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Genetic overlap between multiple sclerosis and several cardiovascular disease risk factors

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Abstract

Background: Epidemiological findings suggest a relationship between multiple sclerosis (MS) and cardiovascular disease (CVD) risk factors, although the nature of this relationship is not well understood.

Objective: We used genome-wide association study (GWAS) data to identify shared genetic factors (pleiotropy) between MS and CVD risk factors.

Methods: Using summary statistics from a large, recent GWAS (total $n > 250,000$ individuals), we investigated overlap in single nucleotide polymorphisms (SNPs) associated with MS and a number of CVD risk factors including triglycerides (TG), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, body mass index, waist-to-hip ratio, type 2 diabetes, systolic blood pressure, and C-reactive protein level.

Results and conclusion: Using conditional enrichment plots, we found 30-fold enrichment of MS SNPs for different levels of association with LDL and TG SNPs, with a corresponding reduction in conditional false discovery rate (FDR). We identified 133 pleiotropic loci outside the extended major histocompatibility complex with conditional FDR < 0.01, of which 65 are novel. These pleiotropic loci were located on 21 different chromosomes. Our findings point to overlapping pathobiology between clinically diagnosed MS and cardiovascular risk factors and identify novel common variants associated with increased MS risk.

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Introduction

Multiple sclerosis (MS) is an autoimmune disease characterized by demyelination of the central nervous system.[1] A recent systematic review of six databases suggests that cardiovascular disease (CVD) risk is increased among patients with MS.[2] However, it is unclear whether an increased CVD risk is secondary to lifestyle and environmental variables, such as medication use, dietary factors, and physical activity, or due to overlapping pathobiology between CVD risk factors and MS.

Large genome-wide association studies (GWASs) provide valuable insights into the role of biologic pathways in disease pathogenesis and have identified genetic polymorphisms associated with a range of human disorders and phenotypes.[3,4] Recent GWAS have identified a total of 110 single nucleotide polymorphisms (SNPs) associated with MS.[5,6] Combining GWAS from multiple disorders and phenotypes provides insights into genetic pleiotropy (defined as a single gene or variant being associated with more than one distinct phenotype) and could elucidate shared pathobiology. Using this approach, we have recently reported genetic overlap between a number of diseases and phenotypes and identified novel common variants associated with schizophrenia, bipolar disorder, prostate cancer, hypertension, primary sclerosing cholangitis, and Alzheimer's disease.[7–15] Here, taking advantage of several large GWASs, we evaluated genetic overlap between MS and a number of CVD risk factors, including systolic blood pressure (SBP),[16] low-density lipoprotein (LDL) cholesterol,[17] high-density lipoprotein (HDL) cholesterol,[17] triglycerides

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(TG),[17] type 2 diabetes (T2D),[18] body mass index (BMI),[19] waist-to-hip ratio (WHR),[20] and C-reactive protein (CRP) level.[21]

Materials and methods

Ethics statement
The relevant institutional review boards or ethics committees approved the research protocol of the individual GWAS used in the current analysis, and all human participants gave written informed consent.

Participant samples
We utilized summary statistics GWAS data (*p*-values and odds ratios) from the International Multiple Sclerosis Genetics Consortium (IMSGC) ($n = 27,148$)[6] and from GWAS evaluating SBP ($n = 203,056$),[16] LDL ($n = 188,577$),[17] HDL ($n = 188,577$),[17] TG ($n = 188,577$),[17] T2D ($n = 22,044$),[18] BMI ($n = 123,865$),[19] WHR ($n = 77,167$)[20] and CRP ($n = 66,185$)[21] (for details, please see Supplementary Table 1). All studies were approved by the respective ethical committees and institutional review boards.

Statistical analysis
Using recently developed statistical methods to evaluate pleiotropic effects,[7–11] we evaluated genetic overlap between MS and CVD risk factors. For given associated phenotypes A and B, pleiotropic "enrichment" of phenotype A with phenotype B exists if the proportion of SNPs or genes associated with phenotype A increases as a function of increased association with phenotype B (see Supplementary Text for details). To assess for pleiotropic enrichment, we constructed fold-enrichment plots of empirical quantiles of nominal $-\log_{10}(p)$ values for SNP association with MS for all SNPs and for subsets of SNPs determined by the nominal *p*-values of their association with CVD factors (BMI, CRP, HDL, LDL, SBP, T2D, TG, and WHR). In fold-enrichment plots, the presence of enrichment is reflected as an upward deflection of the curve for phenotype A if the degree of deflection from the expected null line is dependent on the degree of association with phenotype B. To assess for polygenic effects below the standard GWAS significance threshold, we focused the fold-enrichment plots on SNPs with nominal $-\log_{10}(p) < 7.3$ (corresponding to $p > 5 \times 10^{-8}$). The nominal *p*-values ($-\log_{10}(p)$) are plotted on the *x*-axis, and cumulative relative fold enrichment in MS is plotted on the *y*-axis (Figure 1).

To identify specific loci associated with MS, we computed *conditional False Discovery Rates* (FDRs). The standard FDR framework is based on a mixture model of SNPs associated with the phenotype (either associated; non-null SNPs, or not; null SNPs). The *conditional* FDR is an extension of the standard FDR, which incorporates information from GWAS summary statistics of a second phenotype. Specifically, MS SNPs were stratified on the basis of *p*-values of each of the CVD factors, separately. Then based on the combination of *p*-values for SNPs in MS and each of the CVD factors, we assigned a conditional FDR value (FDR$_{MS|CVD}$, CVD represent one of BMI, CRP, LDL, HDL, T2D, SBP, TG, and WHR) to each SNP for MS by interpolating into a two-dimensional (2D) lookup table (Supplementary Figure 1). We used a conditional FDR threshold of 0.01, which means 1 false discovery per 100. Loci thus identified can be visualized by a conditional Manhattan plot (Figure 2). It is important to note that ranking SNPs by FDR or by *p*-values both give the same ordering of SNPs, whereas the conditional FDR re-orders SNPs resulting in a different *p*-value based ranking if the primary and secondary phenotype are genetically related.

