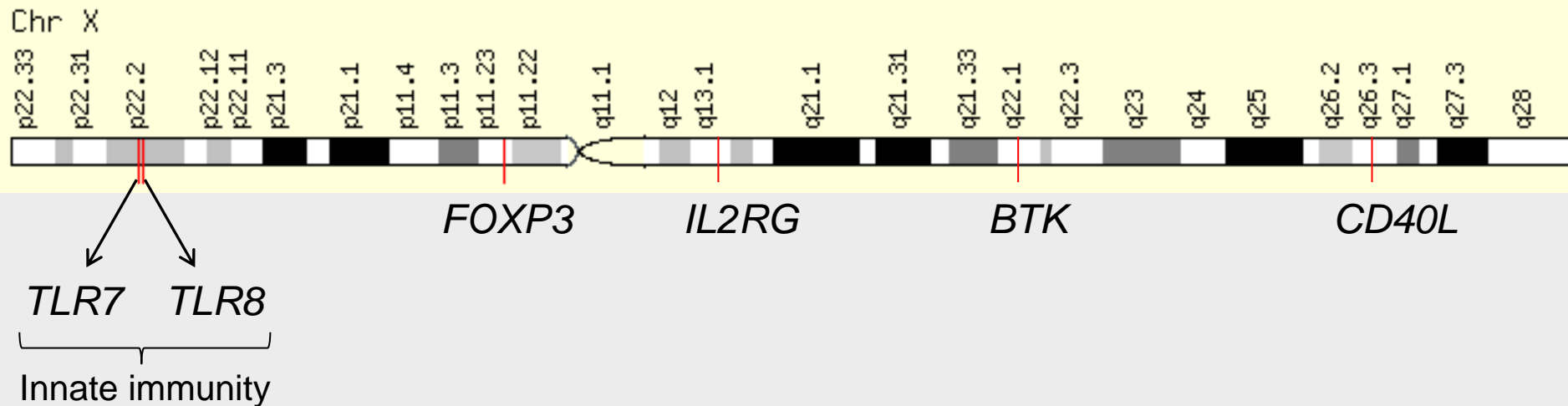
Scleroderma

RA

Myositis

The X chromosome holds many genes involved in immune functions

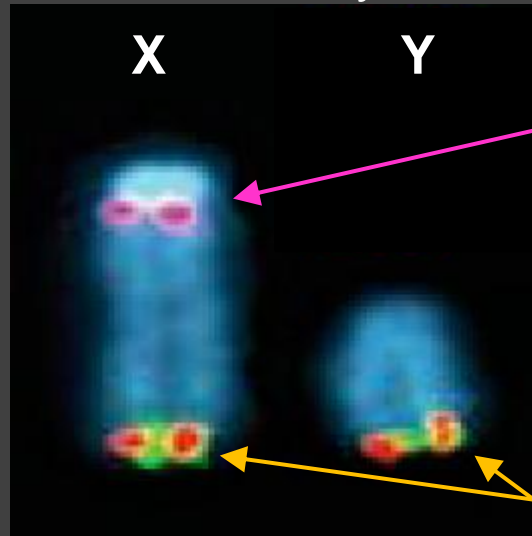
Database: GeneCards.org



TLR7 gene duplication aggravates lupus in male mice



FISH analysis



X centromere specific

16 genes from the X
(*Tlr7*, *Tlr8*,...)

Pisitkun et al., *Science* 2006

- BXSB strain:
 - Yaa translocation : cluster of 16 genes from X to Y chromosome including *Tlr7*
- Double dose of *Tlr7* sufficient to induce the acceleration of lupus in male mice

