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Research Article

Pleiotropic Genetic Effects between Multiple Sclerosis and Musculoskeletal Traits

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Abstract

Background: Musculoskeletal disorders were commonly reported in patients with multiple sclerosis. However, the underlying etiology linking Multiple Sclerosis (MS) and musculoskeletal disorders is not well studied. With large-scale Genome-Wide Association Studies (GWAS) publicly available, we conducted genetic correlation analysis to identify shared pleiotropic genetic effects between MS and musculoskeletal traits. We also conducted Mendelian Randomization (MR) to estimate the causal relationship between MS and increased risks of musculoskeletal disorders.

Methods: Linkage Disequilibrium Score Regression (LDSR) analysis was performed to estimate heritability and genetic correlation. Invariable, multivariable, and bidirectional MR analyses were conducted to estimate the causal relation. These analyses were done by utilizing the recent GWAS summary statistics of MS, fracture, frailty, falls, and several musculoskeletal risk factors, including bone mineral density, lean mass, grip strengths, and vitamin D.

Results: LDSR analysis showed a moderate genetic correlation of MS with falls (RG=0.10, p=0.01) but not with fracture and frailty. Genetic variants (rs13191659) in the LINC00240 gene which is associated with iron status biomarkers were found to be associated with both MS and falls. In MR analyses after excluding outlier SNPs with potential pleiotropic effects and correcting for multiple testing, MS presented no causal association with fracture and frailty but a minimal association with falls. Falls showed causally increased risks of fracture and frailty.

Conclusion: Our study suggests a potential genetic correlation with shared pleiotropic genetic effects between MS and falls. However, we didn't find evidence to support the causal relation between MS and increased risks of falls, fractures, and frailty.

Keywords: LD score regression • Multivariable Mendelian Randomization • Bidirectional Mendelian Randomization • Fracture • Falls • Frailty

Introduction

Multiple Sclerosis (MS) is a demyelinating inflammatory autoimmune disorder presenting continuous and diffuse changes in the white and grey matter, breakdown of myelin, and damage to axons [1]. Despite recent progress characterized by the advent of new disease-modifying therapies, patients with MS still show an increased risk of musculoskeletal disorders such as fractures [2, 3], accidents and falls [4], and frailty [5] compared to controls. A meta-analysis comprising 9 cohort studies proposed significantly Increased fracture risk in MS by 1.58 times compared to controls [6]. Falls are a debilitating consequence of MS and 56% of MS patients experience falls in any 3-month period [4]. Frailty is a marker of poor prognosis in patients suffering from systemic sclerosis [5] and a significantly higher percentage of MS patients were frail compared to controls (28% vs 8%) [5].

Multiple attributable risk factors of musculoskeletal disorders of MS have been proposed, including secondary causes delineating systemic bone loss by chronic inflammatory status [7], muscle weakness due to immobility [8], and ataxia from disruption of neuronal impulses [9], as well as bone and muscle loss due to MS treatment such as corticosteroids use [10], and psychotropic drugs (antidepressants, anxiolytics, and anti-epileptics) use [11, 12], which make MS patients vulnerable to falls. Beyond the secondary causes, intrinsic factors of MS etiology might be associated with the increased musculoskeletal disorders such that early-stage comparatively young MS patients with minimal or no physical disability also presented low BMD [13].

However, most of the findings were typical observational studies not precluding potential biases from undefined residual confounding and reverse causation. Therefore, the elucidation of the effects of genetically unmodifiable predisposing factors will give further insights into preventative strategies for MS patients' care.

In this context, LDSR is a reliable and efficient method of using GWAS summary-level data to estimate the genetic correlation between different phenotypes [14]. In addition, MR is a widely used method using measured variation in genes (genotypes, SNPs) of exposure to examine the causal effect of exposures on disease outcomes in observational studies [15, 16]. We implemented LD score regression as well as invariable, multivariable and bidirectional MR, and estimated genetic pleiotropic effect (correlation) and direct MS effects to 3 musculoskeletal outcomes (fracture, falls, and frailty) adjusting for the confounding effects from well-established bone and muscle disorder-related risk factors such as bone mineral density (BMD), lean body mass (whole body fat-free mass, appendicular lean mass), grip strengths and vitamin D.

We performed the following analyses:

1. LDSR to assess genetic heritability and genetic pleiotropy (correlations) between MS and musculoskeletal traits;
2. Univariable MR to assess the causal effect of genetic determinants of MS on fracture, falls, frailty, and multiple risk factors;
3. Multivariable MR to evaluate independent specific effects of MS to fracture, falls, frailty adjusting for other contributing risk factors;
4. Bidirectional MR to assess the directionality among fracture, falls, frailty.

Methods

Linkage Disequilibrium Score Regression (LDSR)

We used the LD Hub interface which provides an automatic LD score regression analysis pipeline for users [17]. To standardize the input file, quality control is automatically performed on the uploaded file in LD Hub. In order to restrict the analysis to well-imputed SNPs, LD Hub filters the uploaded SNPs to HapMap3 SNPs [18] with 1000 Genomes EUR MAF above 5%, which tend to be well-imputed in most studies. In addition, to make the estimates of the genetic correlation to be reliable, the recent MS GWAS meta-analysis of the International Multiple Sclerosis Genetics Consortium (IMSGC) comprising 47,429 cases ad 68,374 controls [19] needs to meet the following criteria;

1) Z score is at least > 1.5 (optimal > 4),

2) Mean Chi-square of the test statistics > 1.02, and

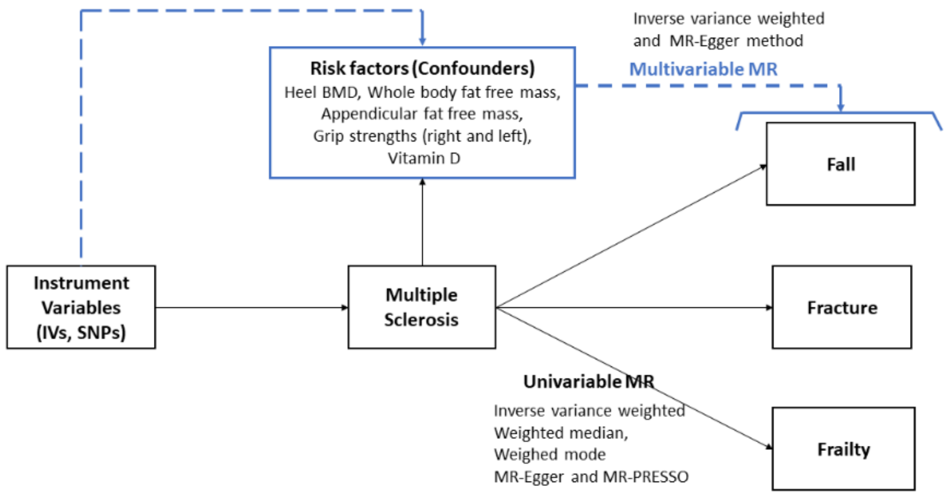
3) the intercept estimated from the SNP heritability analysis is between 0.9 and 1.1 [17].

