

HLA genotyping ANCA vasculitis patients

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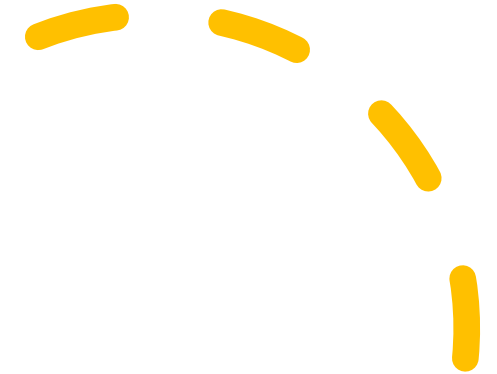
Prof Mark Little TCD

C-ANCA

P-ANCA

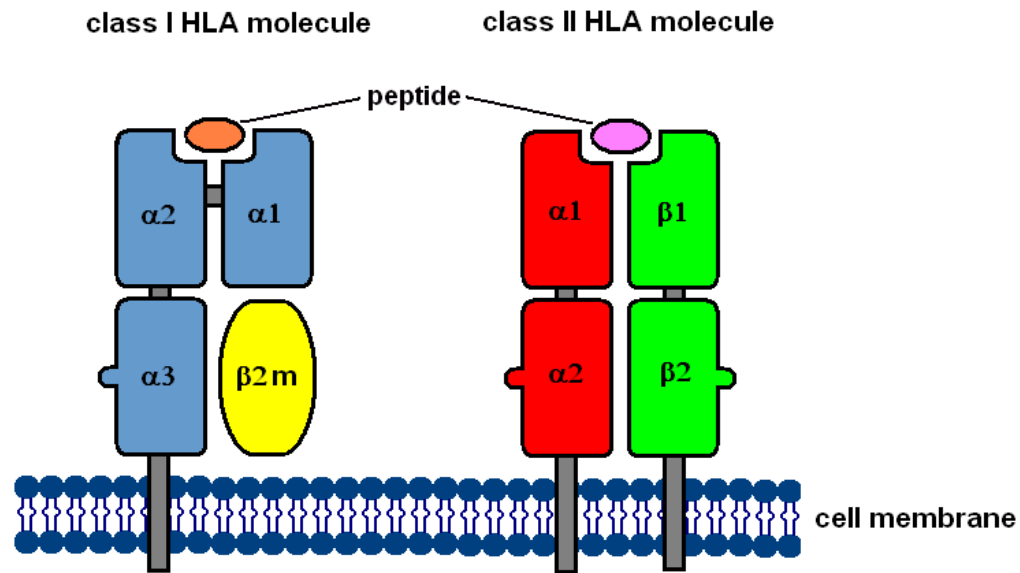
Anti-Neutrophil Cytoplasmic Antibody (ANCA) Vasculitis

- **Rare** multisystem autoimmune disease - inflammation of small blood vessels
 - affecting kidneys, lungs, ENT
- **2 types** of ANCA antibodies directed against neutrophil granule proteins:
 - PR3-ANCA : proteinase 3 (60% cases in Ireland)
 - MPO-ANCA : myeloperoxidase (40% cases)
- **Incidence** : 13-20 cases/million
 - Male 1.2 > 1 Female
 - Median age onset in Ireland: 64 years
 - Improved survival but significant impact on Quality of Life & 2.7-fold increased risk of death



- **Aetiology:** unknown.
 - Silica exposure increases risk.
 - repeated Staph. aureus infections
- **Incidence rates differ** in different populations
 - PR3-ANCA Northern Europe higher incidence
 - vs MPO-ANCA – high East Asia incidence (older onset, kidney involvement)
- **Diagnosis** time can take up to 10 weeks (ANCA Antibodies not specific to vasculitis)
- **Treatment** : immunosuppressants
 - 1 year vs 5 year survival : 94% vs 77%
 - Cause of death : infection > vasculitis
- Need to better stratify patients for treatment & risk of relapse

Human Leukocyte Antigen (HLA)



- Present antigen to T-cells
- Important in immune system to differentiate self- & non-self peptides

HLA associations with Autoimmune Diseases

Class I : HLA-A*29 (uveitis), HLA-B*27 (arthritis)

Class II : HLA-DRB1*15 &

DQB1*06 (multiple sclerosis & narcolepsy),

HLA-DQ (coeliac disease)