Low conditional FDR values can be driven by association with both phenotypes or with the primary phenotype only. To detect true pleiotropic signal (association with both phenotypes), we computed the *conjunctional FDR*, computed as the maximum of the two conditional FDR values (i.e. MS conditional on CVD factors and CVD risk factors conditional on MS). Similar to conditional FDR, we assigned to each MS SNP a conjunctional FDR value using a 2D lookup table (Supplementary Figure 2). We used overall a conjunctional FDR threshold of 0.05, which means 5 expected false discoveries per 100. To illustrate the genomic location of significant loci, we constructed the conjunctional *Manhattan plots* based on the ranking of conjunctional FDR (Figure 3).

Annotation of new MS associated loci
The list of significant SNPs identified by conditional and conjunctional FDR were binned into independent loci using the linkage disequilibrium (LD) structure of the European subpopulation from 1000 Genomes Project at the LD-r2 < 0.2 level and a radius of 1 mega base. In addition, the extended major histocompatibility complex (xMHC) region (chr6:25652429–33368333) was considered as a single locus and SNPs close to the same genes were also binned into a single locus. These loci are numbered (locus #) in Tables 1 and 2 and Supplementary Tables 2, 3 and 4. Genes at
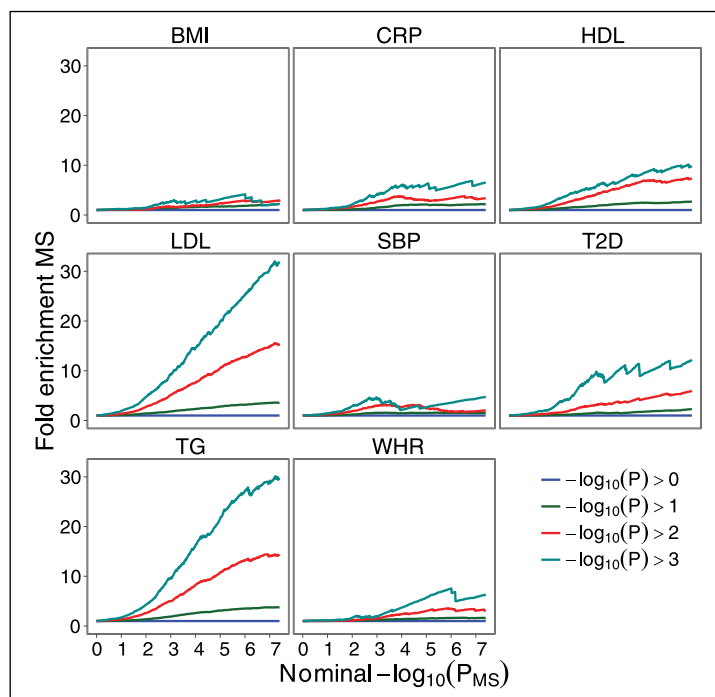
Figure 1. Pleiotropic enrichment of MS and CVD factors.

Fold-enrichment plots of enrichment versus nominal $-\log_{10}$ *p*-values in multiple sclerosis (MS) below the standard GWAS threshold of $p < 5 \times 10^{-8}$ as a function of the association level with body mass index (BMI), C-reactive protein (CRP) level, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, systolic blood pressure (SBP), type 2 diabetes (T2D), triglycerides (TG), and waist-to-hip ratio (WHR) at the level of $-\log_{10}(p) \geqslant 0$, $-\log_{10}(p) \geqslant 1$, $-\log_{10}(p) \geqslant 2$, $-\log_{10}(p) \geqslant 3$ corresponding to $p \leqslant 1$, $p \leqslant 0.1$, $p \leqslant 0.01$, $p \leqslant 0.001$, respectively. Successive upward elevation in terms of all SNPs ($-\log_{10}(p) \geqslant 0$, blue horizontal line) demonstrate pleiotropic enrichment of MS association conditioned CVD factors. The figure also shows that the fold enrichment of MS (y-axis) is also a monotonic increasing function of the nominal *p*-value (x-axis). All data are first genome corrected by intergenic SNPs.
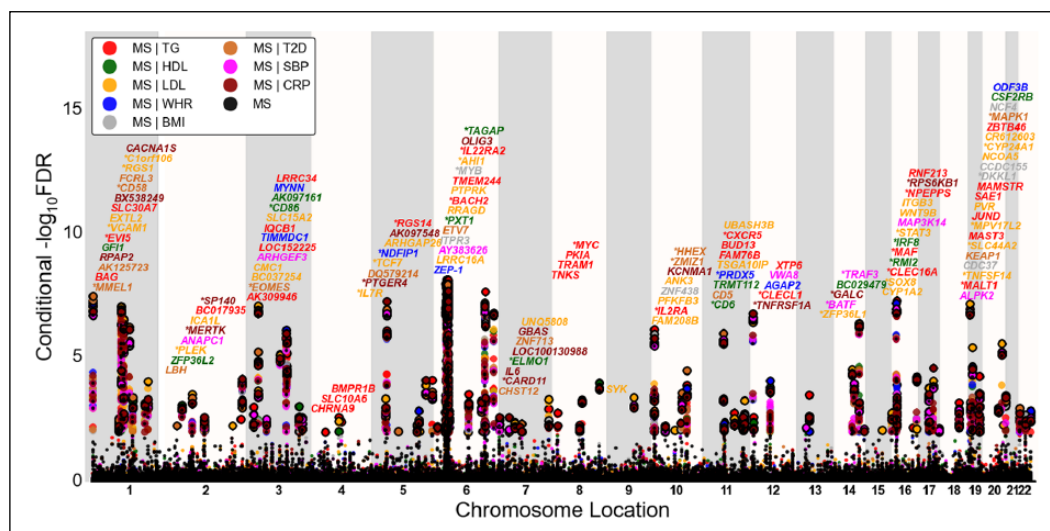

Figure 2. "Conditional FDR Manhattan plot" of multiple sclerosis (MS) on cardiovascular disease risk factors.