We used UK Biobank GWAS datasets provided by LD hub pipeline estimating genetic heritability and correlation with MS.

Mendelian Randomization (MR)

Supplementary Table 1 describes the detailed data source used in MR study. The largest publicly available GWAS summary statistics were used. Most GWAS summary statistics data were downloaded from the "MRC IEU OpenGWAS database" repository (https://gwas.mrcieu.ac.uk/) or each GWAS consortia. The overall study design for MR is displayed in Figure 1.

Figure 1: Conceptual model of univariable and multivariable mendelian randomization



Supplementary Table 1. GWAS summary statistics used in Mendelian Randomization

Traits	Sample size	Consortium	First author	Dataset ID*
Multiple sclerosis (exposure)	115,803: 47,429 cases, 68,374 controls	IMSGC	Patsopoulos NA et al, 2019	ieu-b-18
Musculoskeletal outcomes				
Fractured/broken bones in last 5 years (outcome)	460,389: 44502 cases, 415887 controls	MRC-IEU	NA	ukb-b-13346
Falls last year	461,725	MRC-IEU	NA	ukb-b-2535
Frailty	164,610	NA	Atkins et al, 2019	NA
Risk factors				
heel BMD (eBMD)	142,487	NA	Kemp JP, 2017	ebi-a-GCST006288
Whole body fat free mass	454,850	MRC-IEU	NA	ukb-b-13354
appendicular lean mass	450,243	NA	Pei et al, 2020	ebi-a-GCST90000025
Handgrip strength(right)	461,089	MRC-IEU	NA	ukb-b-10215
Handgrip strength(left)	461,026	MRC-IEU	NA	ukb-b-7478
Vitamin D	417,580	NA	Revez et al, 2020	ebi-a-GCST90000615

*ID in ieu open gwas project (https://gwas.mrcieu.ac.uk/)

Exposure data:

Instrumental variables (IVs) for MS are obtained from the recent GWAS meta-analysis of the IMSGC [19]. Among the 233 genome-wide statistically independent associations with MS susceptibility, 200 effects (SNPs) located in the autosomal non-MHC genome were selected as IVs Supplementary Table 2. The detailed analysis and results description is provided in the previous IMSGC publication [19]. SNPs of genome-wide and suggestive effects jointly explain ~48% of the estimated heritability for MS [19].

Outcome data:

Fracture and falls GWAS data used in the analysis were selected from the UK Biobank data, fractured/broken in last 5 years (ID: ukb-b-13346, n=44,502 cases and 415,887 controls) and falls last year (ID: ukb-b-2535, n=461,725). Frailty GWAS data was from Atkins et al, 2019 (n=175,226) [20,21].

Supplementary Table 2. 200 MS SNPs located in the autosomal non-MHC genome from International Multiple Sclerosis Genetics Consortium

SNP	effect_allele	other_allele	eaf	pval	OR	beta	se
chr1:154983036	G	T	0.040	0.009398	1.125	0.11778304	0.04534955
chr1:32738415	A	G	0.153	5.04E-07	1.145	0.13540464	0.02694736
chr11:118783424	G	A	0.976	3.07E-05	1.250	0.22314355	0.05353461
chr11:14868316	G	A	0.020	1.13E-06	1.387	0.32714314	0.06720923
chr13:100026952	A	C	0.968	4.07E-07	1.777	0.57492655	0.11349502
chr14:88523488	C	T	0.949	2.09E-12	1.347	0.2978799	0.04238266
chr16:11213951	C	T	0.459	4.65E-24	1.206	0.1873091	0.01851451
chr16:11353879	T	C	0.038	6.00E-09	2.064	0.72464585	0.124579
chr2:112492986	C	T	0.212	2.01E-06	1.199	0.18148788	0.03818855
chr3:100848597	C	T	0.922	2.05E-05	1.162	0.15014266	0.03524993
chr3:112693983	T	G	0.984	0.001152	1.289	0.25386672	0.07810069
chr3:121783015	T	G	0.909	7.66E-05	1.142	0.13278111	0.03357471
chr5:40429250	T	A	0.445	2.32E-07	1.095	0.09075436	0.01754834
chr6:119215402	A	C	0.619	8.15E-06	1.083	0.07973497	0.01787297
chr6:130348257	T	C	0.158	3.60E-05	1.127	0.11955924	0.02893665
chr6:14691215	C	T	0.930	2.97E-06	1.327	0.28292076	0.06054522
chr7:50328339	A	G	0.962	4.17E-08	1.589	0.46310489	0.0844543
chr8:129177769	C	T	0.207	1.23E-08	1.129	0.12133229	0.02130313
chr8:95851818	G	T	0.025	0.0009061	1.120	0.11332869	0.03415399
rs10063294	G	A	0.429	1.13E-09	1.104	0.09893995	0.01624663
rs1014486	C	T	0.471	1.36E-10	1.111	0.10526051	0.01639493
rs10191360	T	C	0.476	3.19E-05	1.102	0.09712671	0.02335079
rs10230723	A	T	0.839	2.29E-06	1.113	0.10705907	0.02265327
rs10245867	T	G	0.323	0.00033	1.065	0.0629748	0.01753911
rs1026916	A	G	0.377	1.02E-13	1.138	0.12927234	0.01737932
rs10271373	A	C	0.497	1.65E-05	1.073	0.07045846	0.0163566
rs1076928	T	C	0.459	4.22E-06	1.079	0.07603469	0.01652841
rs1077667	C	T	0.763	8.37E-13	1.164	0.15186235	0.02122478
rs10801908	C	T	0.868	3.54E-16	1.240	0.21511138	0.02638321
rs1087056	A	G	0.420	3.12E-06	1.081	0.07788654	0.01670396
rs10936182	T	G	0.176	8.94E-06	1.102	0.09712671	0.02186878
rs10936602	T	C	0.743	1.68E-08	1.115	0.1088544	0.01929328
rs10951042	C	T	0.387	2.02E-06	1.082	0.07881118	0.01658689
rs10951154	C	T	0.153	6.73E-05	1.106	0.1007499	0.02527826
rs11079784	C	T	0.525	4.34E-11	1.113	0.10705907	0.01624081
rs11083862	A	T	0.447	4.07E-09	1.106	0.1007499	0.01713048
rs11125803	C	T	0.252	0.001722	1.062	0.06015392	0.01919147
rs1112718	A	G	0.591	2.46E-10	1.111	0.10526051	0.01663022
rs11161550	G	A	0.521	6.27E-07	1.091	0.08709471	0.01747939
rs11231749	C	T	0.296	2.34E-05	1.076	0.07325046	0.01731811
rs11256593	T	C	0.561	6.78E-27	1.205	0.18647957	0.01736701
rs11578655	G	T	0.136	0.0004817	1.094	0.0898407	0.02573693
rs11749040	A	G	0.131	3.54E-17	1.217	0.19638881	0.02330413
rs1177228	G	A	0.739	8.57E-09	1.113	0.10705907	0.01859683