Class I – HLA- A/B/C

- all nucleated cells
- Present Intracellular peptides

Class II – HLA- DR/DQ/DP

- on antigen presenting cells
- Present Extracellular antigen

Aim, Objectives & Methods

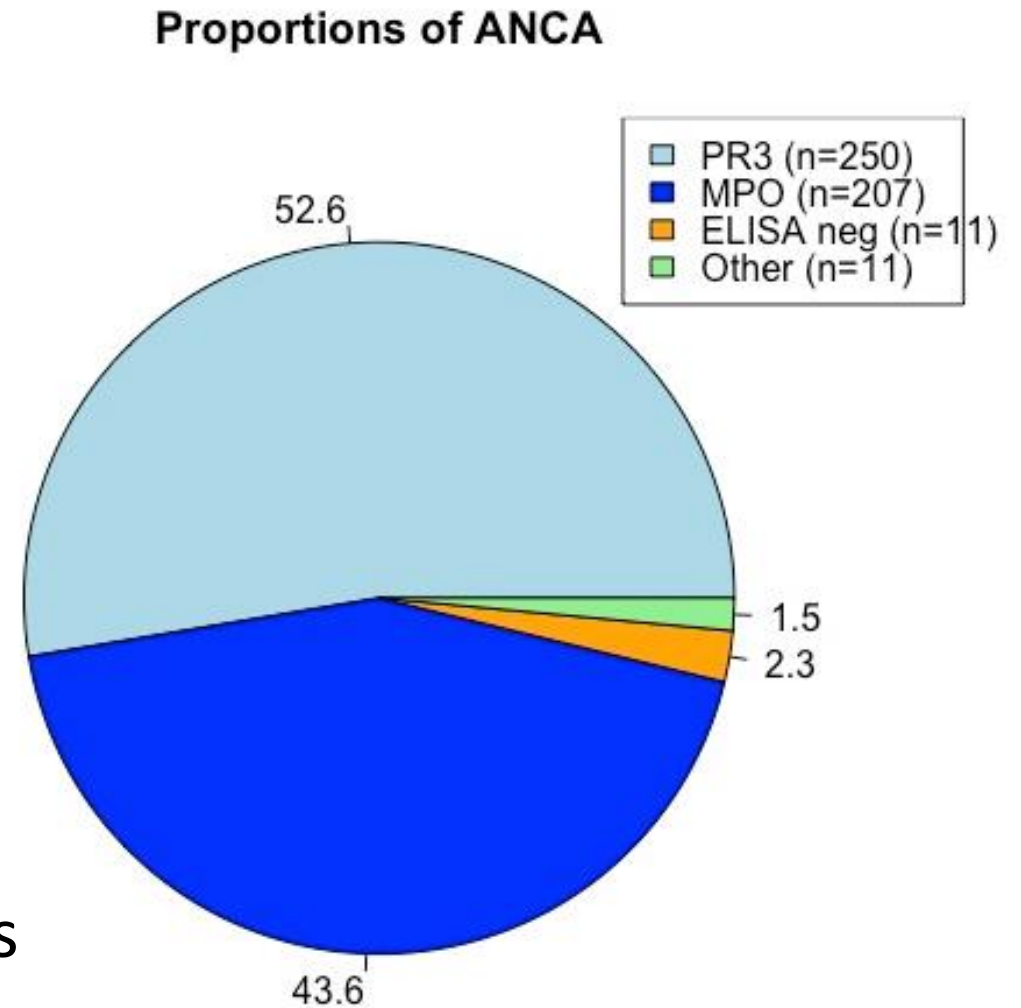
Previous GWAS Studies: Positive HLA associations

- 1. Specific HLA type group of Irish ANCA vasculitis patients
- 2. Compare HLA allele frequency to a control group of Irish bone marrow donors
- 3. Software used to compare HLA frequencies, HLA haplotypes, logistic regression
 - Hapl-o-Mat
 - PyHLA
 - BIGDAWG
 - midasHLA