The unconditioned $-\log_{10}$ (FDR) values for MS alone (black) and conditioned on given cardiovascular disease risk factors triglycerides (TG; MS|TG), low-density lipoprotein (LDL) cholesterol (MS|LDL), high-density lipoprotein (HDL) cholesterol (MS|HDL), systolic blood pressure (SBP; MS|SBP), body mass index (BMI; MS|BMI), waist-to-hip ratio (WHR; MS|WHR), type 2 diabetes (T2D; MS|T2D), and C-reactive protein (CRP; MS|CRP) were plotted against the genomic locations of SNPs. SNPs with conditional $-\log_{10}$ FDR > 2 (i.e. FDR < 0.01) are shown with large points. A black line around the large points indicates the most significant SNP in each LD block, and this SNP was annotated with the closest gene which is listed above the symbols in each locus (except for the xMHC region on chromosome 6). Genes replicated in this study were marked by asterisks ("*"). Details for not previously reported non-MHC loci with $-\log_{10}$ FDR > 2 (i.e. FDR < 0.01) are shown in Table 1.
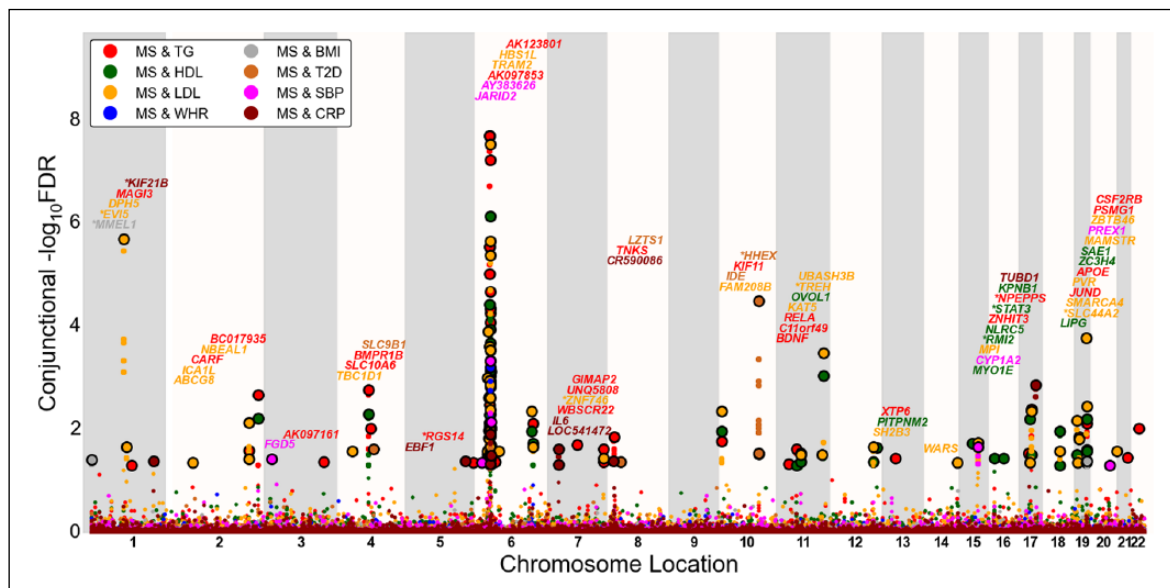
Figure 3. "Conjunctional FDR Manhattan plot" for multiple sclerosis (MS) and cardiovascular disease risk factors. Conjunctional $-\log_{10}$ (FDR) values for MS given the cardiovascular disease risk factors triglycerides (TG; MS & TG), low-density lipoprotein (LDL) cholesterol (MS & LDL), high-density lipoprotein (HDL) cholesterol (MS & HDL), systolic blood pressure (SBP; MS & SBP), body mass index (BMI; MS & BMI), waist-to-hip ratio (WHR; MS & WHR), type 2 diabetes (T2D; MS & T2D), and C-reactive protein (CRP) level (MS & CRP). SNPs with conditional $-\log_{10}$ FDR > 1.3 (i.e. FDR < 0.05) are shown with large points. A black line around the large points indicates the most significant SNP in each LD block, and this SNP was annotated with the closest gene which is listed above the symbols in each locus (except for the MHC region on chromosome 6). The figure shows the localization of 60 loci on a total of 21 chromosomes. Genes previously reported for MS are marked by asterisks ("*"), and details for the not previously reported non-MHC loci with $-\log_{10}$ FDR > 1.3 (i.e. FDR < 0.05) are shown in Table 2.

or closest to each SNP locus were obtained from the HUGO Gene Nomenclature Committee (HGNC) gene database. Any loci that did not contain previously reported MS associated SNPs or genes were deemed as findings adding to the currently known associations in MS (Tables 1 and 2).

The impact of MHC region on enrichment

To test the possibility that the observed enrichment may be driven by the large extended MHC region (chr6: 25652429–33368333, xMHC), we removed the xMHC region–related SNPs, defined as SNPs located within the xMHC or SNPs within 1Mb and in LD ($r^2 > 0.2$) with such SNPs, and then re-performed the analyses.

Non-genetic confounding

To investigate whether non-genetic confounders between MS and CVD risk factors contribute to the observed enrichment, we used a permutation procedure. Specifically, we permuted the p-values of each of the CVD risk factors 100 times and reconstructed the fold-enrichment plots using the average empirical cumulative distributions across all iterations.

Gene expression analysis of new MS loci

We used publicly available gene expression data for 170 MS patients and 60 controls[22] (NCBI Gene Expression Ontology database (GSE41850)) and mapped the suggested genes from our conditional analysis of MS on CVD to the assigned genes in this dataset by gene symbols. We restricted our analyses to the baseline expression level data and applied a two-sided t-test to baseline expression levels of the mapped genes for patients and controls.