rs11809700	T	C	0.277	3.51E-15	1.155	0.14410034	0.01830708
rs11852059	C	A	0.181	3.02E-06	1.101	0.09621886	0.02060602
rs11899404	C	T	0.562	0.0007573	1.109	0.10345871	0.03071862
rs11919880	A	G	0.584	1.97E-05	1.073	0.07045846	0.01650752
rs12133753	C	T	0.854	8.53E-09	1.137	0.12839321	0.02229964
rs12147246	A	G	0.351	4.29E-09	1.104	0.09893995	0.01684771
rs12211604	G	A	0.384	1.86E-07	1.098	0.09349034	0.01793473
rs12365699	G	A	0.847	3.15E-10	1.155	0.14410034	0.02290503
rs12434551	A	T	0.542	1.83E-10	1.110	0.10436002	0.01637028
rs12478539	G	C	0.735	4.37E-11	1.131	0.1231022	0.01867744
rs1250551	T	G	0.310	2.66E-11	1.123	0.11600368	0.01740683
rs12588969	G	C	0.307	0.0001897	1.074	0.07139	0.01912735
rs12609500	C	T	0.757	3.74E-05	1.084	0.0806579	0.019563
rs12614091	A	T	0.769	0.0003583	1.074	0.07139	0.02000261
rs12622670	T	C	0.550	1.04E-10	1.113	0.10705907	0.01657
rs12722559	C	A	0.867	2.78E-07	1.145	0.13540464	0.02635469
rs12832171	C	G	0.598	0.0009452	1.381	0.32280787	0.0976327
rs12925972	C	T	0.526	3.07E-08	1.099	0.09440068	0.01704784
rs12971909	A	G	0.372	8.98E-06	1.090	0.0861777	0.01940773
rs13066789	C	T	0.447	3.17E-05	1.072	0.06952606	0.01670939
rs13136820	C	T	0.329	0.0001235	1.073	0.07045846	0.01835299
rs1323292	A	G	0.810	1.40E-08	1.132	0.12398598	0.02185395
rs13327021	T	C	0.379	4.50E-11	1.120	0.11332869	0.01720594
rs13385171	C	T	0.602	2.42E-05	1.072	0.06952606	0.01646704
rs13414105	C	A	0.809	9.40E-06	1.105	0.09984533	0.02253573
rs1365120	C	T	0.108	5.76E-06	1.127	0.11955924	0.02636359
rs137955	T	C	0.409	0.0001947	1.065	0.0629748	0.01690238
rs1399180	C	T	0.818	0.0003753	1.083	0.07973497	0.02241717
rs140522	T	C	0.340	2.85E-10	1.117	0.11064652	0.01754421
rs1415069	G	C	0.189	4.01E-05	1.098	0.09349034	0.0227642
rs1465697	T	C	0.238	3.48E-11	1.132	0.12398598	0.01871577
rs17051321	T	C	0.216	4.95E-07	1.099	0.09440068	0.0187741
rs1738074	C	T	0.548	9.91E-12	1.120	0.11332869	0.01664688
rs17724508	T	C	0.956	2.59E-08	1.238	0.21349717	0.03834974
rs17741873	G	T	0.809	2.15E-05	1.093	0.08892621	0.02093014
rs17780048	C	T	0.939	0.0002208	1.176	0.16211885	0.04388771
rs1800693	C	T	0.430	1.02E-13	1.135	0.12663265	0.01702444
rs198398	C	T	0.910	5.72E-05	1.188	0.17227122	0.04281016
rs2084007	C	T	0.500	1.15E-06	1.083	0.07973497	0.01639267
rs2150879	G	A	0.441	3.29E-10	1.109	0.10345871	0.01646263
rs2248137	C	G	0.600	7.81E-11	1.119	0.11243543	0.01728655
rs2269434	C	T	0.350	5.24E-07	1.090	0.0861777	0.01717608
rs2286974	A	G	0.592	1.51E-07	1.115	0.1088544	0.0207289
rs2289746	C	T	0.646	1.48E-06	1.089	0.08525984	0.01771111
rs2317231	G	T	0.541	1.90E-09	1.106	0.1007499	0.0167745
rs2327586	T	C	0.256	7.82E-10	1.125	0.11778304	0.01915626
rs2331964	C	T	0.612	5.89E-07	1.089	0.08525984	0.01706976
rs2364485	A	C	0.182	2.19E-05	1.099	0.09440068	0.02224027
rs244656	A	T	0.850	2.57E-05	1.108	0.10255659	0.02436855
rs2469434	C	T	0.398	0.0003094	1.063	0.0610951	0.01693652