Results

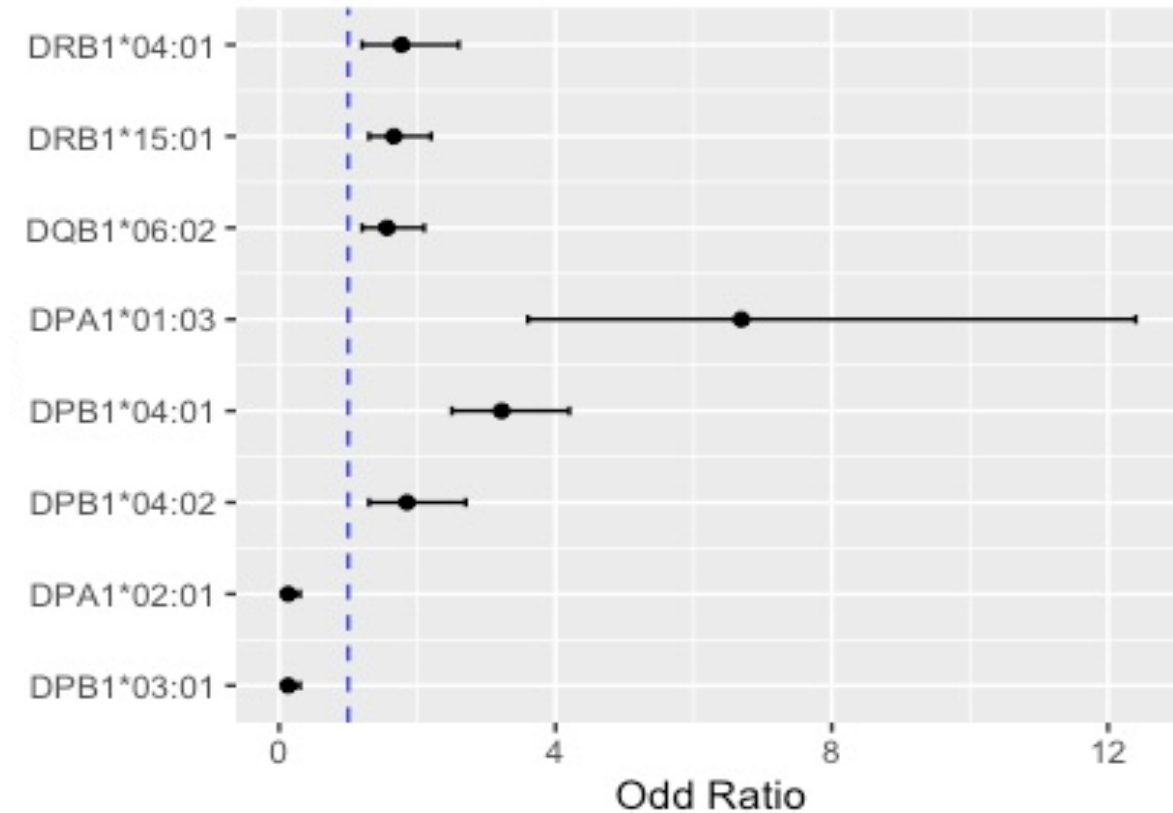
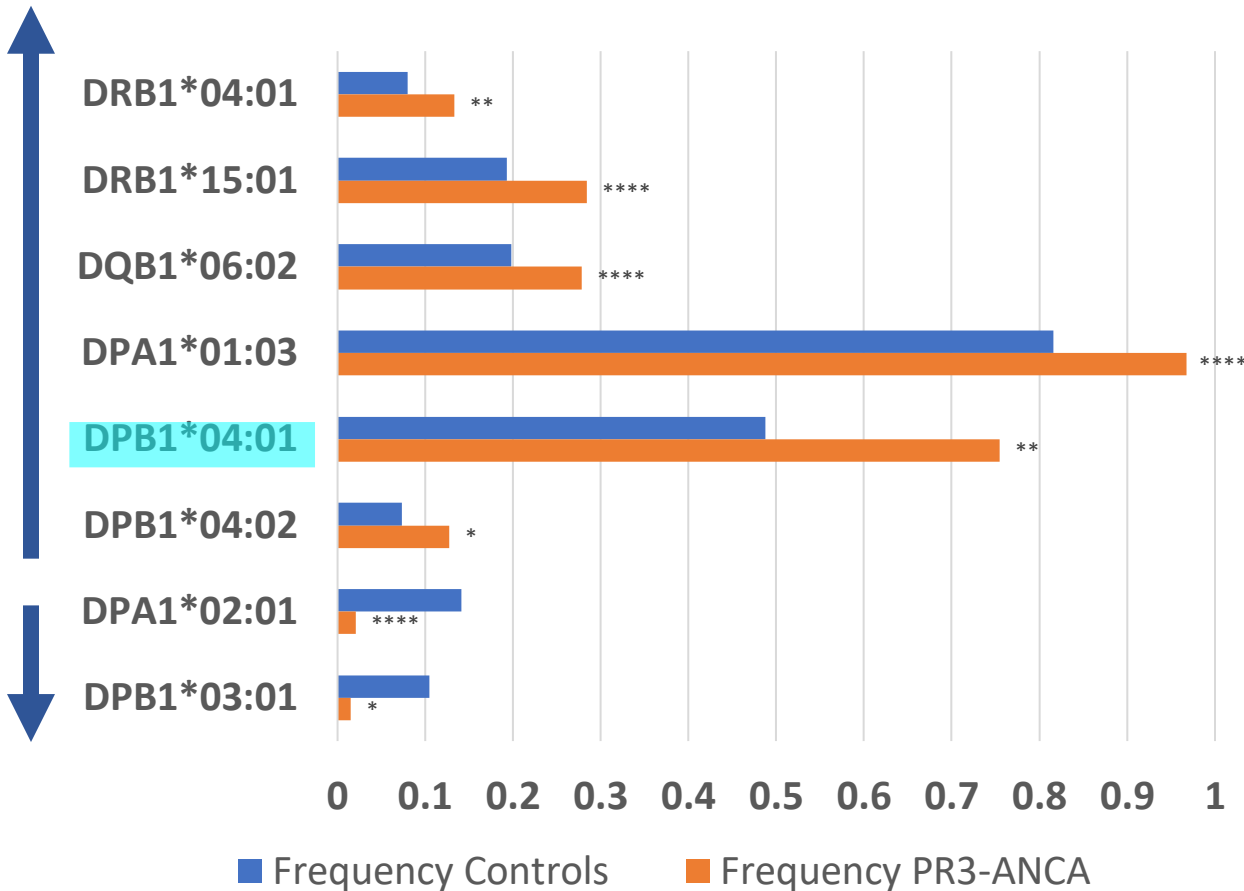
- Total samples tested: 475 patients
- 457 ANCA positive (PR3 or MPO)
- Control group – 6,124 Bone Marrow donors