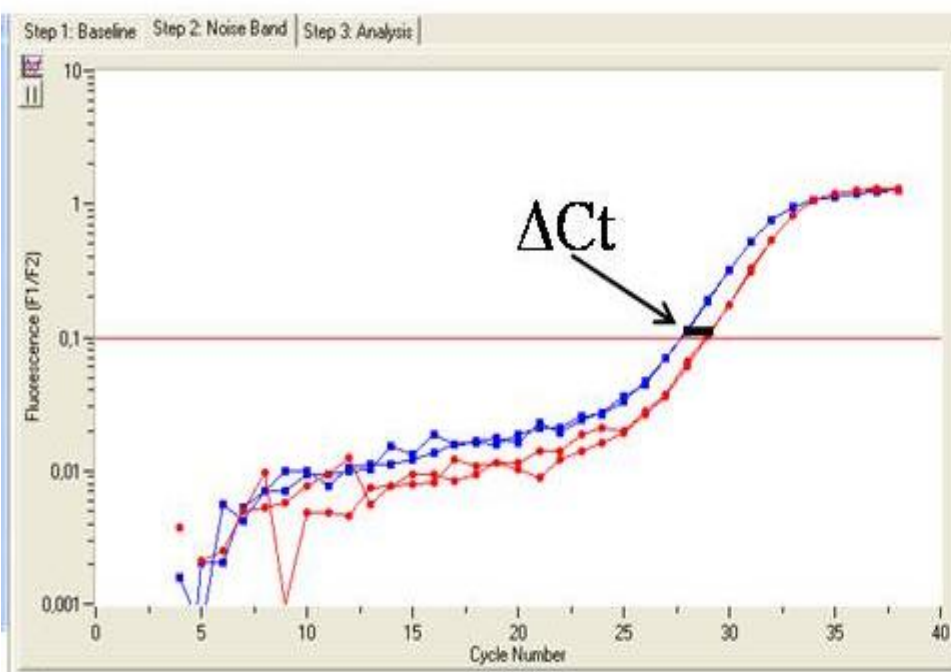
In humans: 2 studies verified *TLR7* copy number variation in SLE with contradictory results :

- Kelley J et al., *Arthritis Rheum*, 2007
- Garcia-Ortiz H et al., *Ann Rheum Dis*, 2010

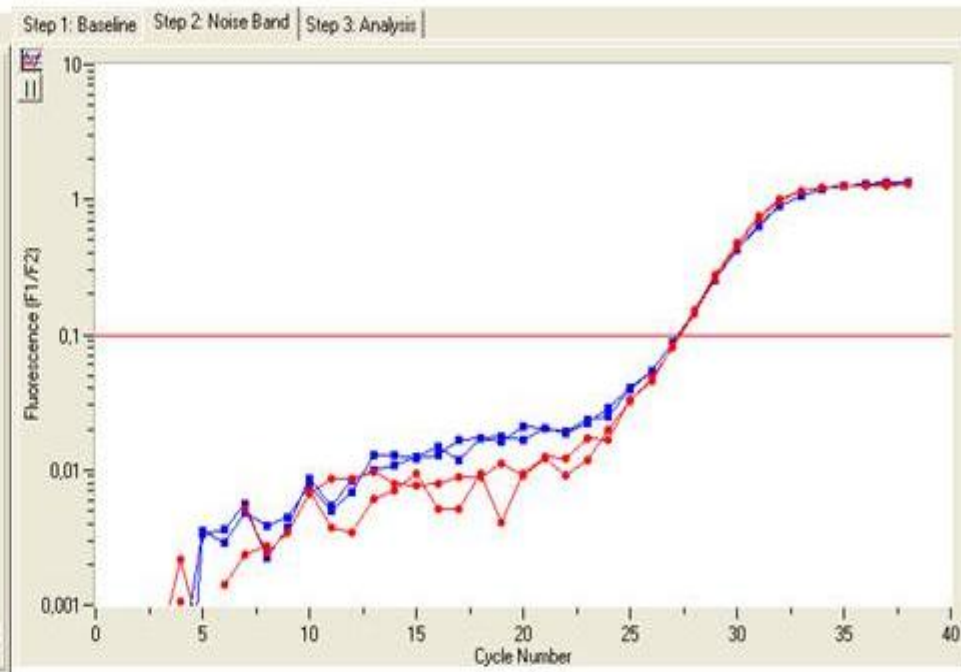
Objective: *TLR7* and *TLR8* CNV profile in humans → association to RA and SSc?

Detecting the additional TLR7 or TLR8 copy with *Relative Standard Curve method*

- **PBMC** sample amplified simultaneously by:
 - TaqMan-PCR **TLR7/8** specific (on X Chr.)
 - TaqMan-PCR **β -globin** specific (housekeeping gene on Chr. 11)