rs249677	A	C	0.623	8.99E-06	1.080	0.07696104	0.01733302
rs2546890	A	G	0.503	1.04E-12	1.124	0.11689375	0.01640589
rs2585447	C	T	0.211	7.89E-08	1.137	0.12839321	0.02391102
rs2590438	G	T	0.331	0.001561	1.055	0.05354077	0.01692669
rs2705616	C	G	0.458	4.35E-07	1.086	0.08250122	0.01632728
rs2726479	C	T	0.566	1.18E-06	1.082	0.07881118	0.01621974
rs2836438	A	G	0.119	2.98E-05	1.104	0.09893995	0.02369825
rs28703878	G	A	0.309	4.51E-10	1.143	0.13365638	0.02143554
rs28834106	T	C	0.764	3.79E-11	1.138	0.12927234	0.01955098
rs2986736	C	T	0.201	3.41E-05	1.112	0.1061602	0.02561656
rs3184504	T	C	0.466	3.45E-05	1.071	0.06859279	0.01656219
rs32658	T	G	0.395	2.95E-05	1.071	0.06859279	0.0164204
rs34026809	G	C	0.927	1.45E-07	1.202	0.18398684	0.03498648
rs34536443	G	C	0.970	0.001633	1.187	0.17142912	0.05442285
rs34681760	C	T	0.643	5.28E-06	1.084	0.0806579	0.017714
rs34695601	T	C	0.760	3.17E-08	1.116	0.10975086	0.01984007
rs34723276	A	G	0.711	1.36E-14	1.218	0.19721017	0.0256115
rs34947566	C	A	0.852	1.61E-06	1.123	0.11600368	0.02418217
rs35218683	T	C	0.276	1.56E-08	1.308	0.26849925	0.0474814
rs354033	G	A	0.740	1.21E-08	1.114	0.10795714	0.01894546
rs35486093	G	A	0.084	1.60E-10	1.197	0.17981843	0.0281163
rs35540610	C	T	0.224	2.88E-12	1.145	0.13540464	0.01938934
rs35703946	G	A	0.858	1.94E-09	1.188	0.17227122	0.0286987
rs3737798	A	G	0.530	1.40E-07	1.091	0.08709471	0.01654142
rs3809627	C	A	0.571	3.25E-08	1.102	0.09712671	0.01757184
rs3923387	T	C	0.425	2.35E-05	1.073	0.07045846	0.0166618
rs405343	T	G	0.174	4.72E-08	1.126	0.11867153	0.02172855
rs4262739	G	A	0.545	5.37E-05	1.069	0.06672363	0.0165202
rs4325907	C	T	0.339	3.68E-09	1.104	0.09893995	0.01677525
rs438613	C	T	0.488	9.43E-17	1.148	0.1380213	0.01660556
rs4409785	C	T	0.189	2.09E-05	1.094	0.0898407	0.02111389
rs4728142	A	G	0.451	0.0002585	1.063	0.0610951	0.01672149
rs4796224	G	A	0.457	1.62E-07	1.089	0.08525984	0.01627602
rs4808760	C	G	0.722	4.84E-13	1.144	0.13453089	0.01860804
rs4812772	C	T	0.720	0.0002541	1.070	0.06765865	0.01849561
rs4820955	A	T	0.436	1.82E-05	1.123	0.11600368	0.02706634
rs483180	C	G	0.673	1.77E-09	1.114	0.10795714	0.01794017
rs4896153	T	A	0.511	1.65E-13	1.148	0.1380213	0.0187161
rs4939490	G	C	0.391	4.25E-15	1.146	0.13627762	0.01736612
rs4940730	A	G	0.508	0.0007057	1.060	0.05826891	0.01720189
rs531612	T	C	0.526	9.41E-08	1.092	0.08801088	0.01648834
rs55858457	T	G	0.376	1.20E-08	1.120	0.11332869	0.01988318
rs56095240	A	T	0.144	1.97E-05	1.127	0.11955924	0.02801121
rs57116599	G	A	0.744	2.59E-09	1.128	0.12044615	0.02022376
rs5756405	A	G	0.472	0.0003876	1.060	0.05826891	0.01642124
rs58166386	G	A	0.300	2.54E-07	1.095	0.09075436	0.01760604
rs58394161	C	T	0.166	0.0008627	1.104	0.09893995	0.02969518
rs59655222	T	C	0.741	3.76E-11	1.131	0.1231022	0.01861451
rs6020055	A	G	0.945	0.001622	1.294	0.2577382	0.08177177
rs6032662	C	T	0.252	2.85E-13	1.143	0.13365638	0.01830583

rs60600003	G	T	0.102	4.20E-07	1.143	0.13365638	0.02641607
rs6072343	A	G	0.153	0.0008908	1.079	0.07603469	0.02288188
rs61708525	G	A	0.315	1.91E-05	1.083	0.07973497	0.01865076
rs61863928	G	T	0.652	5.45E-08	1.115	0.1088544	0.02002485
rs61884005	C	G	0.854	8.02E-08	1.144	0.13453089	0.02506781
rs62013236	C	T	0.846	2.07E-06	1.133	0.12486898	0.02630777
rs62420820	A	G	0.226	2.50E-13	1.147	0.13714984	0.01873912
rs631204	A	C	0.400	2.60E-11	1.116	0.10975086	0.0164603
rs6427540	C	T	0.862	3.20E-05	1.112	0.1061602	0.02552698
rs6496663	C	A	0.318	2.78E-08	1.106	0.1007499	0.01813759
rs6533052	A	G	0.482	8.01E-05	1.067	0.06485097	0.01644252
rs6564681	C	T	0.311	2.23E-07	1.098	0.09349034	0.01805158
rs6589706	A	G	0.450	2.20E-09	1.109	0.10345871	0.01729412
rs6589939	G	A	0.380	8.67E-06	1.078	0.07510747	0.01688592
rs6670198	T	C	0.666	2.03E-16	1.156	0.14496577	0.0176351
rs6672420	A	T	0.505	2.23E-05	1.073	0.07045846	0.01661551
rs67111717	G	A	0.342	4.96E-07	1.100	0.09531018	0.01895643
rs6738544	C	A	0.661	9.49E-05	1.068	0.06578774	0.0168545
rs6742	C	T	0.782	1.30E-07	1.173	0.15956457	0.03022717
rs6789653	G	A	0.668	0.0002778	1.067	0.06485097	0.01783989
rs67934705	G	A	0.567	1.01E-05	1.234	0.21026093	0.04762399
rs6837324	G	A	0.380	3.16E-07	1.090	0.0861777	0.0168525
rs6911131	G	A	0.061	3.09E-05	1.157	0.14583045	0.03499877
rs6990534	G	A	0.670	3.60E-09	1.113	0.10705907	0.0181407
rs701006	G	A	0.592	1.35E-11	1.121	0.11422114	0.01688869
rs71329256	C	G	0.529	6.34E-15	1.154	0.14323417	0.01837044
rs719316	T	C	0.536	7.05E-05	1.067	0.06485097	0.01631638
rs7222450	A	G	0.464	1.78E-08	1.103	0.09803374	0.01740616
rs7260482	C	A	0.263	1.84E-06	1.093	0.08892621	0.01864185
rs72922276	G	A	0.902	6.88E-06	1.141	0.13190507	0.0293294
rs72928038	A	G	0.179	9.01E-11	1.174	0.16041672	0.02474538
rs72989863	G	A	0.629	7.01E-05	1.070	0.06765865	0.01701699
rs73414214	C	A	0.904	0.0003744	1.109	0.10345871	0.02908185
rs735542	A	G	0.672	2.38E-06	1.089	0.08525984	0.01807061
rs760517	C	T	0.584	6.62E-06	1.088	0.08434115	0.01871937
rs7731626	G	A	0.625	2.85E-06	1.097	0.09257918	0.0197762
rs7855251	T	C	0.731	4.23E-08	1.116	0.10975086	0.02002399
rs7975763	T	C	0.215	7.80E-09	1.129	0.12133229	0.0210182
rs7977720	T	C	0.470	3.96E-10	1.107	0.10165365	0.01625003
rs802730	T	C	0.722	3.24E-10	1.121	0.11422114	0.0181683
rs8062446	T	C	0.400	8.41E-07	1.089	0.08525984	0.01730952
rs883871	A	G	0.160	7.08E-06	1.115	0.1088544	0.02423689
rs9308424	G	A	0.698	1.34E-06	1.096	0.09166719	0.01896408
rs9568402	T	A	0.125	0.0003227	1.095	0.09075436	0.02523505
rs9591325	T	C	0.950	4.16E-10	1.237	0.21268909	0.03404168
rs9610458	T	C	0.536	4.57E-12	1.121	0.11422114	0.01650993
rs962052	C	T	0.300	0.0002103	1.070	0.06765865	0.01825498
rs9808753	G	A	0.156	0.0004805	1.085	0.08157999	0.023366
rs983494	G	A	0.784	3.40E-07	1.107	0.10165365	0.01993284
rs9843355	G	A	0.838	4.73E-10	1.143	0.13365638	0.02146122

