ASHG VIRTUAL MEETING 2020 OCTOBER 27-30

Identifying genetic modifiers of age at onset of multiple sclerosis.

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Disclosure Slide

Financial Disclosure for:

E. Misicka – no disclosures

Gap in the Knowledge – MS Age of Onset

- Multiple sclerosis (MS) age at onset (AAO) is a strong predictor of **timing to disease milestones:**
  - Earlier AAO is associated with reaching **disability milestones** at younger ages.
  - A **higher frequency of relapse** prior to transition to progressive MS.
  - Earlier AAO is associated with **transition** to progressive MS at younger ages.

- Are there genetic factors predicting MS AAO? Yes, but little is known about them.
  - ***HLA-DRB1*15:01*** (an established MS risk variant) confers earlier AAO.
  - A **genetic risk score** (GRS) comprised of **variants outside the Major Histocompatibility Complex** (MHC) confers earlier AAO.

Sawcer et al. 2011, *Nature;* Briggs et al. 2019, *Mult Scler Relat Disord;* Sorosina et al. 2015, *Mult Scler;* Barcellos et al. 2002, *Brain;* Masterman et al. 2000, *Ann Neurol;* Voskuhl and Gold, 2012, *Nat Rev Neurol*

Study Design

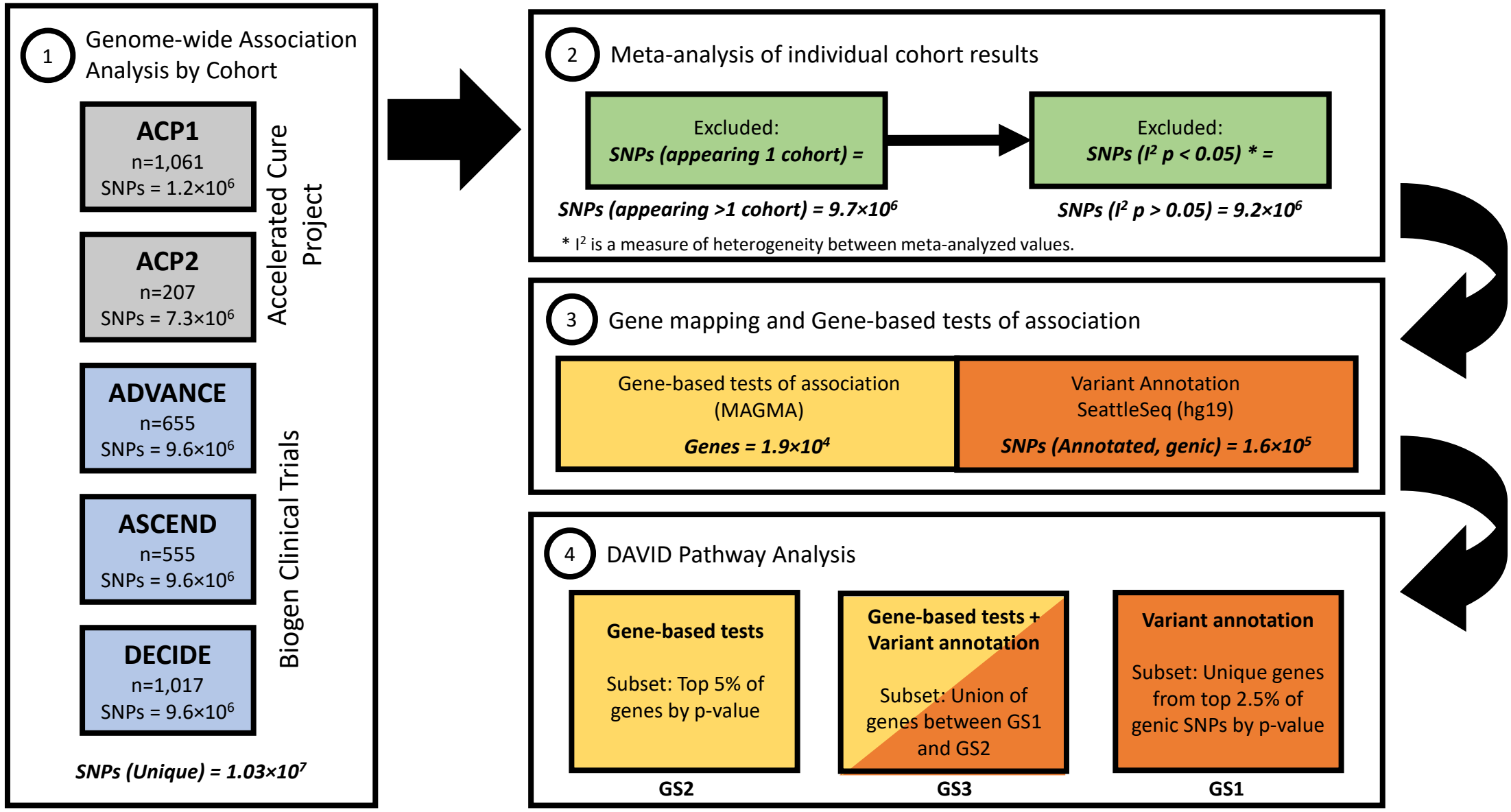
1. Performed **genome-wide association study (GWAS) analysis** across five **non-Hispanic white cohorts** to ascertain genetic variants associated with AAO

