Shared genetic and environmental risk factors for the autoimmune diseases Type 1 Diabetes Mellitus and Multiple Sclerosis.

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Background

Multiple Sclerosis (MS) and type 1 diabetes mellitus (T1DM) are both autoimmune diseases with inflammatory, auto-antigen-specific T-cell, and decreased T-cell suppressor components, but they affect different organs and have marked differences in pathogenesis and clinical manifestations. Despite this, they co-occur more often than expected by chance, pointing to shared susceptibility factors. Low sun exposure and low vitamin D (vitD) levels are common risk factors, as demonstrated by clear latitude gradients of disease prevalence.

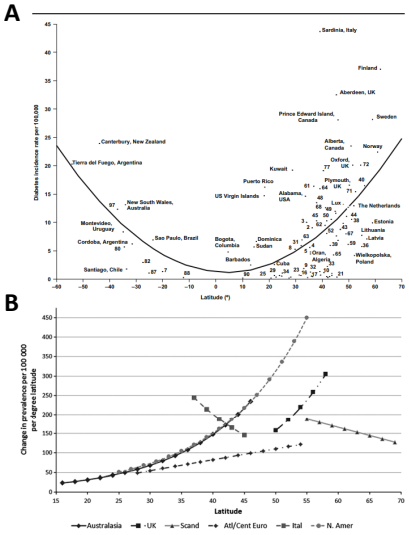


Figure 1: Type I Diabetes Mellitus (A, ref 12) and Multiple Sclerosis (B, ref 13) both display a latitude gradient of disease prevalence.

Genome-wide association studies and candidate gene studies have identified shared genetic risk factors, including variants in the vitamin D pathway gene, CYP27B1. In addition, both MS and T1DM risk variants are over-represented in the vitD receptor (VDR) cistrome of myeloid cells ($p<0.005$, FDR<0.05). There are also several marked differences between MS and T1DM in terms of genetic and environmental risk. For instance, the HLADRB1*1501 allele confers protection in T1DM (OR 0.03) but has an opposing effect on risk in MS where it is a risk allele of large effect present in over 55% of individuals with MS (OR 3.92). In addition, MS and T1DM are associated with different viral infections; MS is associated with EBV infection, whereas T1DM is associated with coxsackievirus B1 infection.

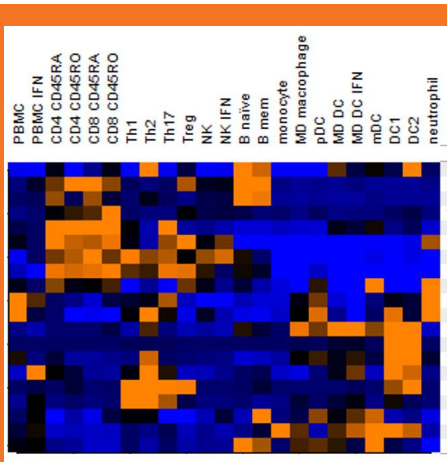
Methods

As associated SNPs from different studies may tag the same genetic regions, we compared linkage disequilibrium (LD) regions around each MS and T1DM associated SNP using the rAggr software program (LD cuttoff of $R^2>0.8$, maximum distance 500kb) to better define the shared risk genes.

We also sought rare variants (prevalance <0.01 in 1000 genome project) that may be contributing to disease risk using whole genome sequencing (WGS) of genomic DNA from individuals with both T1D and MS. Sequencing libraries were prepared using the TruSeq DNA PCR-free Library Preparation Kit and sequenced on the Illumina HiSeq X Ten platform. Multiple algorithms were used to filter the variants for impact on the genome including CADD, polyphen and SIFT. The overlap with the identified MS and T1DM risk genes was also determined. In addition, individuals were genotyped for HLADRB1*1501 using the tagging SNP rs9271366 and confirmed by sanger sequencing.

Patient	Age	Sex	DRB1*1501 status
p124	23	M	-/-
p150	30	M	-/-
p238	38	M	-/-
p901	38	F	-/-
p902	35	F	-/-

Table 1: Individuals with both MS and T1DM genotyped using whole genome sequencing.