rs9863496	C	T	0.251	0.0003101	1.068	0.06578774	0.01824036
rs9878602	T	G	0.515	2.60E-07	1.087	0.08342161	0.01619727
rs9900529	C	G	0.255	1.11E-06	1.099	0.09440068	0.0193799
rs9909593	G	A	0.492	1.16E-05	1.074	0.07139	0.0162806
rs9955954	A	G	0.785	1.54E-08	1.116	0.10975086	0.01940074
rs9992763	G	T	0.451	4.51E-08	1.094	0.0898407	0.0164254

Risk factors of musculoskeletal outcomes: Known bone and muscle strength-related factors were used as covariates in MVMR. They are eBMD (heel) [22], appendicular lean mass, whole body fat-free mass, right handgrip strength, left grip strength [23], vitamin D [24], frailty [21], and falls [25].

Statistical analysis

Univariable MR between MS and three musculoskeletal traits as well as risk factors:
Univariable MR was applied to assess the causal relation between MS and 3 musculoskeletal outcomes (fracture, falls, and frailty) and 5 risk factors. Furthermore, MR effects of musculoskeletal risk factors on 3 musculoskeletal outcomes were assessed to examine the potential genetically determined risk paths of 3 musculoskeletal outcomes. Multi-instrument MR approaches were applied, which were to perform a single instrumental variable MR first; and then combined MR results from multiple instrumental variables via fixed-effect meta-analysis to estimate the overall causal relationships.

MVMR between MS and three musculoskeletal outcomes adjusting for risk factors:
We also applied regression-based MVMR to account for many variants having pleiotropic effects that are associated with multiple risk factors in order to provide coefficients (effect sizes) representing the direct causal effects of MS to three musculoskeletal outcomes in turn with the other risk factors being fixed. MVMR is an extension of MR to deal with genetic variants that are associated with multiple risk factors. We selected risk factors that are known to be biologically related to musculoskeletal traits and where there is a potential network of causal effects (mediation) from one risk factor to another. The causal estimate is obtained by regression of the associations with the outcome on the associations with the risk factors, with the intercept set to zero and weights being the inverse-variances of the associations with the outcome [26]. Risk factors presenting significant univariable MR association with each musculoskeletal trait were included in MVMR.

Bidirectional MR among the three musculoskeletal outcomes:

Bidirectional MR was performed among fracture, fall and frailty to examine their mutual effects and directionality considering their highly correlative nature. In bidirectional MR, instruments for both exposure and outcome are used to evaluate whether the “exposure” variable causes the “outcome” or whether the “outcome” variable causes the “exposure”[27].

Heterogeneity analysis

We used Cochran’s Q statistic to check the heterogeneity of IVs. Heterogeneity indicates a possible violation of the necessary IVs or modeling assumptions of which pleiotropy is a likely major cause. Cochran’s Q statistic derived from the inverse variance weighted (IVW) estimate should follow a $\chi 2$ distribution with degrees of freedom equal to the number of SNPs minus 1. Excessive heterogeneity is an indication that either the modeling assumptions have been violated, or that some of the genetic variants violate the IV assumptions—e.g. by exerting a direct effect on the outcome, not through the exposure [28]. This is termed 'horizontal pleiotropy' [29].
When Q statistics showed significant heterogeneity, we applied MR-PRESSO method to reassess the MR effects after filtering out outlier SNPs and compared the fixed MR results with IVW-based results [29].

Sensitivity analysis

The sensitivity analysis including IVW, weighted median, weighted mode, and MR-Egger tests were performed in univariable MR analysis. For MVMR, IVW and MR-Egger tests were evaluated. To exclude instrumental variables that may have horizontal pleiotropy and cause false positive findings of the causal inference, we also applied the MR-Egger regression test [30] to verify horizontal pleiotropy in each MR. Among the multiple methods, we set the results from IVW and MR-PRESSO outlier removal results as primary findings.
All the MR analyses were conducted in R (version 4.0.0) using TwoSampleMR, MendelianRandomizatio, MRPRESSO packages, and the MR-Base platform [31-33]. The causal effect size (beta) with standard error and p-values were presented as appropriately. We applied a false discovery rate (FDR) to correct for multiple testing. The criteria used to select final instrument variables in each MR were GWAS significance: p<5e-8, LD clumping: r2>0.001 within a 10MB window, and proxy SNPs in 1000 Genomes EUR r2=0.8 [33].