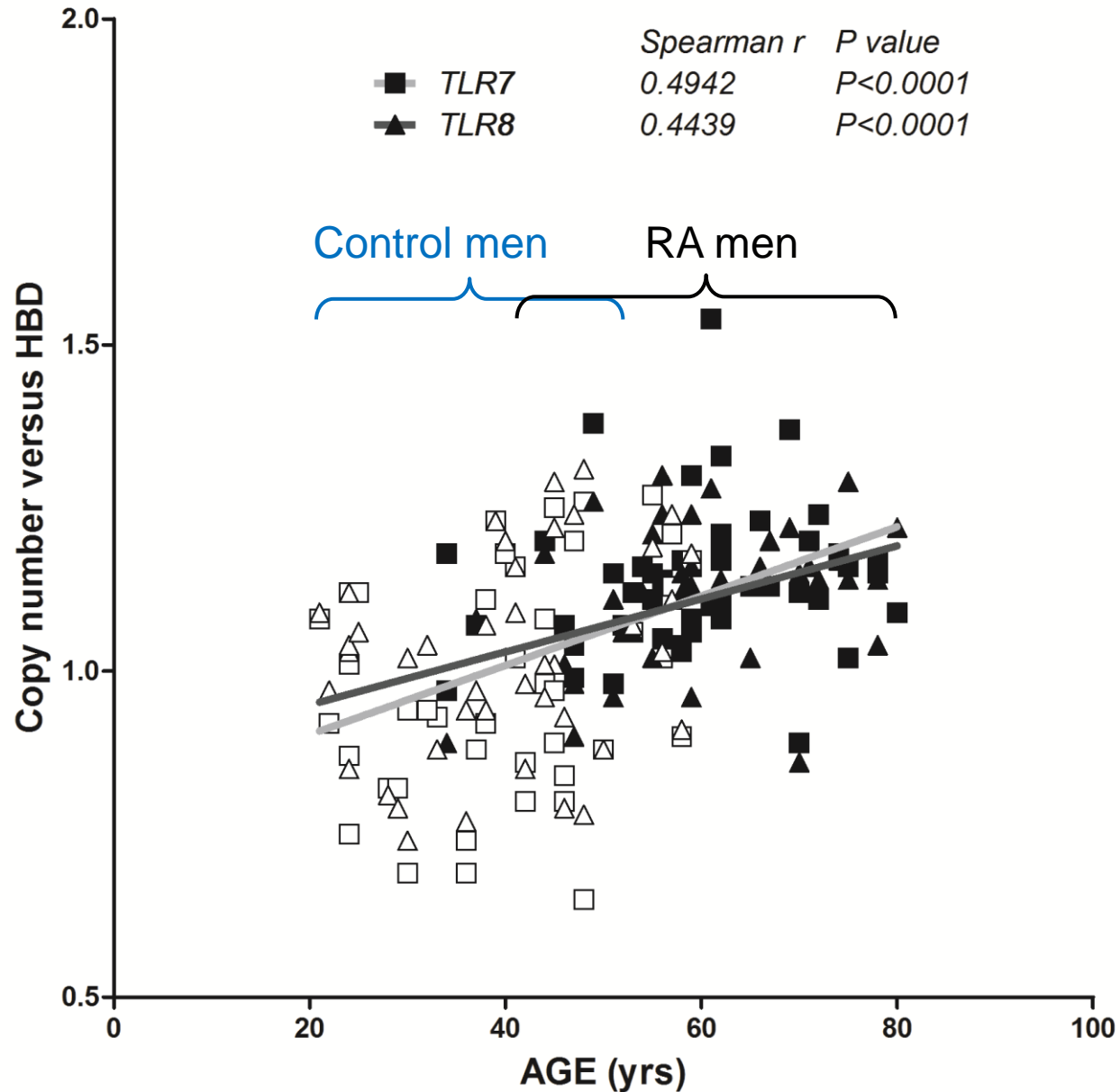


Man

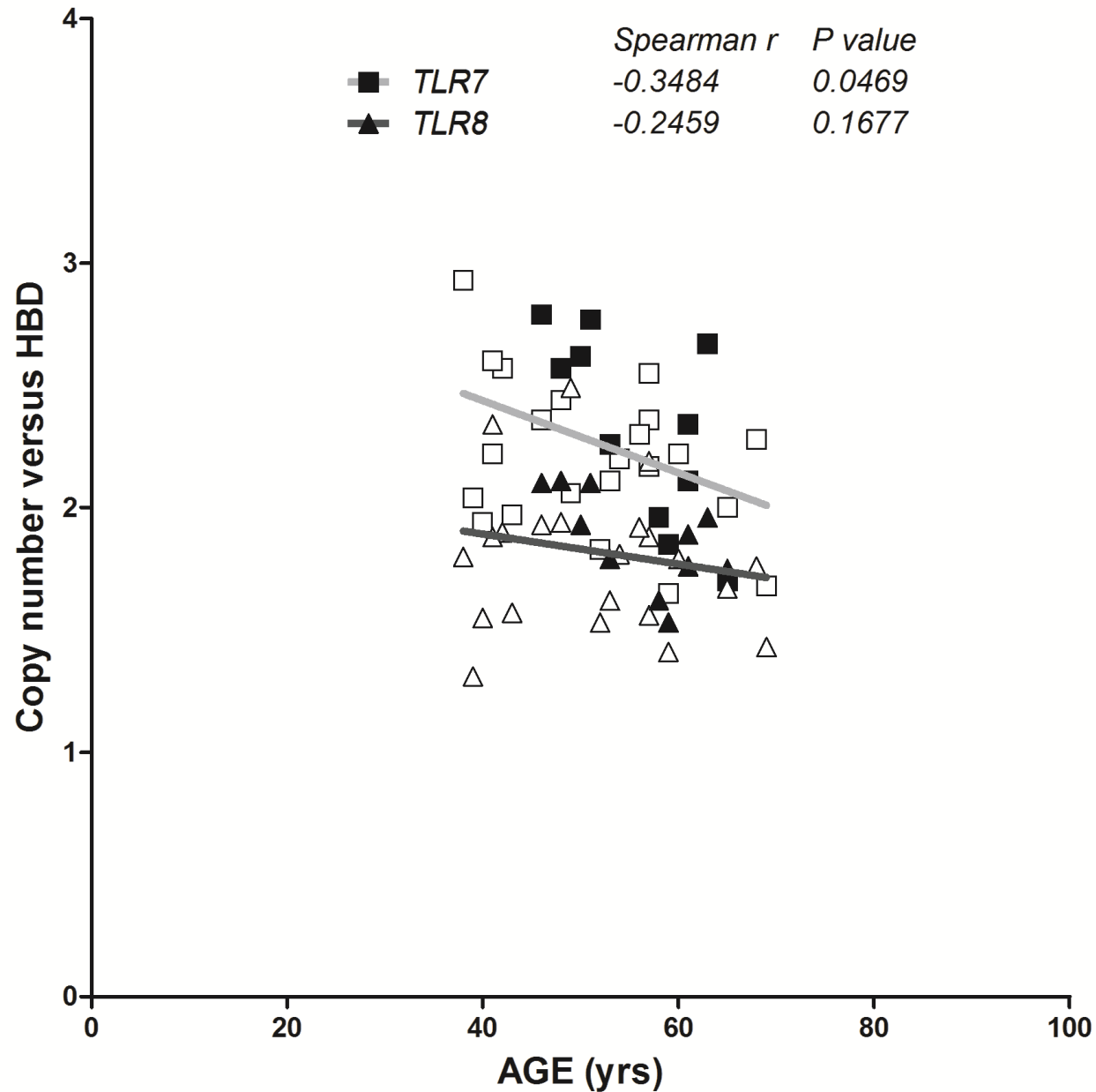


Woman

TLR7 and TLR8 copy numbers increase with age in men

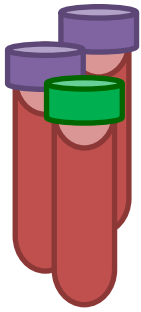


Opposite trend observed in women

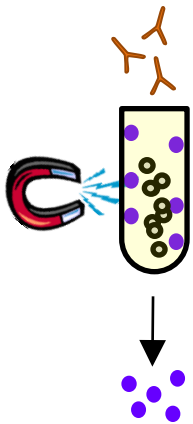