GENE	Multiple Sclerosis risk SNP (ref)	Type 1 Diabetes Mellitus risk SNP (ref)
CLEC16A	rs12927355 (1)	rs12708716 (5,9), rs12927355 (10,6)
CD69	rs10466829 (2)	rs10492166 (10)
BACH2	rs72928038 (1)	rs72928038 (6)
TNFAIP3 $	rs67297943 (1)	rs6920220 (4,6)
IL7R $	rs6897932 (2), rs6881706 (1)	rs11954020 (6)
AGAP2	rs201202118 (1)	Rs10877012 *# (11)
CLEC2D	rs10466829 (2)	rs10492166 (10)
IKZF1 $	rs201847125 (1)	rs62447205 (6)
TYK2	rs34536443 (1)	rs34536443 (6)
BAD	rs694739 (1)	Rs694739 # (8)
PRDX5	rs694739 (1)	Rs694739 # (8)
METTL1	rs201202118 (1)	Rs10877012 *# (11)
CYP27B1	rs201202118 (1)	Rs10877012 *# (11)
TSFM	rs201202118 (1)	Rs10877012 *# (11)
SOCS1	rs12927355 (1)	rs12708716 (5,9), rs12927355 (10,6)
IL2R $	rs7090512, rs3118470 (2), rs2104286 (1), rs12722489, rs7089861 (3)	rs7090530 (10), rs12251307 (9), rs61839660, rs10795791, rs41295121 (6)
RMI2	rs12927355 (1)	rs12708716 (5,9), rs12927355 (10,6)
CLECL1	rs10466829 (2)	rs10492166 (10)
CTSH $	rs59772922 (1)	rs3825932 (7), rs34593439 (6)
DEXI	rs12927355 (1)	rs12708716 (5,9), rs12927355 (10,6)

$ T1DM and MS risk SNPS not in strong LD ($R^2<0.8$);* Candidate gene study; # GWAS p value <1e-5, >5e-8

Figure 1: Immune Cell subset gene expression by RNAseq of genes associated with risk of both Multiple Sclerosis and Type 1 Diabetes Mellitus. There are 15 candidate genes corresponding to 6 loci with risk SNPS in strong LD ($R^2>0.8$) and a further 5 genes (indicated by the $ symbol) where the risk SNPs for the 2 diseases are not in strong LD ($R^2<0.8$). This pattern of gene expression across the different immune cell subsets indicates that the shared genetic risk across MS and T1DM likely involves multiple arms of the immune system.

All 5 individuals with both MS and T1DM sequenced were homozygous negative for the HLADRB1*1501 allele. This absence is significantly different than the prevalence of this allele in the MS population (Chi-Square p= 0.038).

Patient	High Impact	Medium Impact	Scaled CADD Score >15	polyphen: possibly damaging	polyphen: probably damaging	SIFT: deleterious	TF binding site (ENCODE)	MS risk genes containing variants	T1DM risk genes containing variants
p124	17	428	203	50	70	104	95	PLEKHG5, PTPRK, RPS6KB1, TNFAIP3	GLIS3, GSDMB
p150	23	529	260	75	75	151	111	EVI5, IL7R	FUT2, IL7R
p238	23	424	213	65	75	126	87	TET2, WWOX	DGKA, GLIS3, PTPN22
p901	18	400	204	53	74	128	75	CYP24A1, PLEKHG5, TNFRSF1A	
p902	27	413	223	61	77	114	72	PTPRK, SLC2A4RG	

Table 2: Summary of rare variants identified by whole genome sequencing of individuals with both MS and T1DM.

The WGS identified several high impact rare variants in each of the individuals including variants resulting in loss or gain of stop codons, changes in initiator codon, splice donor or splice acceptor site changes. A higher number of medium Impact rare variants were identified and these included variants resulting in missense mutations or splice region changes. Three of the 5 individuals had rare variants identified in genes associated with T1DM risk and all 5 had rare variants identified in MS risk genes.

Conclusions

These data implicate shared, specific immune dysregulation between T1DM and MS. Further study into the risk genes common to these two diseases may provide additional insight into the autoimmune process and reveal common pathways for therapeutic targeting. Downstream analysis of the consequence of the rare variants identified in by WGS may provide novel explanations as to why these individuals have developed multiple autoimmune diseases. The absence of the DRB1*1501 allele in individuals with both MS and T1DM warrants further investigation in a larger cohort and if validated may provide a link between the HLA genotype and the differences in pathogenesis and clinical manifestation in the two diseases.

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