Results

Genetic heritability and correlations by LDSR

Regression weight LD Scores for 1,293,150 SNPs of MS GWAS data were read. After merging with reference panel LD, a total of 1,109,876 SNPs remained for the final analysis. LDSR demonstrated that MS has statistically significant moderate genetic correlation with falls (rG: 0.105, p=0.010) but not with fracture (rG: -0.017, p=0.711) or frailty (rG: 0.151, p=0.082). Both handgrip strengths and both leg fat-free masses also showed a genetically significant correlation with MS. However, the whole-body fat free mass didn’t have a significant correlation with MS. The heritability of these traits estimated from GWAS summary statistics was low or considered not high; fracture (h2:0.019), falls (h2: 0.033), and frailty (h2: 0.015) Table 1. We found genetic variant rs13191659 mapped to LINC00240 (Long Intergenic Non-Protein Coding RNA 240) gene is commonly associated with MS and fall (MS GWAS p <5e-8 and fall GWAS<5e-5). LINC00240 is known for total iron binding capacity [37], folic acid deficiency anemia and mean corpuscular volume [38].

Table 1. Genetic correlations between MS and musculoskeletal phenotypes and heritability by LD score regression

Trait	rG	SE_rG	Z_rG	P_rG	h2_obs	h2_obs_se	h2_int	h2_int_se
Fractured/broken bones in last 5 years	-0.017	0.047	-0.371	0.711	0.019	0.002	1.007	0.008
Falls in the last year	0.105	0.041	2.591	0.01	0.033	0.002	1.004	0.008
Frailty	0.151	0.087	1.741	0.082	0.062	0.028	1.056	0.009
Heel Bone Mineral Density (BMD)	-0.003	0.025	-0.132	0.895	0.297	0.033	1.08	0.035
Whole body fat - free mass	0.035	0.025	1.395	0.163	0.291	0.012	1.114	0.029
Hand grip strength (right)	-0.066	0.027	-2.454	0.014	0.104	0.004	1.058	0.013
Hand grip strength (left)	-0.067	0.028	-2.427	0.015	0.104	0.004	1.055	0.012

Leg fat - free mass (right)	0.06	0.025	2.359	0.018	0.266	0.011	1.099	0.026
Leg fat - free mass (left)	0.062	0.025	2.435	0.015	0.268	0.011	1.092	0.025
Arm fat - free mass (right)	0.019	0.025	0.766	0.444	0.267	0.011	1.091	0.026
Arm fat - free mass (left)	0.03	0.025	1.191	0.234	0.267	0.011	1.097	0.025

Mendelian Randomization

The univariable MR analysis showed a minimal causal effect of the total 109 MS SNPs to fracture, falls, and frailty in the IVW method. After excluding SNP outliers by MR-PRESSO and applying multiple testing corrections, without meaningful ORs, MS doesn't seem to causally increase risks of falls (OR:1.004, 95% CI: 1.001-1.006, q=0.018); fracture (OR:1.002, 95% CI:1.000-1.003, q=0.056) and frailty score (beta:0.009, 95% CI: 0.001-0.017, q=0.147) and none of the univariable MR effects between MS and musculoskeletal risk factors were significant Table 2 and Supplementary Table 3. Sensitivity analyses with different MR methods such as MR Egger, Weighted median, and weighted mode methods didn't replicate the significant results in all the MR analyses. All pleiotropy tests (MR Egger intercept test) were insignificant Supplementary Table 3. Single SNP MR Effects between MS and falls are presented in Supplementary Table 4 and 5. In MVMR analysis, except for adjustment of frailty, adjustment of each risk factor didn't modify the overall MR results between MS and fall Table 3. MVMR between MS and fracture as well as MS and frailty presented similar results that adjustment for each risk factor had no meaningful modification in overall MR results Supplementary Table 6 and Table 7.

Table 2. Univariable MR effects between exposure (MS) and outcomes (musculoskeletal outcomes and risk factors)

Exposure	Outcomes	MR effects between MS and outcomes							
		MR method	#SNP	beta	se	*p value*	Q value	OR	95% CI
Multiple Sclerosis	**Musculoskeletal traits**								
	Fracture	IVW	109	0.002	0.001	0.001		1.002	1.000 - 1.004
		MR - PRESSO*		0.002	0.001	0.007	0.056	1.002	1.001 - 1.003
	Fall	IVW	109	0.004	0.001	0.005		1.004	1.002 - 1.006
		MR - PRESSO		0.004	0.001	0.002	0.018	1.004	1.001 - 1.006
	Frailty (continuous)	IVW	152	0.012	0.004	0.007		-	0.004 - 0.020
		MR - PRESSO		0.009	0.004	0.021	0.147	-	0.001 - 0.017
	Musculoskeletal risk factors								
	Heel BMD	IVW	110	-0.002	0.005	0.722	0.722	-	-
	Whole body fat free mass	IVW	109	0.004	0.004	0.258	0.722	-	-
	Appendicular fat free mass	IVW	110	0.006	0.007	0.402	0.722	-	-
	Grip strength(right)	IVW	109	-0.004	0.003	0.183	0.722	-	-
	Grip strength(left)	IVW	109	-0.002	0.003	0.389	0.722	-	-
	Vitamin D	IVW	105	-0.003	0.003	0.427	0.722	-	-

Table 3. Multivariable MR between MS and fall adjusting for musculoskeletal risk factor

	MR effects between MS and fall				
	beta	se	pval	OR	95% CI
Unadjusted model	0.004	0.001	0.005	1.004	1.002 - 1.006
Adjusted by frailty	0.002	0.001	0.058	1.002	1.000 - 1.004
Adjusted by appendicular fat free mass	0.004	0.001	0.004	1.004	1.002 - 1.006
Adjusted bywhole body fat free mass	0.004	0.001	0.004	1.004	1.002 - 1.006
Adjusted by heel BMD	0.004	0.001	0.004	1.004	1.002 - 1.006
Adjusted by right grip strength	0.003	0.001	0.011	1.003	1.000 - 1.005
Adjusted by left grip strength	0.003	0.001	0.008	1.003	1.000 - 1.005
Adjusted by all except frailty	0.003	0.001	0.022	1.003	1.000 - 1.005
Adjusted by all	0.002	0.001	0.184	1.002	1.000 - 1.004

Supplementary Table 3. Sensitivity analsys: MR results between MS and musculoskeletal outcomes and risk factors