Results

Pleiotropic enrichment of MS conditioned by association with related phenotypes

As illustrated by the conditional fold-enrichment plots, we found a strong enrichment of MS SNPs conditioned on the nominal p-values of association with several CVD risk factors (Figure 1). Across all evaluated CVD phenotypes, we found that the polygenic pleiotropic enrichment was strongest for LDL and TG (approximately 30-fold with respect to whole genome SNPs). We additionally performed a normal test of the empirical cumulative distribution of MS SNP p-values conditioned on the association level of CVD

Table 1. Conditional FDR. independent MS loci given CVD risk factors (MS|CVD) in non-MHC regions excluding previously reported MS loci.

Locus #	SNP[a]	Chr_loc[b]	Pos	Gene	OR_L	OR_U	P	FDR	Min condFDR[c]	P expression	Driving phenotype
1	rs4465231	1p36.22	9347278	SPSB1	1.0427	1.1274	4.93E−05	3.07E−02	9.66E−03	NA[d]	HDL
2	rs17519972	1p22.1	92828505	RPAP2	1.0719	1.1489	9.42E−08	**1.33E−04**	3.25E−05	**5.96E−03**	CRP
3	rs6662618	1p22	92935411	GFI1	1.0965	1.1754	2.76E−10	**4.69E−07**	3.09E−07	**1.10E−02**	T2D
4	rs12756986	1	101604753	S1PR1	1.068	1.1594	3.52E−06	**3.05E−03**	1.97E−03	NA	HDL
	rs17123757	1	101622960	S1PR1	1.0664	1.1551	3.21E−06	**3.05E−03**	1.75E−03	NA	CRP
5	rs3761959	1q21	157669278	FCRL3	0.8596	0.9422	9.97E−07	**1.08E−03**	3.46E−04	**1.45E−03**	LDL
6	rs6733372	2p23.1	30510444	LBH	1.0373	1.1008	3.47E−05	2.12E−02	5.42E−03	**2.47E−02**	T2D
7	rs1439287	2q13	111871897	ACOXL	1.0367	1.1148	9.46E−05	5.26E−02	8.82E−03	NA	TG
8	rs12373588	2q12.1	112466265	ANAPC1	0.8779	0.9557	1.52E−05	1.19E−02	3.69E−03	NA	SBP
9	rs2052401	2q23	162962045	DPP4	0.884	0.9615	5.88E−05	3.68E−02	7.15E−03	NA	LDL
10	rs2036927	2q33	203640713	ICA1L	0.8718	0.9596	1.08E−04	5.26E−02	3.50E−03	NA	HDL
11	rs2943633	2	227054881	MIR548AR	0.8839	0.9614	5.83E−05	3.68E−02	2.08E−03	NA	TG
12	rs9821630	3	16970938	PLCL2	0.8628	0.9484	7.50E−06	**6.75E−03**	1.75E−03	NA	HDL
	rs433317	3	28060456	CMC1	1.0393	1.1025	1.97E−05	1.45E−02	8.43E−03	8.16E−01	LDL
13	rs338610	3p24.1	28081442	CMC1	0.8376	0.9303	2.84E−07	**3.86E−04**	2.11E−04	8.16E−01	HDL
14	rs1500710	3p14.3	56914065	ARHGEF3	0.8841	0.9526	6.45E−06	**5.57E−03**	2.02E−03	8.78E−02	TG
15	rs771767	3	101748638	ZPLD1	1.0713	1.1401	1.02E−08	**1.57E−05**	1.02E−05	NA	CRP
	rs1398607	3	101755738	ZPLD1	1.0794	1.1687	1.03E−08	**1.57E−05**	3.91E−06	NA	TG
16	rs1920296	3q21.1	121543577	IQCB1	0.8523	0.9336	7.21E−08	**1.08E−04**	5.67E−06	5.80E−02	TG
	rs4285028	3q21.1	121660664	SLC15A2	1.0701	1.1419	3.75E−08	**5.75E−05**	2.28E−05	NA	HDL
17	rs10936599	3q26.31	169492101	MYNN	0.8514	0.9451	9.93E−06	**8.16E−03**	2.69E−03	NA	TG
	rs1997392	3q26.2	169509652	LRRC34	0.8561	0.9468	1.02E−05	**8.16E−03**	1.42E−03	3.54E−01	TG
18	rs6832151	4p14	40303633	CHRNA9	0.8724	0.9562	3.58E−05	2.56E−02	5.65E−03	NA	TG
19	rs13106574	4q22.1	87769929	SLC10A6	1.0477	1.1304	4.81E−05	3.07E−02	1.69E−03	NA	TG
20	rs6819188	4q23	95694977	BMPR1B	0.8694	0.9561	4.72E−05	3.07E−02	1.69E−03	3.74E−01	TG
21	rs12515731	5	79666489	ZFYVE16	1.0801	1.2586	8.35E−05	4.40E−02	9.18E−03	NA	T2D
22	rs853158	5q31	142605172	NR3C1	1.0412	1.1149	5.88E−05	3.68E−02	9.31E−03	NA	HDL
23	rs11755724	6	7118990	RREB1	1.0413	1.1099	2.83E−05	2.12E−02	5.71E−03	NA	WHR
24	rs9358854	6p22.1	25411464	LRRC16A	1.041	1.1175	2.88E−05	2.12E−02	6.72E−03	NA	TG
	rs9358858	6p22.1	25446308	LRRC16A	1.0428	1.111	1.91E−05	1.45E−02	7.71E−03	NA	LDL
25	rs932316	6p22.3	25641200	SCGN	0.8613	0.9538	5.16E−05	3.07E−02	2.43E−03	NA	HDL
26	rs210131	6p21.31	33535466	BAK1	1.1096	1.2146	2.33E−08	**3.76E−05**	1.92E−05	4.78E−01	SBP
	rs394199	6	33553580	GGNBP1	0.8625	0.9356	1.67E−08	**2.44E−05**	6.58E−06	NA	SBP
	rs942637	6p21.31	33653111	ITPR3	1.0883	1.1837	1.64E−07	**2.04E−04**	9.16E−05	**1.07E−02**	BMI
	rs471942	6p21.31	33696785	IP6K3	1.0918	1.2145	5.11E−06	**4.57E−03**	2.99E−03	6.00E−01	WHR
27	rs854917	6q15	90127390	RRAGD	1.0463	1.1195	1.85E−05	1.45E−02	8.33E−03	NA	HDL
28	rs6933404	6q23.3	137959235	OLIG3	1.0482	1.1257	2.24E−05	1.45E−02	5.62E−03	8.09E−01	CRP
29	rs632057	6	139834012	CITED2	0.8891	0.9674	2.34E−04	1.02E−01	7.42E−03	NA	TG
30	rs6952809	7p22	2448493	CHST12	1.0403	1.1085	3.39E−05	2.12E−02	7.57E−03	9.67E−01	T2D
31	rs2066992	7p21-p15	22768249	IL6	0.7198	0.9039	1.90E−05	1.45E−02	2.55E−03	NA	CRP
32	rs921911	7	50241812	C7orf72	1.0523	1.1361	2.31E−05	1.75E−02	5.22E−03	NA	LDL
33	rs10271662	7p11.2	55962907	ZNF713	1.0461	1.1244	3.81E−05	2.56E−02	4.60E−03	NA	T2D
	rs4543497	7p12	56047215	GBAS	0.863	0.954	4.71E−05	3.07E−02	8.87E−03	NA	TG
34	rs10111980	8p23.1	9418167	TNKS	1.0428	1.1142	3.06E−05	2.12E−02	1.11E−03	**3.97E−04**	TG
35	rs10106461	8q13.1	71405288	TRAM1	0.8702	0.9528	1.41E−05	**9.87E−03**	6.14E−03	NA	LDL
36	rs290986	9q22	93563536	SYK	1.0641	1.1436	1.04E−06	1.08E−03	2.18E−04	NA	HDL
37	rs2275774	10p15.1	5799613	FAM208B	1.0416	1.1233	1.29E−04	6.26E−02	3.94E−03	NA	TG
38	rs7905327	10p15.1	6176112	PFKFB3	1.0463	1.129	1.81E−05	1.45E−02	9.20E−03	NA	HDL
39	rs1442539	10q21	62481380	ANK3	1.0417	1.1147	4.86E−05	3.07E−02	4.60E−03	**8.84E−03**	HDL
40	rs7912269	10q22	78727604	KCNMA1	1.0776	1.1972	2.26E−05	1.75E−02	4.19E−03	NA	CRP
41	rs12289836	11q13	65436888	RELA	0.8857	0.9669	2.70E−04	1.02E−01	9.86E−03	NA	HDL
42	rs606978	11q13.1	65711517	TSGA10IP	1.0394	1.1062	3.44E−05	2.12E−02	8.16E−03	9.13E−01	HDL
43	rs4409785	11q21	95311422	FAM76B	0.8432	0.9405	5.67E−06	**5.57E−03**	1.45E−03	NA	LDL
44	rs491111	11q23.3	116238034	BUD13	1.046	1.1239	1.02E−05	**8.16E−03**	2.69E−03	1.59E−01	TG
45	rs7941030	11q24.1	122522375	UBASH3B	0.8691	0.9509	8.24E−06	**6.75E−03**	3.21E−04	3.50E−01	HDL
46	rs2059405	12q13.11	46175469	ARID2	0.8561	0.953	6.57E−05	3.68E−02	9.93E−03	NA	LDL
47	rs17594362	13q14.11	42139245	VWA8	1.0568	1.1499	3.15E−05	2.12E−02	6.32E−03	NA	SBP
48	rs806321	13	50841323	DLEU1	1.0466	1.1118	4.29E−06	**3.74E−03**	6.03E−04	NA	TG
49	rs17119756	14	84634432	LINC00911	0.8311	0.9439	4.77E−05	3.07E−02	4.23E−03	NA	TG