CELL SORTING

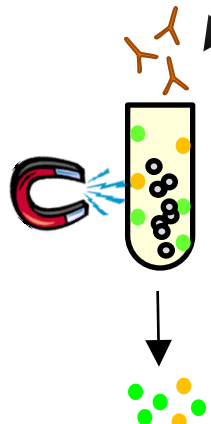
Fresh blood



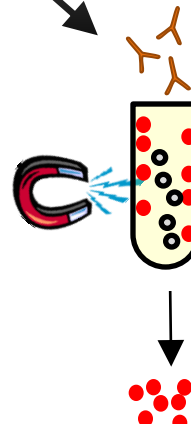
RoboSep™
STEMCELL Technologies



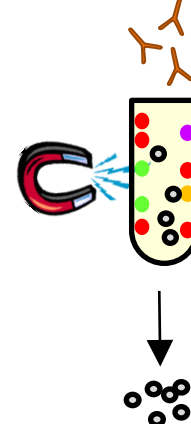
B cells



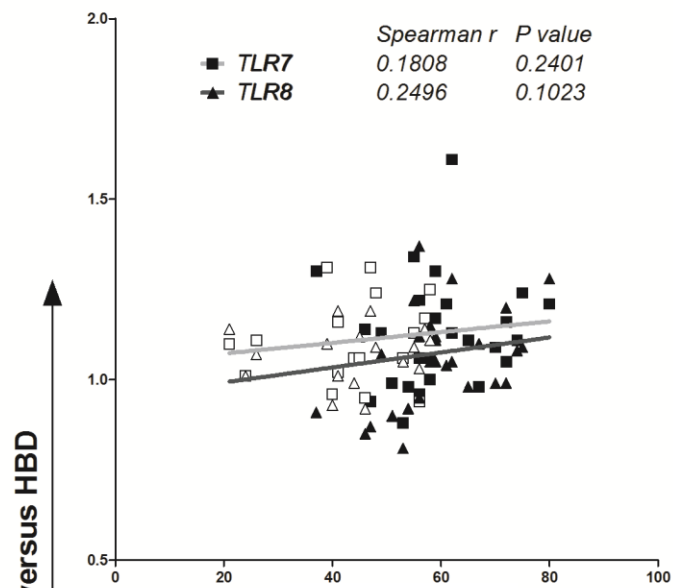
T, NKT cells