2. **Meta-analyzed GWAS results**

3. Performed **gene-based tests of association**

4. Performed **pathway enrichment analyses**

Study Design and Methods



Methods II: Investigation of MS Risk Variants

- ***HLA-DRB1*15:01*** was regressed on AAO
  - **Additive and dominant models**

- Non-MHC Variants
  - Each variant was **individually regressed on AAO**
  - An **unweighted** GRS was regressed on AAO.
  - **GRS quintiles** (1-5) were regressed on AAO

- Combinations of *HLA-DRB1*15:01* and GRS burden were regressed on AAO.

- **All models were adjusted for population substructure and sex.**

RESULTS: Investigation of MS Risk Variants

Genetic Predictor	β (95% CI)	p
*HLA-DRB1*15:01*	**-0.67** (-1.16, -0.19)	6.2×10^{-3}
Carrier of *HLA-DRB1*15:01*	**-1.00** (-1.58, -0.41)	9.0×10^{-4}
GRS	**-0.10** (-0.13, -0.06)	9.8×10^{-9}

Table 1A. Results of linear regression of genetic factors on MS AAO.

- Consistent and significant trends were observed for earlier AAO with increasing load of MS risk variants (**Table 1A**)
- Distribution of AAO and GRS was similar across cohorts (**Figures 1** and **2**).

Figure 1. Distribution of AAO

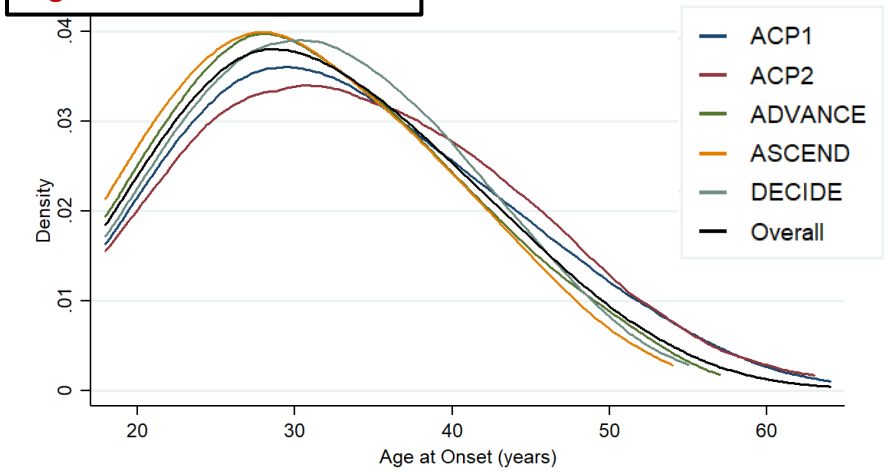
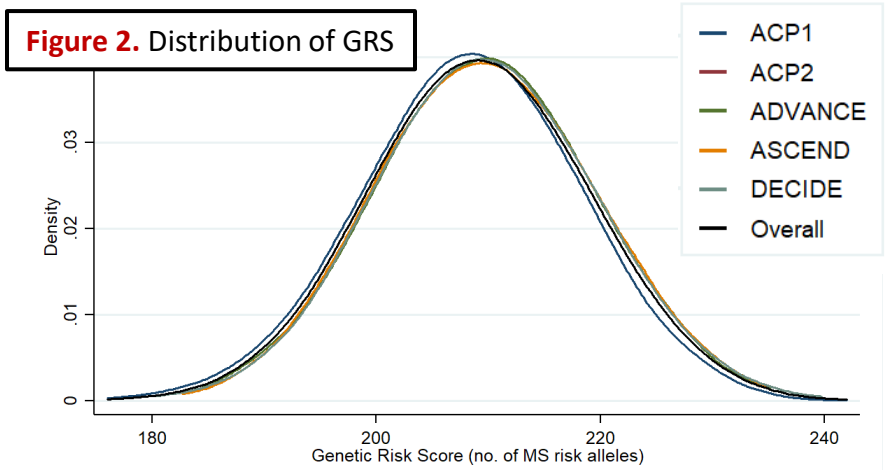