(Continued)

Table 1. (Continued)

Locus #	SNP[a]	Chr_loc[b]	Pos	Gene	OR_L	OR_U	P	FDR	Min condFDR[c]	P expression	Driving phenotype
50	rs4886406	15q24.1	75057203	CYP1A2	0.8712	0.9609	1.59E−04	7.40E−02	5.71E−03	4.31E−01	HDL
51	rs11864333	16p13.13	11475576	PRM1	1.0444	1.1097	7.82E−06	**6.75E−03**	3.15E−03	NA	T2D
52	rs2012068	17p13.3	2255351	SGSM2	0.8805	0.9604	5.89E−05	3.68E−02	6.69E−03	NA	LDL
53	rs4792814	17q21	43403005	MAP3K14	0.8694	0.9496	4.80E−06	**4.57E−03**	9.55E−04	NA	LDL
54	rs1373089	17q21	44915265	WNT9B	0.8937	0.9579	1.12E−05	**8.16E−03**	1.47E−03	NA	HDL
55	rs12603582	17q21.32	45377577	ITGB3	0.8608	0.9551	7.88E−05	4.40E−02	2.84E−03	**2.79E−02**	HDL
56	rs8081176	17q25.3	78283987	RNF213	0.8735	0.9543	1.59E−05	1.19E−02	6.68E−03	**2.21E−08**	TG
57	rs12456021	18q21.31	56213390	ALPK2	0.8663	0.9455	7.87E−06	**6.75E−03**	2.95E−03	2.86E−01	SBP
58	rs243354	19p13.3	4412713	CHAF1A	0.8798	0.9651	2.53E−04	1.02E−01	9.86E−03	NA	HDL
59	rs7255066	19q13.2	45146103	PVR	0.8566	0.9447	4.45E−06	**3.74E−03**	1.63E−04	NA	HDL
60	rs307896	19q13.32	47661493	SAE1	1.0574	1.1396	1.04E−06	**1.08E−03**	5.77E−05	NA	TG
61	rs2032809	19q13.3	47736216	MIR3191	0.8952	0.9697	2.79E−04	1.02E−01	9.84E−03	NA	TG
62	rs281380	19q13.33	49214470	MAMSTR	0.8826	0.9607	5.32E−05	3.07E−02	1.78E−03	NA	TG
63	rs7260291	19q13.33	49886010	CCDC155	1.0525	1.1432	1.15E−05	**9.87E−03**	4.88E−03	NA	BMI
64	rs967990	20	56335741	PMEPA1	0.8876	0.9624	5.39E−05	3.07E−02	6.69E−03	NA	HDL
65	rs2072711	22q13.1	37268555	NCF4	1.0615	1.1455	3.96E−06	**3.74E−03**	1.94E−03	5.95E−01	TG
	rs2413436	22q12.2	37312561	CSF2RB	1.0437	1.1088	8.78E−06	**6.75E−03**	3.42E−03	NA	LDL

