Risk factors, Diagnosis & Assessing disease activity

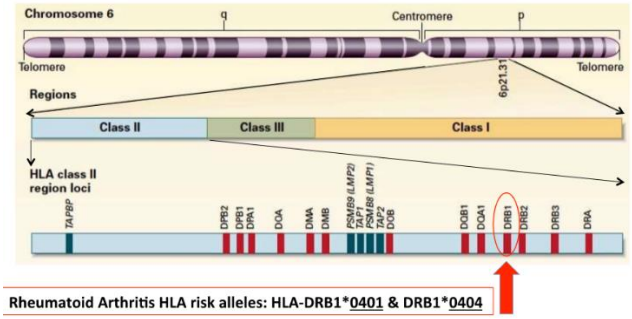
Risk factors:

Principle of autoimmunity: genetic susceptibility + environmental trigger = break down of immune tolerance (self-reactive Abs or T-cells) >>> autoimmune disease

Genetic factor:

- Through studies of **identical twins**, **genetics** account for **50 – 60 %** disease susceptibility >>> **remaining** is **environmental** cause
- Genetic **risk factors**: HLA – **12.7%** & non-HLA – **4%**

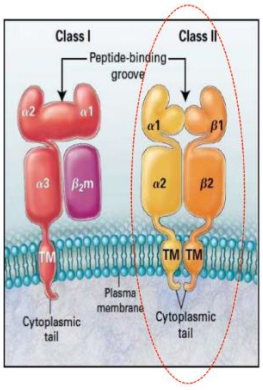
HLA:



HLA gene located on the **p-arm of chromosome 6** >>> **class 2 molecule** region >>> **DRB1** gene (encodes HLA-DR **antigen presenting molecule**) >>> **high-risk alleles** – DRB1*0401 & DRB1*0404

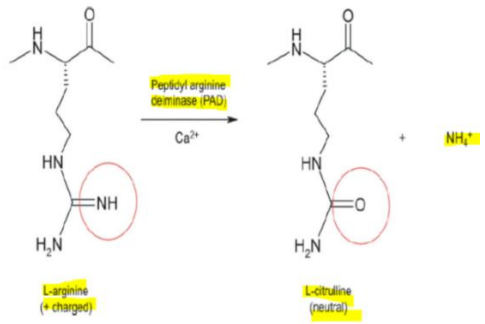
HLA-DBR1 alleles encode **five highly conserved aa sequence** called **shared epitope**

Shared epitope:



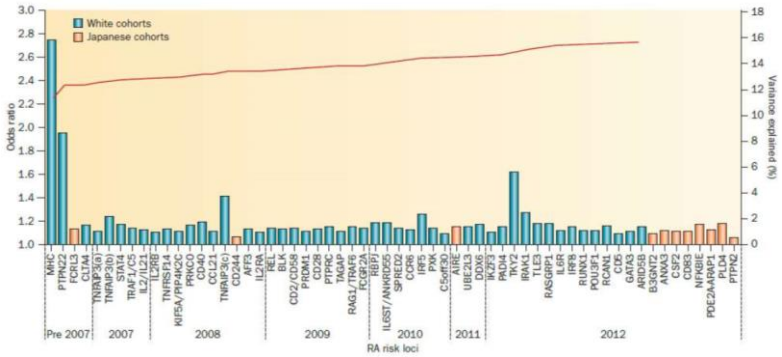
- Highly conserved **5 aa sequence** encoded by ALL **allelic variants** >>> **QKRAA** occupies positions from **70 to 74** of the **HLA-DR beta chain** (beta-1 domain) >>> this sequence surrounds **peptide binding groove**
- Predict the severity of disease: if we have **2 copies of high-risk alleles**, increased risk of **joint damage**, **erosions** & **extra-articular manifestations**
- Predict the production of anti-citrullinated protein antibody (**ACPA**/anti-CCP):
  - Expression of **ACPA** is the most consistent association with **shared epitope**
  - **ACPA** is used for **diagnosis** of ACPA positive RA
  - **Subclassify** patients as a **prognostic marker** for disease **severity**

Citrullination:



- **Post-translational** conversion of **arginine** to **citrulline** by peptidyl-arginine deiminase (**PADI**) enzyme **intra-** or **extra-**cellularly >>> **induction of PADI expression** & **peptide citrullination** are NOT specific to **RA** AND occurs in sites of **tissues stress** or **inflammation** >>> human have **4 isoforms** of PADI – PADI **2** & **4** abundant in **inflamed synovium**
- **Generate neoepitope** >>> serve as targets for **autoreactive antibodies** >>> **immune tolerance breakdown** leads to the generation of **ACPA**
- **Citrulline** binds **shared epitope** more **avidly**
- Arginine is '**positively**' charged whereas citrulline is '**neutral**' AND **ammonium** is produced as by-product

Non-HLA:



HLA-DR has the **highest odds ratio**

Multiple genetic risk factors for non-HLA risks:

PTPN22 double the risk of RA development compared to people who do NOT have this gene >>> seen ONLY in White cohorts AND not generalizable in **Asian** population

PADI4 (PADI enzyme) double the risk of RA development and primarily in **Asian** population

Odds ratio:

OR is the measure of association between an **exposure** & an **outcome** >>> odds that an outcome occurring in a group EXPOSED to a variable of interest, compared to the odds of outcome occurring in a group NOT EXPOSED to the same variable of interest

- OR = 1, exposure (HLA-DR beta 1) does NOT affect odds of outcome (non-contributory to RA)
- OR > 1, exposure (HLA-DR beta 1) associates with higher odds of outcome (risk factor)
- OR < 1, exposure (HLA-DR beta 1) associates with lower odds of outcome (protective)

Epigenetic factor:

Definition: Modification of **chromosome** that leads to altered gene expression, without changing DNA sequence >>> may be **heritable**

Possible mechanism:

- Post-translational histone modification
- DNA methylation in **fibroblast-like synoviocytes** & **T-cells**
- MicroRNAs (miRNA)

Hormonal factor:

Oestrogen exposure: **higher prevalence** of RA in **females**

Possible mechanism:

- **B-cells** (produce autoantibodies) are more **resistant to apoptosis** when exposed to **oestrogen**
- **Fibroblast-like synoviocytes** (FLS) have **oestrogen receptors** >>> interaction with oestrogen stimulates the production of **metalloproteinases** that is responsible for degradation of **bones**, **cartilage** and **adjacent structures**
- **Macrophage** expose to **oestrogen** >>> produce a lot of **TNF-alpha**

Pregnancy: **1st** & **2nd trimester** gives **75% remission**, **3rd trimester** gives **90% remission** and **postpartum** leads to **90% flare**

Possible mechanism:

- The combination between **oestrogen** & **progesterone** upregulates **anti-inflammatory IL10** >>> reduce the secretion of **TNF-alpha**
- mother carries foreign DNA hence changed cell-mediated immunity >>> **remission**

Environmental factor:

Pollution related:

- RA is first described in 1800 >>> not demonstrated before 19th century (**industrial revolution**) hence described as '**new world pathogen** or **allergen**' >>> hypothesis: the **environmental pollution** during **industrial revolution** triggers RA
- **Smoking**: the process of **citrullination** is seen in the lung of smokers (**PADI2** related) >>> this could form a target of autoimmunity
- **Bronchial stress**: exposure to **silica** & **traffic pollution** also lead to **citrullination**

Infection & **their products related**:

- Oral infection (e.g. periodontal disease)
    - **Periodontal disease** contributes to **RA formation** >>> periodontal is closely associated with Aggregatibacter Actinomycetemcomitans (**AA** more related than PG) and Porphyromonas gingivalis (**PG** – related to **PADI4**)
    - **Smoking** contributes to high incidence of **chronic oral inflammation**
- Toll-like receptor activation: direct cause of arthritis >>> **streptococcus** (e.g.) activate **TLR2** that consequently increase **PADI** expression >>> **citrullination**

Emerging theories:

Microbiome:

- In gnotobiotic (germ-free) experiment, it demonstrates that **gut bacteria** can induce **autoimmunity** in genetically **predisposed** model
- In two germ-free mouse experiment, it demonstrates that **bacteria** can induce **arthritis**
- **Dietary changes** or **antibiotics** may disrupt the **normal homeostasis of microbiome equilibrium** >>> **pathogenic bacteria overgrow** HENCE **chronic inflammation of gut mucosal** >>> **citrullination**

Neurological:

- With stroke suffers, they develop RA **asymmetrically** >>> ONLY on the **unparalysed** side of the body
- Experiment demonstrated that:
    - This protective effect is NOT associated with nerves
    - **Denervation of limbs** leads to the **alternation of microvasculature** >>> it impairs the transfer of **potentially pathogenic antibodies** into the joints that was the original cause of RA development
    - This finding could be a potential **therapeutic target**

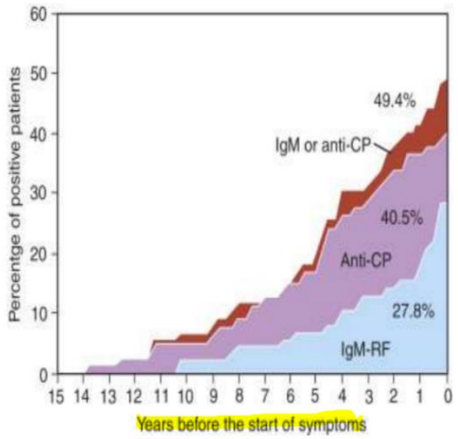
Diagnosis:

Abs in RA:

- Rheumatoid factor (RF):
    - High-affinity **autoantibody** against Fc portion (epitope) of **IgG**
    - Prior to RA onset, elevation of **IgM** & **IgA** isotypes
- ACPA:
    - **Antibody** against citrullinated proteins

- **Detected** via anti-cyclic citrullinated (**CCP**) peptide >>> the targets for this assay including **alpha-enolase**, **keratin**, **type II collagen** & **fibronectin**, etc.
- Prior to RA onset, **rapid rise** of **ACPA** & its **avidity**, **epitope spreading** (at the beginning, ACPA may only target **alpha-enolase** BUT as time spreads, ACPA also spreads its specificity against e.g. **keratin**) AND **isotype changes**

Abs development years before the onset of RA:



Retrospective study:

X-axis: 0 is the time that **disease develops**

Y-axis: **percentage of positive patients** who have **measurable level of antibodies**

Prior to onset of disease, we have detectable level of anti-CP (citrullinated protein) >>> NO **clinical symptoms** >>> conclude: **disease** in **serum** develops before **clinical symptoms**

As time goes on, the **titres of Abs** & **percentage of positive patients rises**

Specificity & sensitivity test:

	Disease Present	Disease Absent
Test Positive	**'A'** True Positive (*Sensitivity*)	**'B'** False Positive
Test Negative	**'C'** False Negative	**'D'** True Negative (*Specificity*)

Sensitivity = A / (A+C) >>> **snNout** (test with high sensitivity, a **negative** result rules out the **diagnosis**) >>> i.e. Infrequently **miss** patients who have the disease OR few false **negative** result

- **Specificity** = D / (B+D) >>> **spPin** (test with high specificity, a **positive** result rules in the **diagnosis**) >>> i.e. Infrequently **determine** patients AS having a disease when they do NOT OR few false **positive** result
- Positive likelihood ratio = sensitivity / (1 – specificity) OR (true positive / false **positive**) >>> ratio divides the **probability** that a patient **with** the **disease** will **test positive** by the **probability** that a patient **without** the disease will **test positive**
- Negative likelihood ratio = (1 – sensitivity) / specificity OR (false **negative** / true negative) >>> ratio divides the **probability** that a patient **without** the **disease** will **test negative** by the **probability** that a patient **with** the disease will **test negative**

Diagnostic utility of autoantibodies:

	IgM Rheumatoid Factor	Anti-Citrullinated Protein Antibodies
Sensitivity, % (95% CI)	**70** (66–73)	67 (64–70)
Specificity, % (95% CI)	79 (74–83)	**95** (94–96)
Positive Likelihood Ratio (95% CI)	3.3 (2.7–3.9)	**14.4** (11.6–18.0)
Negative Likelihood Ratio (95% CI)	0.39 (0.35–0.42)	0.35 (0.32–0.38)