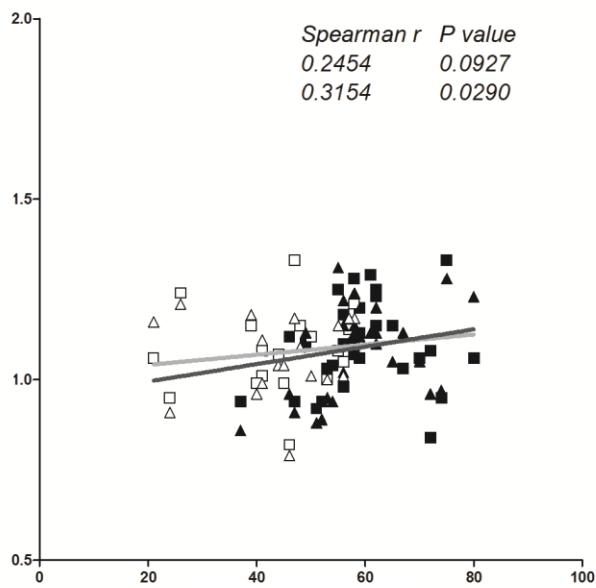
Granulocytes
(Neutrophils)



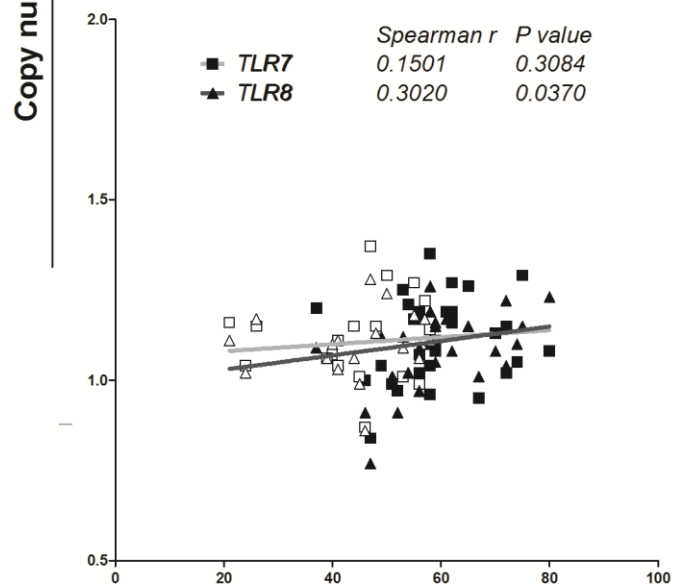
Monocytes;
Macrophages; NK;...



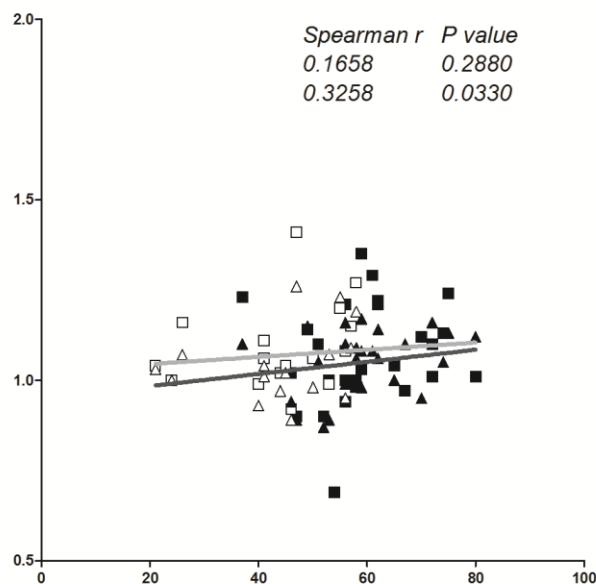
B cells



T cells



Granulocytes



Monocytes Macrophages NK...