Figure 2. Distribution of GRS



RESULTS: Investigation of MS Risk Variants

- **Table 1B** shows the results for linear regressions for GRS quintiles on AAO, as well as the simultaneous regression of *HLA-DRB1*15:01* and the GRS quintiles.
- In general, an **increasing load of genetic variants confers earlier AAO**
- Individuals with the highest genetic risk burden were on average **5 years younger** at MS onset than those with the lowest genetic risk burden

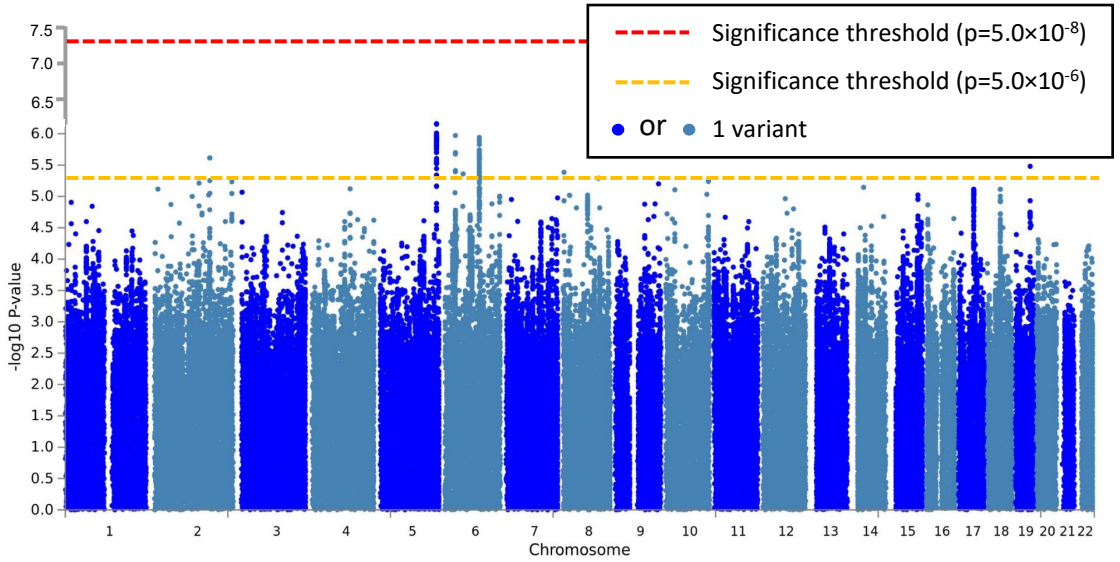
Genetic Predictor			β (95% CI)	p	p trend
GRS Quintile	Q1 (n=717)		Ref	-	2.1×10^{-7}
	Q2 (n=722)		-1.84 (-2.74, -0.94)	6.8×10^{-5}	
	Q3 (n=658)		-2.27 (-3.20, -1.34)	1.7×10^{-6}	
	Q4 (n=700)		-2.32 (-3.24, -1.41)	6.7×10^{-7}	
	Q5 (n=698)		-2.49 (-3.41, -1.57)	1.2×10^{-7}	
Combinations of genetic risk factors	0 copies of *HLA-DRB1*15:01*	Q1 (n=388)	Ref	-	2.9×10^{-8}
		Q2 (n=384)	-1.93 (-3.17, -0.70)	2.1×10^{-3}	
		Q3 (n=332)	-2.08 (-3.36, -0.80)	1.5×10^{-3}	
		Q4 (n=399)	-2.64 (-3.87, -1.42)	2.4×10^{-5}	
		Q5 (n=396)	-2.44 (-3.67, -1.21)	1.0×10^{-4}	
	1 copy of *HLA-DRB1*15:01*	Q1 (n=273)	-1.32 (-2.68, 0.04)	0.057	
		Q2 (n=300)	-2.72 (-4.04, -1.40)	5.5×10^{-5}	
		Q3 (n=289)	-3.47 (-4.80, -2.13)	3.6×10^{-7}	
		Q4 (n=256)	-3.37 (-4.76, -1.99)	1.8×10^{-6}	
		Q5 (n=271)	-3.55 (-4.91, -2.19)	3.2×10^{-7}	
	2 copies of *HLA-DRB1*15:01*	Q1 (n=56)	0.01 (-2.45, 2.46)	0.996	
		Q2 (n=38)	-3.53 (-6.44, -0.61)	0.018	
		Q3 (n=37)	-3.47 (-6.42, -0.52)	0.021	
		Q4 (n=45)	-1.22 (-3.92, 1.48)	0.376	
		Q5 (n=31)	**-4.98** (-8.19, -1.78)	2.3×10^{-3}	