Sensitivity of **IgM rheumatoid factor**: 100 individuals with RA & they **all** have **rheumatoid factor** >>> **70** out 100 will be tested **positive** AND miss **30**

Specificity of **IgM rheumatoid factor**: 100 individuals tested **positive** for **rheumatoid factor** >>> **79** out 100 would have RA AND 21 **false positive**

Sensitivity of **ACPA**: lower compared to **IgM rheumatoid factor**

Specificity of **ACPA**: **false positive** due to 1. Psoriatic arthritis 2. **AT**

AT = active tuberculosis

The higher the positive OR negative likelihood ratio, the more useful the test is >>> If we have RA, we are **14.4** times more likely to have **positive** test (ACPA test) than someone who does NOT have RA

Autoantibodies in RA (classification method 1): diagnosis + **re-stratify** patients

1. Sero**positive**: RF/ACPA positive >>> **prognostic** indicator of **radiographic** progression (deformities, erosions, etc.), **extra-articular** manifestations & **functional** impairment >>> HOWEVER, autoantibodies do NOT assess disease **activity**
2. Sero**negative**: this also include the false negatives (1 – sensitivity) >>> 30% from RF & 33% from ACPA

2010 ACR/EULAR (classification method 2):

- (who should be tested) newly presenting patients who have:
  - ≥ 1 joint with definite **clinical synovitis**
  - Synovitis can NOT be better explained by another disease
- **Originally** used for enrolling patients into a homogenous group for clinical studies
- **However**, we do NOT have **diagnostic criteria** for RA >>> clinicians **defer** to these criteria to facilitate diagnosis
- Classification criteria: $\geq 6/10$ implies **definite** RA

	Score
A. Joint involvement§	
1 large joint¶	0
2–10 large joints	1
1–3 small joints (with or without involvement of large joints)#	2
4–10 small joints (with or without involvement of large joints)	3
>10 joints (at least 1 small joint)**	5
B. Serology (at least 1 test result is needed for classification)††	
Negative RF *and* negative ACPA	0
Low-positive RF *or* low-positive ACPA	2
High-positive RF *or* high-positive ACPA	3
C. Acute-phase reactants (at least 1 test result is needed for classification)‡‡	
Normal CRP *and* normal ESR	0
Abnormal CRP *or* abnormal ESR	1
D. Duration of symptoms§§	
<6 weeks	0
≥6 weeks	1

Joint involvement = # & distribution of joints, RA has a preference of affecting **small joints** over large joints >>> the more joints affected, the higher score

Serology = autoantibodies test >>> RF/ACPA

Acute phase reactants = markers of inflammation >>> ESR/CRP measurements

NOTE: at least one of EACH three conditions required

Duration of symptoms = if the duration of symptoms is less than 6 weeks, it means less severe

Assessing disease activity:

Assessments:

- **Joint counts**: determine if the joint is swollen, tender OR both
- **Global assessment**: assessed by a single question with 0 – 10 responses
- **Pain score**: evaluate pain level out of 10
- **Morning stiffness**: > 1hr, indicates **active inflammatory oedema**
- **Laboratory**: erythrocyte sedimentation rate (ESR) & c-reactive protein (CPR)
- **Disability** & **fatigue**: can be an indicator BUT may also be due to SBD or depression
- **Radiological damage**: erosions indicate aggressive progress >>> radiographs have to be done **serially** ensuring NO new damage

DAS-28 ESR/CPR:

- Exam **28** joints using ESR or CRP >>> the joint score is combined with **global assessment** & **lab results**
- **Cutoff criteria**: assess disease activity

DAS28 < 2.6: Remission ←
DAS28 >= 2.6 and <= 3.2: Low Disease Activity
DAS28 > 3.2 and <= 5.1: Moderate Disease Activity
DAS28 > 5.1: High Disease Activity

Even with clinical **remission** (<2.8) patients, **subclinical inflammation** remains possible >>> could be detected through **image studies**