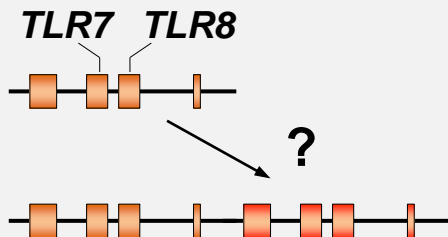
Age (years)

TLR7 and TLR8 CNVs show same trend with age in blood subpopulations of men

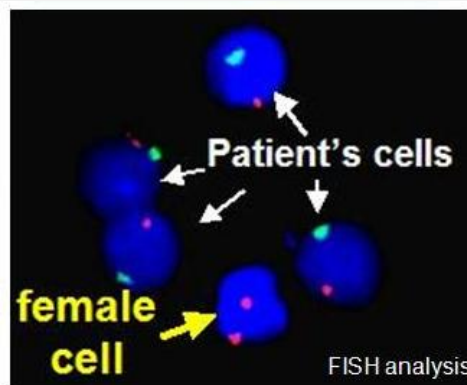
CONCLUSION / PERSPECTIVES

TLR7 and TLR8 CNVs are age- and sex-mediated

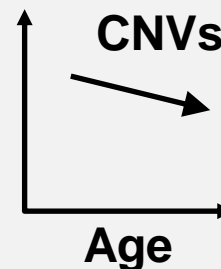
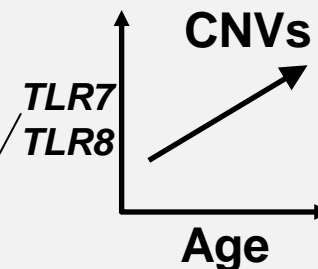
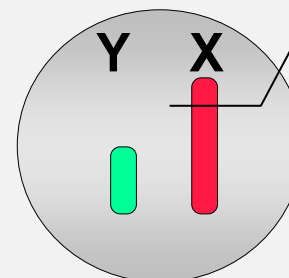
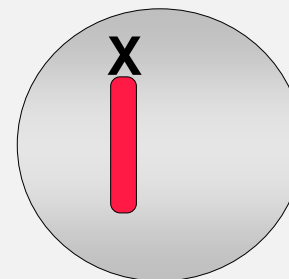
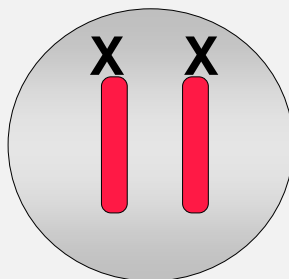
Somatic duplication