Table 1B. Results of linear regression of multiple categorical genetic factors on MS AAO.

RESULTS: Meta-Analysis of GWA Results

- No variants were significant at $p<5.0\times10^{-8}$ (**Figure 3**). At **$p<5.0\times10^{-6}$**, seven independent risk loci were nominally associated with AAO (**Table 2**).

Figure 3. Manhattan plot of meta-analyzed GWA results



Chr	BP	rsID	MAF	A1	A2	N	N(Cohorts)	β (SE)	p	Gene	SNP Function
2	170812811	rs145201293	0.30	T	TTA	2,434	3	1.3 (0.28)	2.4×10^{-6}	*UBR3*	Indel
5	173141042	rs17076315	0.35	A	G	2,434	4	-1.3 (0.26)	6.9×10^{-7}	*LINC01484*	Intron variant
6	32626537	rs28672722	0.26	T	G	3,495	5	1.2 (0.25)	1.1×10^{-6}	*HLA-DQB1*	Upstream transcript variant
6	56121561	rs149847639	0.015	A	C	1,210	2	-7.5 (1.60)	4.4×10^{-6}	*COL21A1*	Intron variant, genic upstream transcript variant
6	106149587	rs17066212	0.022	A	G	2,434	4	-3.9 (0.80)	1.1×10^{-6}	-	Intergenic
8	3655967	rs74402157	0.028	T	G	2,434	3	-4.5 (0.98)	4.1×10^{-6}	*CSMD1*	Intron variant, genic upstream transcript variant
19	46163870	rs34132828	0.24	A	G	3,495	5	1.2 (0.25)	3.3×10^{-6}	-	Intergenic

Table 2. Risk loci for meta-analysis of 5 cohorts at $p<5.0\times10^{-6}$

RESULTS: Gene-based tests of association

- **~19,000 genes** were tested. Of these genes, **1,067 were associated with AAO at p<0.05** (**Table 3**).