FDR: false discovery rate; MS: multiple sclerosis; CVD: cardiovascular disease; MHC: major histocompatibility complex; SNP: single nucleotide polymorphisms; HDL: high-density lipoprotein; CRP: C-reactive protein; T2D: type 2 diabetes; LDL: low-density lipoprotein; TG: triglycerides; SBP: systolic blood pressure; BMI: body mass index; WHR: waist-to-hip ratio.
[a]The most significant MS SNP in each LD block based on the minimum condFDR (min condFDR) for each phenotype.
[b]Chromosome location.
[c]The CVD risk factor which provided the signal, that is, minimal condFDR (driving phenotype).
[d]NA indicates not available.
Bold face indicates <= 0.05

Table 2. Conjunctional FDR. MS and CVD risk factors loci (MS & CVD) in non-MHC regions excluding previously reported MS loci.

Locus #	SNP[a]	A1	A2	Chr_loc[b]	pos	Gene	Min cnjFDR	Driving phenotype
1	rs10909880	G	C	1p36	2727804	TTC34	3.77E−02	BMI
2	rs11588410	G	C	1p21.2	101486061	DPH5	2.16E−02	LDL
3	rs11102646	G	A	1p12	114068933	MAGI3	4.98E−02	TG
4	rs4148211	A	G	2p21	44071743	ABCG8	4.32E−02	LDL
5	rs2036927	A	G	2q33	203640713	ICA1L	7.20E−03	LDL
	rs17406900	T	C	2q33.3	203784202	WDR12	2.51E−02	TG
	rs7573079	C	T	2q33	203927551	NBEAL1	3.65E−02	LDL
6	rs2943633	G	A		227054881	MIR548AR	2.08E−03	TG
7	rs13070927	A	G	3p25.1	14919646	FGD5	3.63E−02	SBP
8	rs907314	A	T	4p14	38254453	PTTG2	2.57E−02	LDL
9	rs13106574	A	G	4q22.1	87769929	SLC10A6	1.69E−03	TG
10	rs6819188	T	A	4q23	95694977	BMPR1B	9.29E−03	TG
11	rs4698874	G	A	4q24	103930511	SLC9B1	2.35E−02	T2D
12	rs4704963	T	C	5q34	158247378	EBF1	4.07E−02	CRP
13	rs1267499	G	A	6p24	14715882	JARID2	4.26E−02	SBP
14	rs2076890	G	A	6p22.1	25420744	LRRC16A	3.05E−02	LDL
15	rs394199	T	C	6	33553580	GGNBP1	7.03E−03	SBP
16	rs9381257	T	C	6	43798902	VEGFA	4.14E−02	TG
17	rs9367490	T	C	6	52459940	TRAM2-AS1	2.57E−02	LDL
18	rs632057	C	T	6	139834012	CITED2	7.42E−03	TG
19	rs7776857	C	A	6	22754768	IL6	4.83E−02	CRP
	rs2066992	G	C	7p21	22768249	IL6	2.37E−02	CRP
20	rs2293489	C	T	7q11.23	73107279	WBSCR22	1.98E−02	TG

Table 2. (Continued)

Locus #	SNP[a]	A1	A2	Chr_loc[b]	pos	Gene	Min cnjFDR	Driving phenotype
21	rs6944136	G	C	7q36.1	150370546	GIMAP2	4.14E−02	TG
22	rs13262031	C	A	8	9389241	TNKS	1.64E−02	TG
	rs10111980	T	C	8p23.1	9418167	TNKS	1.39E−02	TG
23	rs2616214	T	C	8p22	20611119	LZTS1	4.12E−02	T2D
24	rs2275774	A	G	10p15.1	5799613	FAM208B	4.32E−03	LDL
25	rs10835211	A	C	11p14.1	27701365	BDNF	4.56E−02	TG
26	rs11039035	C	T	11	46967415	C11orf49	2.38E−02	TG
27	rs2306365	G	A	11q13	65427346	RELA	2.86E−02	TG
	rs6591188	T	C	11q13	65467953	KAT5	3.05E−02	LDL
	rs557675	G	A	11q13	65566719	OVOL1	4.21E−02	HDL
28	rs7941030	T	C	11q24.1	122522375	UBASH3B	3.21E−04	LDL
29	rs3184504	G	A	12q24.12	111884608	SH2B3	2.14E−02	LDL
30	rs940904	C	T	12q24.31	123491572	PITPNM2	2.20E−02	HDL
31	rs12871645	G	A	13	50931565	DLEU1	3.56E−02	TG
	rs2400899	G	A	14q32.2	100828487	WARS	4.32E−02	LDL
32	rs2306791	C	T	15q21	59453384	MYO1E	1.85E−02	HDL
33	rs4886406	G	A	15q24.1	75057203	CYP1A2	2.14E−02	SBP
	rs6495126	T	C	15q22-qter	75175026	MPI	1.77E−02	LDL
34	rs158481	A	T	16q13	57075253	NLRC5	3.61E−02	HDL
35	rs2306589	G	T	17q21.1	34848874	ZNHIT3	2.86E−02	TG
36	rs1292053	T	C	17q23.1	57963537	TUBD1	1.35E−03	CRP
37	rs8090363	G	T	18q21.1	47133828	LIPG	1.08E−02	HDL
	rs4939883	C	T	18q21.1	47167214	LIPG	2.57E−02	LDL
38	rs8102273	G	C	19p13.3	11180047	SMARCA4	4.32E−02	LDL
39	rs12608504	C	T	19p13.2	18389135	JUND	1.39E−02	TG
40	rs7255066	C	A	19q13.2	45146103	PVR	1.63E−04	LDL
41	rs405509	T	C	19q13.31	45408836	APOE	4.14E−02	TG
42	rs10408163	G	A	19q13.33	47597102	ZC3H4	1.11E−02	TG
	rs307896	A	G	19q13.32	47661493	SAE1	6.22E−03	HDL
	rs466477	G	A	19q13.32	47679798	SAE1	7.42E−03	TG
43	rs281380	C	A	19q13.33	49214470	MAMSTR	3.45E−03	LDL
44	rs926629	C	T	20q13.13	47335736	PREX1	4.97E−02	SBP
45	rs6010669	G	A	20q13.33	62445688	ZBTB46	2.57E−02	LDL
46	rs2836878	T	C	21q22.3	40465534	PSMG1	3.44E−02	TG
47	rs5756391	C	T	22q12.2	37298344	CSF2RB	9.29E−03	TG