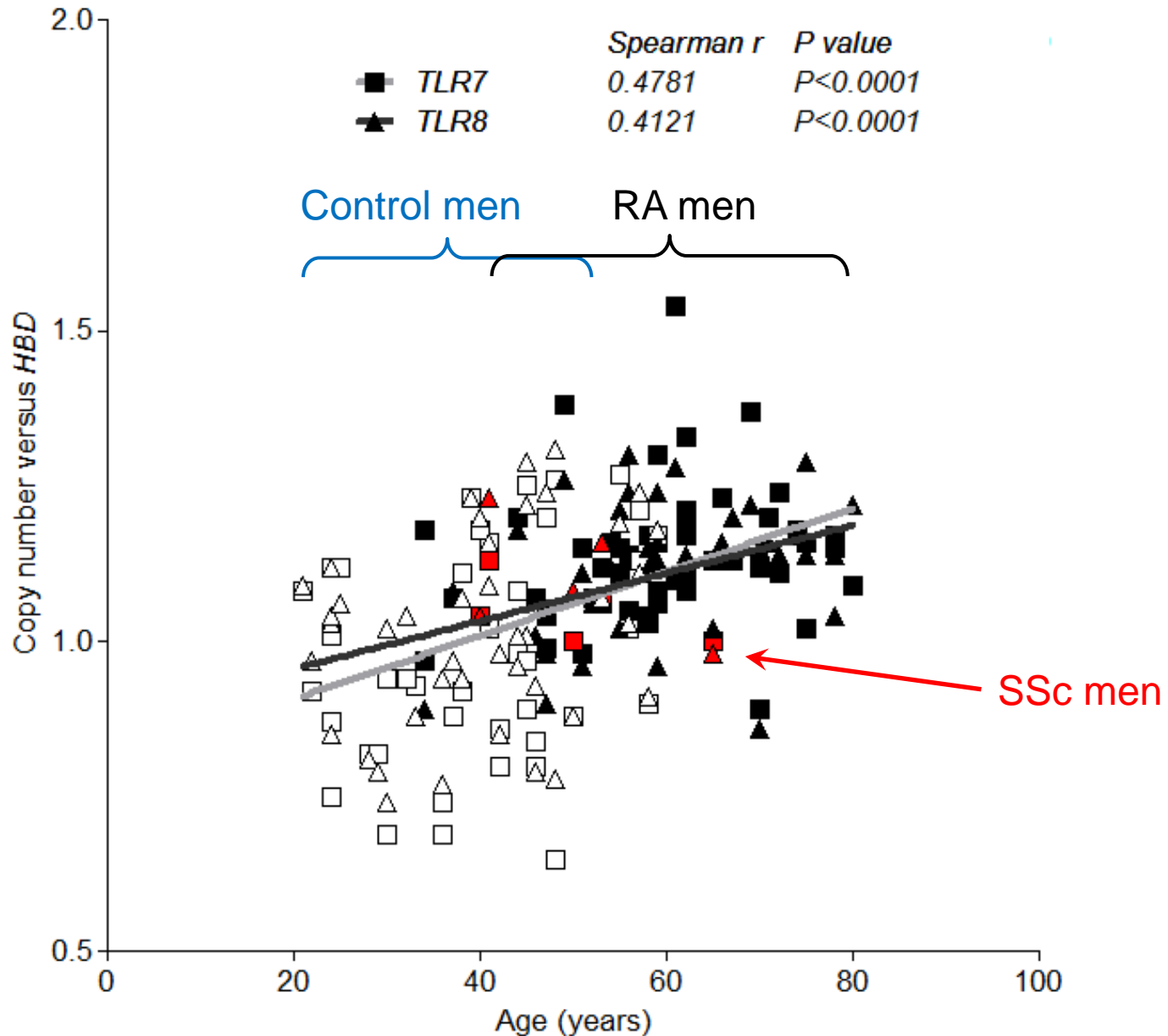
Female microchimerism
(maternal or from twin sister)



Aneuploidy acquired with age
(X monosomy)



TLR7 and TLR8 copy numbers increase with age in men



ACKNOWLEDGMENTS

FRANCE

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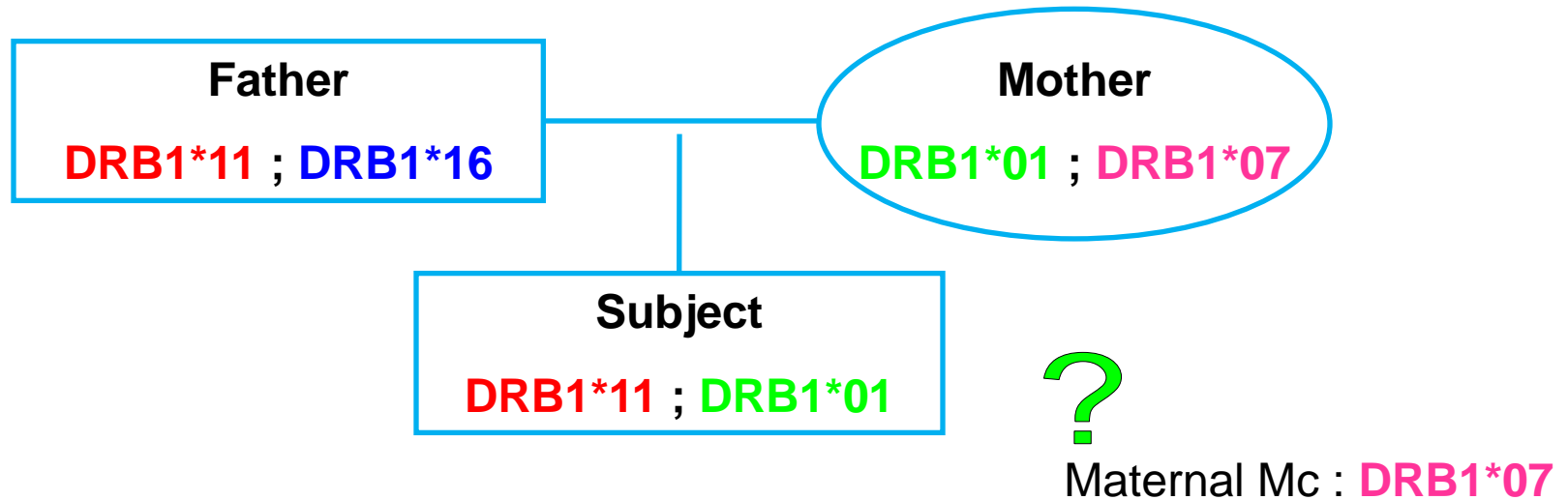
Elisabeth Eynier