PR3-AAV - Allele frequencies

250 of 475 patients (53%)

- 6 increased in PR3-AAV
- 2 decreased



Increased in patients:

-DPB1*04:01 (75% patient vs 48% controls)

Negatively associated in patients:

- DPA1*02:01/DPB1*03:01

(2% patients vs 14% controls)

Allele	Count (freq) 2n = 12,248	Count (freq) 2n = 500	Odds Ratio (95% Confidence Interval)	P-value adjusted	Sig
DPA1*01:03	10047 (0.82)	485 (0.969)	6.9 (4.0 – 11.7)	7.54E-16	****
DPB1*04:01	5933 (0.484)	380 (0.758)	3.3 (2.7 – 4.1)	1.88E-29	****

PR3- AAV: HLA Haplotypes

	Control 2n = 12,248	PR3-ANCA 2n=500			
	Count (frequency)	Count (frequency)	OR (95% Confidence Interval)	P-value	Sig
DPA1~DPB1					
01:03~04:01	5920 (0.483)	378 (0.753)	3.16 (2.4 - 4.2)	<2.22E-16	****
01:03~03:01^a	1106 (0.09)	6 (0.013)	0.13 (0.04 - 0.32)	2.86e-13	****
DRB1~DQA1~DQB1~DPA1~DPB1					
15:01~01:02~06:02~01:03~04:01	1709 (0.140)	117 (0.238)	1.98 (1.46 - 2.66)	3.39e-08	****