FDR: false discovery rate; MS: multiple sclerosis; CVD: cardiovascular disease; MHC: major histocompatibility complex; SNP: single nucleotide polymorphisms; BMI: body mass index; LDL: low-density lipoprotein; TG: triglycerides; SBP: systolic blood pressure; T2D: type 2 diabetes; CRP: C-reactive protein; HDL: high-density lipoprotein.

[a]The most significant MS SNP in each LD block based on the minimum conjunctional FDR (min cnjFDR) for each phenotype.

[b]Chromosome location.

[c]The CVD risk factor which provided the signal, that is, minimal cnjFDR (driving phenotype).

phenotypes (with $-\log_{10}$P(CVD)$> 1, 2, 3$, and 4 vs the depleted category, $-\log_{10}$P(CVD)< 1) and found that all four tests were significant ($p < 0.05$) for HDL and LDL, but only one or two tests were significant for other CVD phenotypes (Supplementary Table 5).

MS gene loci identified with conditional FDR

As shown in the conditional FDR Manhattan plot for MS and each of the related CVD risk factors (Figure 2), we identified a total of 133 non-MHC loci, of which 65 are novel compared to the GWAS (see Table 1 for not

previously reported non-MHC loci and Supplementary Table 2 for all loci).

Overlapping gene loci in MS and CVD risk factors identified with conjunctional FDR

As indicated by the "Conjunction FDR Manhattan plot" (Figure 3), we detected loci significantly associated with both MS and the CVD risk factors on all chromosomes (including chromosome 6) except chromosome 21 (see Table 2 and Supplementary Tables 3 and 4). In general, we observed an *opposite* direction of effect between MS and TG, LDL, WHR, and T2D and the *same* direction of effect between MS and BMI (Supplementary Figure 3 and Supplementary Table 4).

Differential impact of xMHC on pleiotropic enrichment

We found that removing the xMHC-related SNPs resulted in substantial attenuation of the enrichment of MS conditioned on TG and HDL (Supplementary Figure 4). For the strata with $-\log_{10}P>3$, the fold enrichment for TG was reduced from 30 to about 2-fold and for HDL from 10 to 2-fold.

Control of non-genetic artifacts

Figure 4 shows the comparison of distributions of genotypic variance $(2 \times (p \times (1-p))$, p: reference allele frequencies from the 1000 Genomes Project) of SNPs having conditional FDR<0.05 for each conditioned trait with all SNPs analyzed. The majority of SNPs with conditional FDR<0.05 are common SNPs, that is, tagging more genotypic variances, for all conditional analysis. In Supplementary Figure 5, we show the fold-enrichment plot based on 100 permutations of the p-values of each conditioned trait. The observed pleiotropic enrichment between MS and CVD risk factors disappeared after randomizing the genotype–phenotype relationship of the conditioned traits, indicating that the observed enrichment between MS and CVD risk factors is not a result of confounders, such as sample overlap or technical artifacts.

Gene expression analysis of new MS loci

Out of the 279 unique genes suggested by the conditional analysis of MS on CVD at the level condFDR (MS|CVD) <0.01, we found available baseline expression data for 129 genes. Across all 129 genes, we found a significant association between baseline expression level and MS status for 28 genes ($p<0.05$, Table 1).
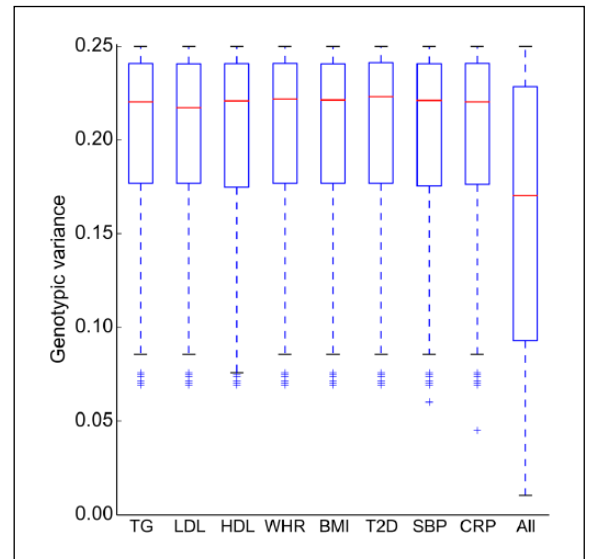

Figure 4. Distribution of tagged genotypic variance of identified SNPs at conditional FDR<0.05.
Comparison of the distribution of tagged genotypic variance (*y*-axis) by SNPs identified by conditional FDR<0.05 of MS conditional on cardiovascular risk factors (*x*-axis): triglycerides (TG), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, waist-to-hip ratio (WHR), body mass index (BMI), type 2 diabetes (T2D), systolic blood pressure (SBP), and C-reactive protein (CRP) level, with all SNPs analyzed (All).