	MS (exposure) to fracture and confounders (outcome)					Heterogeneity test				Horizontal pleiotropy test	
	nSNP		beta	se	*p value*		Q	Q_df	Q_pval		
Musculoskeletal outcomes										Egger regression intercept:	-8.50E-07
Fracture	109	IVW	0.002	0.000	0.001					Standard error:	0.0003

				7							
		MR-egger	0.002	0.002	0.383	MR Egger	118	107	0.22	Directionality p-value:	0.997
		Weighted median	0.002	0.001	0.051	IVW	118	108	0.241		
		Weighted mode	0.001	0.002	0.620						
Frailty	152	IVW	0.012	0.004	0.007					Egger regression intercept:	0.002
		MR-egger	-0.017	0.016	0.296	MR Egger	297.2	150	9.20E-12	Standard error:	0.002
		Weighted median	0.011	0.005	0.051	IVW	299.1	151	8.10E-12	Directionality p-value:	0.331
		Weighted mode	0.029	0.016	0.065						
Fall	109	IVW	0.004	0.001	0.005	MR Egger	129.7	107	0.06731	Egger regression intercept:	0.0002
		MR-egger	0.002	0.005	0.751	IVW	129.7	108	0.07421	Standard error:	0.001
		Weighted median	0.004	0.002	0.038					Directionality p-value:	0.649
		Weighted mode	0.002	0.004	0.682						
Risk factors											
Heel BMD	110	IVW	-0.002	0.005	0.722	MR Egger	274.6	108	1.74E-16	Egger regression intercept:	-0.003
		MR-egger	0.027	0.020	0.186	IVW	280.2	109	4.97E-17	Standard error:	0.002
		Weighted median	0.010	0.005	0.057					Directionality p-value:	0.141
		Weighted mode	0.017	0.010	0.083						
Whole body fat free mass	109	IVW	0.004	0.004	0.258	MR Egger	857.9	107	1.61E-117	Egger regression intercept:	-0.002
		MR-egger	0.019	0.013	0.155	IVW	868.8	108	3.81E-119	Standard error:	0.001
		Weighted median	0.003	0.002	0.230					Directionality p-value:	0.246
		Weighted mode	0.003	0.005	0.577						
Appendicular fat free mass	110	IVW	0.006	0.007	0.402	MR Egger	1299	108	2.34E-203	Egger regression intercept:	-0.003
		MR-egger	0.036	0.024	0.140	IVW	1320	109	6.51E-207	Standard error:	0.003
		Weighted median	0.003	0.004	0.462					Directionality p-value:	0.194
		Weighted mode	0.007	0.005	0.225						
Grip strength (right)	109	IVW	-0.004	0.003	0.183					Egger regression intercept:	-0.002
		MR-egger	0.011	0.009	0.237	MR Egger	323.1	107	1.49E-23	Standard error:	0.001
		Weighted median	-0.002	0.002	0.477	IVW	330.4	108	2.23E-24	Directionality p-value:	0.124
		Weighted mode	-0.003	0.006	0.626						
Grip strength (left)	109	IVW	-0.002	0.003	0.389	MR Egger	331	107	1.03E-24	Egger regression intercept:	-0.002
		MR-egger	0.015	0.010	0.130	IVW	341.4	108	5.08E-26	Standard error:	0.001
		Weighted median	0.000	0.002	0.990					Directionality p-value:	0.069
		Weighted mode	0.006	0.007	0.404						
Vitamin D	105	IVW	-0.003	0.003	0.427	MR Egger	252.9	103	1.30E-14	Egger regression intercept:	-0.0002
		MR-egger	-0.001	0.012	0.925	IVW	252.9	104	2.02E-14	Standard error:	0.001
		Weighted median	0.001	0.003	0.693					Directionality p-value:	0.9
		Weighted mode	0.003	0.007	0.673						

Supplementary Table 4. Harmonized MR results between MS(exposure) and falls (outcomes)

	Exposure		Outcome							
SNP	effect_allele exposure	other_allele exposure	effect_allele outcome	other_allele outcome	Beta exposure	Beta outcome	Se outcome	Pval outcome	pval.exposure	se.exposure
rs10063294	A	G	A	G	-0.099	-0.002	0.001	0.170	1.130E-09	0.016
rs1014486	C	T	C	T	0.105	-0.002	0.001	0.160	1.360E-10	0.016
rs10191360	T	C	T	C	0.097	-0.003	0.001	0.027	3.190E-05	0.023
rs10245867	G	T	G	T	-0.063	0.001	0.001	0.270	3.300E-04	0.017
rs1026916	G	A	G	A	-0.130	-0.003	0.001	0.034	1.020E-13	0.017
rs10271373	C	A	C	A	-0.071	-0.001	0.001	0.310	1.650E-05	0.016
rs1076928	C	T	C	T	-0.076	0.000	0.001	0.790	4.220E-06	0.017
rs1077667	C	T	C	T	0.152	-0.001	0.001	0.320	8.370E-13	0.021
rs10801908	C	T	C	T	0.215	0.001	0.002	0.430	3.540E-16	0.026
rs10951042	C	T	C	T	0.079	0.000	0.001	0.930	2.020E-06	0.017
rs10951154	T	C	T	C	-0.100	-0.001	0.002	0.450	6.730E-05	0.025
rs11125803	T	C	T	C	-0.060	0.000	0.001	0.970	1.722E-03	0.019
rs1112718	A	G	A	G	0.106	0.001	0.001	0.620	2.460E-10	0.017
rs11231749	C	T	C	T	0.073	0.002	0.001	0.150	2.340E-05	0.017
rs11256593	T	C	T	C	0.186	0.000	0.001	0.760	6.780E-27	0.017
rs11578655	T	G	T	G	-0.090	-0.004	0.002	0.052	4.817E-04	0.026
rs11749040	G	A	G	A	-0.197	-0.003	0.002	0.091	3.540E-17	0.023
rs1177228	G	A	G	A	0.107	-0.001	0.001	0.440	8.570E-09	0.019
rs11809700	C	T	C	T	-0.144	-0.002	0.001	0.100	3.510E-15	0.018
rs11852059	A	C	A	C	-0.097	-0.003	0.002	0.028	3.020E-06	0.021
rs11899404	T	C	T	C	-0.103	-0.001	0.001	0.640	7.573E-04	0.031
rs11919880	A	G	A	G	0.071	0.000	0.001	0.740	1.970E-05	0.017
rs12147246	G	A	G	A	-0.099	0.001	0.001	0.370	4.290E-09	0.017
rs12211604	A	G	A	G	-0.093	0.000	0.001	0.930	1.860E-07	0.018
rs12365699	G	A	G	A	0.144	-0.002	0.002	0.330	3.150E-10	0.023
rs1250551	G	T	G	T	-0.115747609	0.000319875	0.00122769	0.79	2.66E-11	0.017368
rs12622670	T	C	T	C	0.107	-0.001	0.001	0.670	1.040E-10	0.017
rs12925972	C	T	C	T	0.095	0.002	0.001	0.054	3.070E-08	0.017
rs12971909	G	A	G	A	-0.086	-0.001	0.001	0.570	8.980E-06	0.019
rs13066789	C	T	C	T	0.069	0.000	0.001	0.720	3.170E-05	0.017
rs13327021	C	T	C	T	-0.113	-0.002	0.001	0.180	4.500E-11	0.017
rs1365120	T	C	T	C	-0.120	0.001	0.002	0.460	5.760E-06	0.026
rs1399180	T	C	T	C	-0.080	-0.001	0.002	0.420	3.753E-04	0.022