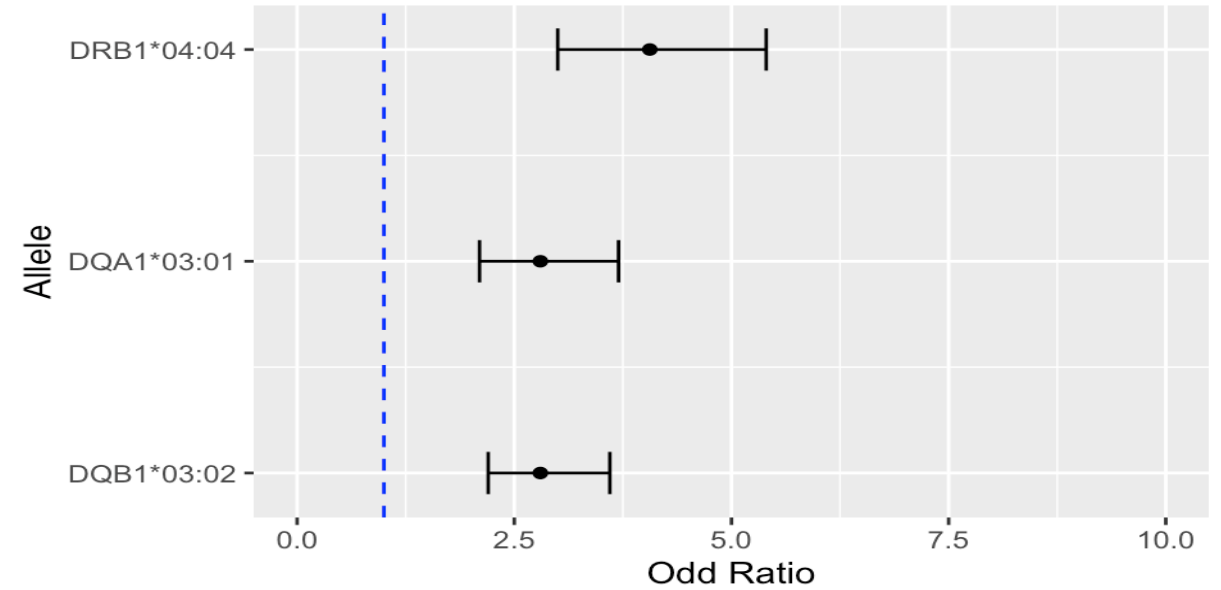
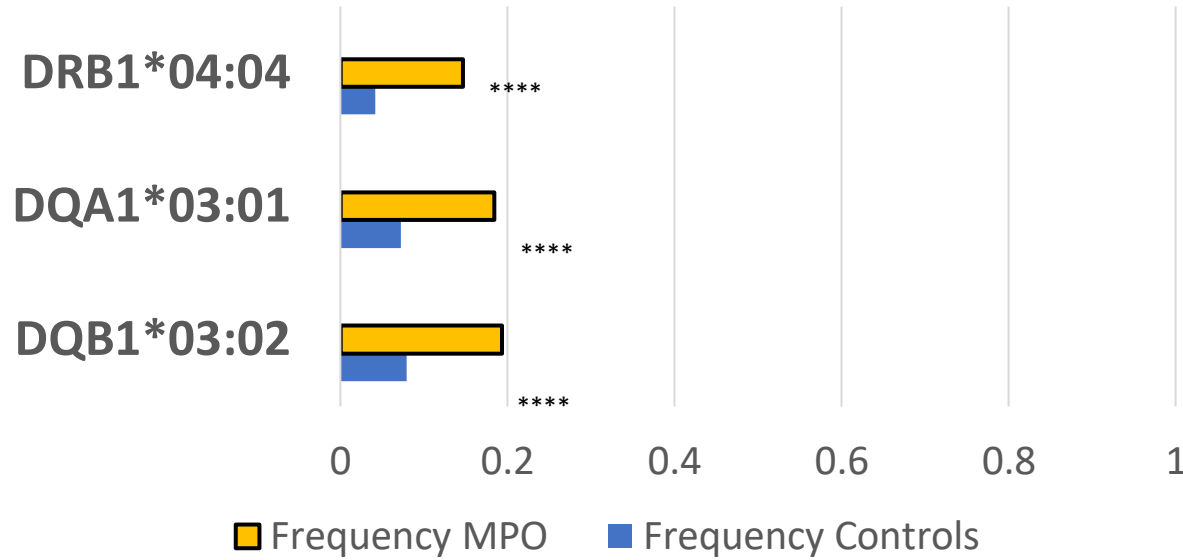
Conditional analysis

- independent signals in the haplotype

Allele	Control Count (frequency)	Case Count (frequency)	OR (95% Confidence Interval)	P.value
DPB1*04:01	4485 (0.36)	240 (0.48)	8.77 (4.9 - 17.7)	1.45E-09

MPO-AAV : Allele frequencies

207 of 475 patients (38%)



Increased in patients:

- DRB1*04:04 (14% vs 4% controls)
- DQA1*03:01 (18% vs 7% controls)
- DQB1*03:02 (19% vs 8% controls)

No Negatively associated allele

	Allele	Control 2n=12,248 Count (frequency)	MPO-AAV case 2n=414 Count (frequency)	OR 95% CI	P.Value
↑	DRB1*04:04	519 (0.042)	52 (0.147)	4.1 (3.0 – 5.4)	<2.22E-16
	DQA1*03:01	888 (0.072)	78 (0.184)	2.8 (2.1 – 3.7)	3.83E-17
	DQB1*03:02	965 (0.079)	80 (0.193)	2.8 (2.2 – 3.6)	1.10E-15

DRB1~DQA1~DQB1~DPA1~DPB1	Count (frequency)	Case (frequency)	OR 95% CI	P.Value	
04:04~03:01~03:02~01:03~04:01	212 (0.017)	17 (0.048)	2.9 (1.62 - 4.8)	8.32E-07	****

Future Directions:



Amino acid analysis



Add HLA types to diagnostic/therapeutic pipeline by PARADISE consortium

THANK YOU

Everyone in IBTS – MBG lab, R&D, L&D:

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Questions?

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