Assistance Publique
Hôpitaux de Marseille



Q-PCR HLA specific



HLA typing of subjects + their mothers must be obtained, to target the ***Non Inherited Maternal Antigen*** by Q-PCR

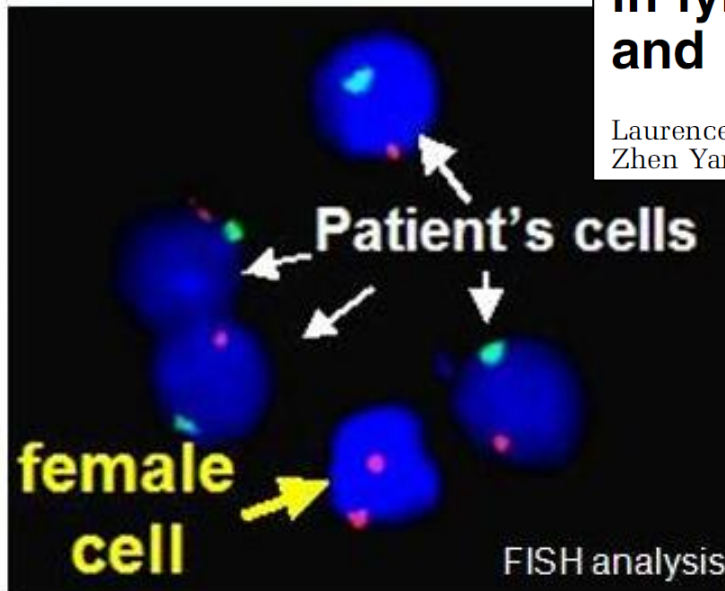
Female Mc (maternal or from twin sister) could be the cause of TLR7/8 copy number increase...

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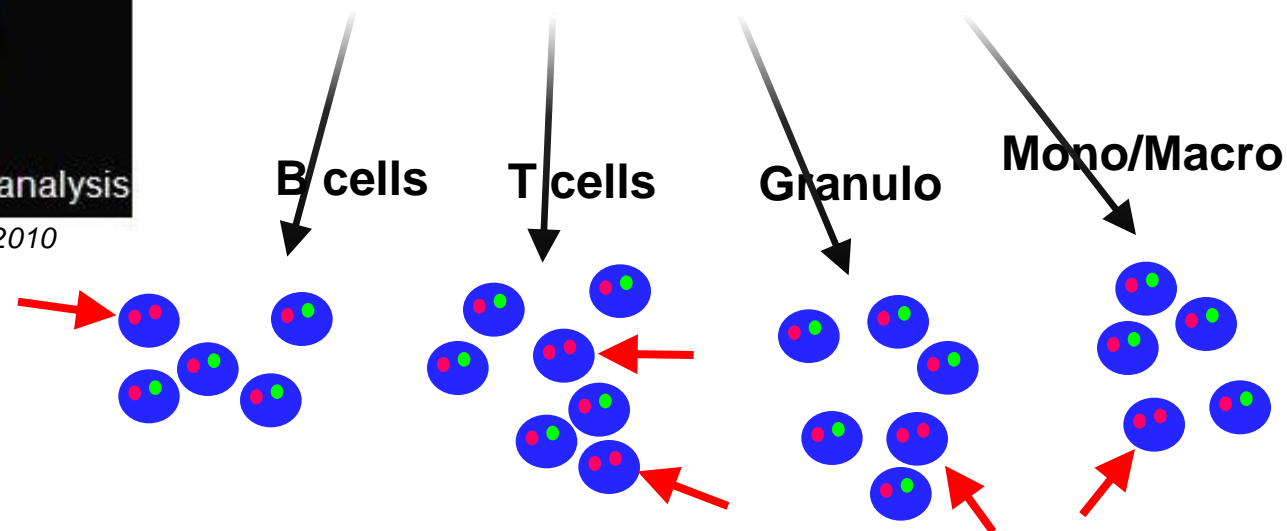


Maternal microchimerism in healthy adults in lymphocytes, monocyte/macrophages and NK cells

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Adapted from de Bellefon et al., Chimerism, 2010



Cells from a vanished twin as a source of microchimerism 40 years later in a male with a scleroderma-like condition

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