Pathway analysis for conjunction loci

We investigated the probable pathways involving the loci identified by the conjunctional analysis of MS with CVD factors using PANTHER[23] and Reactome.[24] The most enriched biological pathways were metabolic process (GO:0008152), cellular process (GO:0009987), and immune system process (GO:0002376) (see Supplementary Figures 6 and 7 and Supplementary Table 6 for details). We found that 32 genes mapped to known pathways in PANTHER, among which the Apoptosis signaling pathway, the Integrin signaling pathway, the Inflammation mediated by chemokine and cytokine signaling pathway, and the T-cell activation pathway showed three hits and others showed one or two hits (Supplementary Figure 6). Consistent with the PANTHER results, we observed that the immune-related pathways were significant ($p<0.05$) by Reactome (Supplementary Table 6). Moreover, several signaling pathways within the immune system, such as Interferon alpha/beta signaling and cytokine signaling, were also detected.

Discussion

Here, we observed polygenic pleiotropy between MS and several CVD risk factors, identifying 133 independent loci associated with MS conditioned on CVD

risk factors. Furthermore, we identified 60 genes associated with *both* MS and CVD risk factors. Considered together, our findings implicate overlapping genetic factors between MS and several CVD risk factors.

The current results suggest that multiple loci in the xMHC region are overlapping between MS, and TG and HDL. These loci seem mainly located in the xMHC region, and due to the high and complex LD pattern in this region, it is difficult to interpret the results in a functional setting. Interestingly, the polygenic overlap observed between MS and LDL and also some of the overlap between MS and the other CVD factors seem less dependent on the xMHC region (Supplementary Figure 4). This strongly suggests that there are also non-MHC genes shared between MS and CVD risk factors. This may further indicate that genetic factors may play a role in the immune activation found in several CVDs. On a methodological note, unlike epidemiological studies, co-heritability analyses,[25,26] or LD regression,[27] one strength of our current approach is the ability to detect genetic effects even when there is no correlation of the signed effects (mixed directionality of effect); the method presented in this work can detect SNPs that have a non-null effect in one trait and that also tend to have a non-null effect in another trait, independent of directionality.[14,28] Taken collectively, these findings illustrate that the genetic relationship between CVD risk factors and MS may not be straightforward; considerable work will be required to carefully characterize the biological mechanisms underlying how each cholesterol-associated genetic variant influences MS pathobiology.

Although the method robustly identifies new variants, the functional mechanisms behind SNP associations to disease remain elusive. However, the fact that these polymorphisms influence both MS and CVD risk suggests the possibility of shared mechanisms for these shared variants. Such functional effects should be studied for each risk polymorphism individually and strategies for such investigations are dependent on the genes that are putatively affected by this polymorphism. The current results suggest a complex pattern of pathological pathways, involving both xMHC and other parts of the genome. Furthermore, the results of the conjunctional FDR identify specific overlapping gene variants between MS and CVD risk factors. Inflammatory processes play an important role in MS, and associations to both human leukocyte antigen (HLA) class I and II loci are well established.[29] Recently, a large number of non-HLA markers have also been associated with MS risk, and immunologically relevant genetic loci were significantly overrepresented among these.[6] Most of

the pleiotropic loci between MS and the CVD risk factors were located on chromosome 6, suggesting involvement of HLA genes also in several CVD risk factors. This is in line with previous findings of the involvement of immunological mechanisms also in CVD. On the other hand, immune-related mechanisms have been implicated in the pathology of several CVD[30–32] and vascular pathology.[33,34] Our approach further elucidates other possible common mechanisms between MS and CVD risk factors. For instance, this may be related both to vascular and lipid biology or inflammatory processes shared between MS etiology and CVD risk factors, although the exact mechanisms may vary between polymorphisms. The interesting recent reports of a relation between BMI and MS susceptibility[35,36] also support the existence of common mechanisms between this CVD risk factor and MS.

The current findings of new genetic variants in MS conditional on CVD risk factors show the feasibility of using a genetic epidemiology framework that leverages overlap in genetic signals from independent GWASs to improve statistical power for gene discovery. In the original MS GWAS sample, >52 loci were significantly associated with MS susceptibility. By combining the original MS sample with independent GWAS of selected CVD risk factors, we identified abundant pleiotropic signal (total of 133 loci). Several of these genetic risk loci have not been previously reported in MS, whereas one of the loci not reported in the GWAS was genome-wide significant in the later immunoChip analysis. Our findings demonstrate the increased power of the combined analytical approach. It is important to note that by applying the conjunctional FDR method,[37] we minimized concerns that our results were solely driven by a strong signal in one phenotype.

Our analysis is based on results from GWAS of different phenotypes, and there might be some overlapping individuals included in several of the primary studies. Since the analysis is restricted to summary statistics, we could not identify the specific individuals. However, we performed standard single phenotype GWAS genomic corrections[38] for genetic stratification before our pleiotropy analysis. The fact that the pleiotropic loci were located at different sites for different CVD risk factors suggests that our findings are not driven by conditional genetic effects and rather by true increases in risk for MS or association to the CVD risk factors. Moreover, when the genotype–phenotype association in CVD risk factors was perturbed, the observed enrichment disappeared (Supplementary Figure 5), indicating that the identified pleiotropic structure is not the result of overlapping samples or

non-genetic confounders. It is known that relative rare variants suffer more from technical artifacts; however, the SNPs we identified are concentrated in common variants (Figure 4), further suggesting that our results are not artifacts. It is also important to note that our conditional FDR is capable of identifying the majority of the established MS risk loci, thereby showing the power and specificity of the method. This study only analyzed SNPs reported by both MS and CVD risk factors GWASs which excluded the large number of SNPs analyzed in the latest immuno-Chip study of MS.[5] Thus, the low replication rates of the SNPs reported by immunoChip study of MS are reasonable. Finally, we hypothesize that when new and larger GWAS data appear for these phenotypes, more pleiotropic loci are expected to be identified.

This work has clinical implications. The present results revealed a large number of genetic loci associated with MS. Careful work will be required to further characterize the candidate genes detected in this study and how these impact MS risk on an individual basis. Although no single variant may be informative clinically, identifying shared loci with cardiovascular risk factors will elucidate more of the polygenic architecture of a complex disease and may offer novel insights into lipid-lowering primary and secondary prevention trials in MS.

Conflict of interest
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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