SYMBOL	CHR	START	STOP	nSNPS	P (0kb)
SSB	2	170648443	170668574	63	2.57×10^{-5}
TRAFD1	12	112563305	112591407	23	2.67×10^{-5}
HECTD4	12	112597992	112819896	225	2.78×10^{-5}
MMP8	11	102582526	102597781	85	2.85×10^{-5}
NAA25	12	112464500	112546826	78	4.50×10^{-5}

Table 3. Top 5 results from gene-based tests of association.

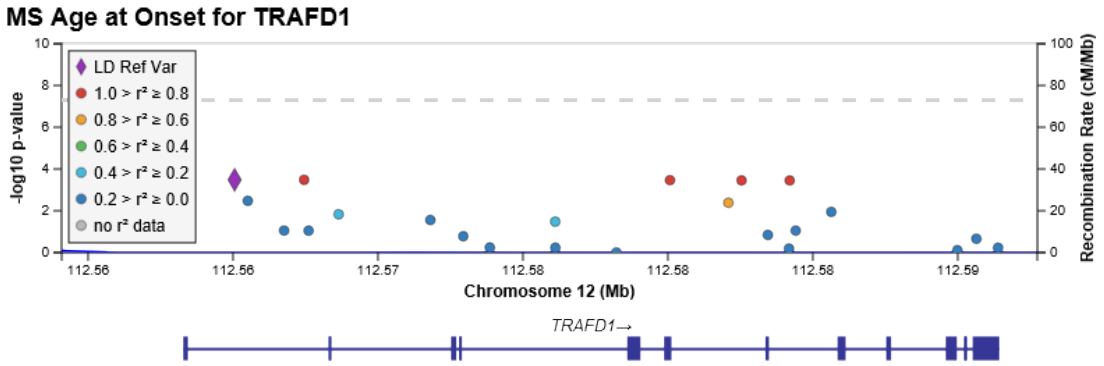


Figure 4. LocusZoom plot of TRAFD1, a negative feedback inhibitor of the innate immune response.

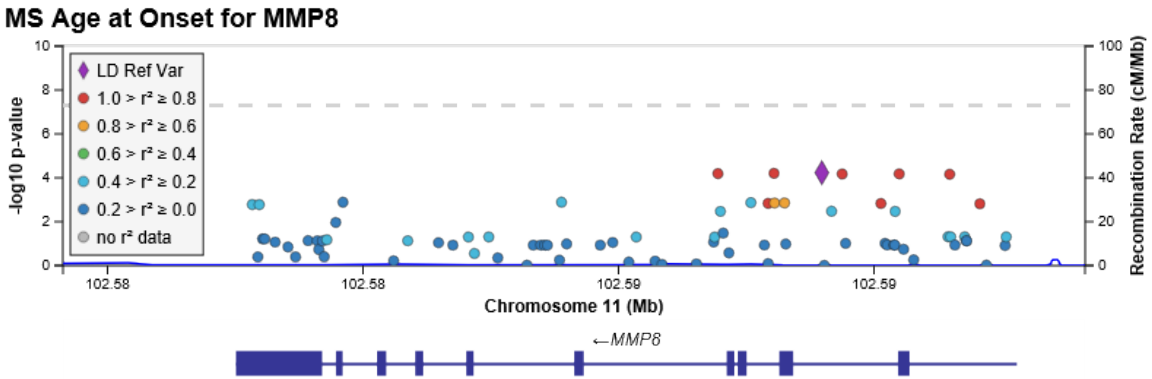


Figure 5. LocusZoom plot for *MMP8,* a matrix metalloproteinase that breaks down collagen in neutrophils and leukocytes.

Results: Pathway Analysis

- Gene Sets
  - **GS1** – Variant Annotations – **950 genes**
    - Unique genes from top 2.5% of genic, associated variants
  - **GS2** – Gene-based Test Results – **872 genes**
    - Top 5% of genes from MAGMA analysis
  - **GS3** – Combined GS1 and GS2 – **1,574 genes**

Table 4. Top 5 enriched pathways for all gene sets

List	Database	Pathway	Fold Enrichment	P-Value	FDR	Genes
GS1	REACTOME	NCAM1 interactions (R-HSA-419037)	4.5	6.7×10^{-4}	0.0099	*NCAM1, COL9A2, COL9A3, COL6A6, COL6A5, CNTN2, ST8SIA2, CACNA1C, COL5A1*
	REACTOME	Generation of second messenger molecules (R-HSA-202433)	4.7	1.3×10^{-3}	0.019	*FYB, HLA-DQB1, PAK2, HLA-DRB1, CD247, HLA-DQA2, HLA-DQA1, HLA-DRA*
	KEGG	Cell adhesion molecules (CAMs) (hsa04514)	2.4	1.7×10^{-3}	0.022	*HLA-DQB1, PTPRM, HLA-DRB1, NRXN3, NLGN1, HLA-C, NRXN1, CDH3, HLA-DQA2, HLA-DQA1, NCAM1, NRCAM, CNTN2, CNTN1, CNTNAP2, HLA-DOB, HLA-DRA*
	REACTOME	Translocation of ZAP-70 to Immunological synapse (R-HSA-202430)	5.8	3.1×10^{-3}	0.045	*HLA-DQB1, HLA-DRB1, CD247, HLA-DQA2, HLA-DQA1, HLA-DRA*
	REACTOME	Collagen biosynthesis and modifying enzymes (R-HSA-1650814)	3.1	4.0×10^{-3}	0.059	*COL9A2, COL9A3, COLGALT2, COL21A1, ADAMTS14, COL6A6, COL6A5, COL22A1, COL25A1, COL5A1*
GS2	BioCarta	Complement Pathway (h_compPathway)	6.8	3.2×10^{-4}	0.0040	*MASP1, C4A, C4B, CFB, C5, C1S, C2*
	REACTOME	Activation of C3 and C5 (R-HSA-174577)	12.8	3.3×10^{-4}	0.0050	*C4A, C4B, CFB, C5, C2*
	REACTOME	Abortive elongation of HIV-1 transcript in the absence of Tat (R-HSA-167242)	6.2	6.3×10^{-4}	0.0095	*POLR2H, NELFCD, SUPT4H1, NELFE, CTDP1, POLR2C, POLR2B*
	KEGG	Staphylococcus aureus infection (hsa05150)	3.8	9.7×10^{-4}	0.013	*MASP1, C4A, C4B, CFB, C5, C1S, C2, HLA-DOB, HLA-DQA1, HLA-DRA*
	BioCarta	Lectin Induced Complement Pathway (h_lectinPathway)	7.4	3.2×10^{-3}	0.039	*MASP1, C4A, C4B, C5, C2*
GS3	KEGG	Staphylococcus aureus infection (hsa05150)	2.9	1.3×10^{-3}	0.017	*HLA-DQB1, MASP1, C4A, HLA-DRB1, C4B, CFB, C5, C1S, C2, HLA-DQA2, HLA-DOB, HLA-DQA1, HLA-DRA*
	REACTOME	Activation of C3 and C5 (R-HSA-174577)	7.6	2.4×10^{-3}	0.037	*C4A, C4B, CFB, C5, C2*
	REACTOME	NCAM1 interactions (R-HSA-419037)	2.9	6.1×10^{-3}	0.090	*NCAM1, COL9A2, COL9A3, COL6A6, COL6A5, COL3A1, CNTN2, ST8SIA2, CACNA1C, COL5A1*
	BioCarta	Complement Pathway (h_compPathway)	3.9	6.2×10^{-3}	0.076	*MASP1, C4A, C4B, CFB, C5, C1S, C2*
	REACTOME	Generation of second messenger molecules (R-HSA-202433)	3.0	7.5×10^{-3}	0.11	*FYB, HLA-DQB1, PAK2, HLA-DRB1, PLCG1, CD247, HLA-DQA2, HLA-DQA1, HLA-DRA*

Conclusions

- There is an evident gradient between increasing genetic risk burden and earlier AAO of MS, suggesting that **a higher genetic risk burden accelerates onset of MS.**

- We also present data suggesting that the complement system plays a role in MS AAO, along with other aspects of innate and adaptive immune function.