rs140522	C	T	C	T	-0.111	-0.003	0.001	0.040	2.850E-10	0.018
rs1465697	C	T	C	T	-0.124	0.001	0.001	0.490	3.480E-11	0.019
rs17051321	C	T	C	T	-0.095	0.000	0.001	0.920	4.950E-07	0.019
rs1738074	T	C	T	C	-0.114	0.000	0.001	0.720	9.910E-12	0.017
rs17724508	T	C	T	C	0.213	0.000	0.003	0.970	2.590E-08	0.038
rs17741873	G	T	G	T	0.089	0.002	0.002	0.120	2.150E-05	0.021
rs1800693	T	C	T	C	-0.127	0.001	0.001	0.400	1.020E-13	0.017
rs2084007	C	T	C	T	0.079	0.001	0.001	0.470	1.150E-06	0.016
rs2150879	G	A	G	A	0.104	-0.001	0.001	0.510	3.290E-10	0.016
rs2269434	T	C	T	C	-0.086	-0.002	0.001	0.210	5.240E-07	0.017
rs2286974	G	A	G	A	-0.109	-0.001	0.001	0.370	1.510E-07	0.021
rs2289746	C	T	C	T	0.085	0.000	0.001	0.730	1.480E-06	0.018
rs2317231	G	T	G	T	0.101	0.000	0.001	0.730	1.900E-09	0.017
rs2469434	T	C	T	C	-0.061	-0.001	0.001	0.400	3.094E-04	0.017
rs2546890	A	G	A	G	0.117	0.001	0.001	0.600	1.040E-12	0.016
rs2585447	T	C	T	C	-0.129	0.001	0.002	0.720	7.890E-08	0.024
rs2590438	T	G	T	G	-0.054	0.002	0.001	0.079	1.561E-03	0.017
rs2836438	G	A	G	A	-0.099	0.002	0.002	0.240	2.980E-05	0.024
rs28703878	A	G	A	G	-0.134	-0.001	0.001	0.700	4.510E-10	0.021
rs2986736	T	C	T	C	-0.106	-0.001	0.001	0.550	3.410E-05	0.026
rs3184504	T	C	T	C	0.068	0.000	0.001	0.800	3.450E-05	0.017
rs32658	G	T	G	T	-0.069	-0.001	0.001	0.340	2.950E-05	0.016
rs34681760	C	T	C	T	0.080	0.000	0.001	0.920	5.280E-06	0.018
rs34695601	T	C	T	C	0.109	0.001	0.001	0.650	3.170E-08	0.020
rs354033	G	A	G	A	0.108	0.001	0.001	0.530	1.210E-08	0.019
rs35486093	A	G	A	G	-0.179	-0.004	0.002	0.081	1.600E-10	0.028
rs35540610	C	T	C	T	0.135	0.001	0.001	0.290	2.880E-12	0.019
rs35703946	G	A	G	A	0.173	0.000	0.002	0.980	1.940E-09	0.029
rs3737798	A	G	A	G	0.087	0.002	0.001	0.043	1.400E-07	0.017
rs3809627	A	C	A	C	-0.097	0.000	0.001	1.000	3.250E-08	0.018
rs3923387	T	C	T	C	0.071	0.002	0.001	0.076	2.350E-05	0.017
rs405343	G	T	G	T	-0.118	-0.002	0.002	0.280	4.720E-08	0.022
rs4325907	T	C	T	C	-0.099	0.001	0.001	0.500	3.680E-09	0.017
rs438613	C	T	C	T	0.138	0.001	0.001	0.370	9.430E-17	0.017
rs4728142	G	A	G	A	-0.061	-0.001	0.001	0.500	2.585E-04	0.017
rs4796224	A	G	A	G	-0.085	0.001	0.001	0.300	1.620E-07	0.016
rs55858457	G	T	G	T	-0.113	-0.002	0.001	0.200	1.200E-08	0.020

rs57116599	A	G	A	G	-0.120	0.001	0.001	0.660	2.590E-09	0.020
rs59655222	T	C	T	C	0.123	0.000	0.001	0.970	3.760E-11	0.019
rs6032662	C	T	C	T	0.134	0.002	0.001	0.140	2.850E-13	0.018
rs60600003	T	G	T	G	-0.134	-0.001	0.002	0.590	4.200E-07	0.026
rs6072343	G	A	G	A	-0.076	-0.002	0.002	0.360	8.908E-04	0.023
rs61708525	G	A	G	A	0.080	0.001	0.001	0.290	1.910E-05	0.019
rs61863928	G	T	G	T	0.109	-0.002	0.001	0.170	5.450E-08	0.020
rs62013236	C	T	C	T	0.125	0.000	0.002	0.970	2.070E-06	0.026
rs62420820	A	G	A	G	0.137	-0.002	0.001	0.170	2.500E-13	0.019
rs6496663	A	C	A	C	-0.101	0.002	0.001	0.071	2.780E-08	0.018
rs6564681	T	C	T	C	-0.094	0.001	0.001	0.490	2.230E-07	0.018
rs6589706	A	G	A	G	0.104	-0.001	0.001	0.390	2.200E-09	0.017
rs6670198	C	T	C	T	-0.145	-0.001	0.001	0.530	2.030E-16	0.018
rs67111717	G	A	G	A	0.096	0.001	0.001	0.670	4.960E-07	0.019
rs6738544	C	A	C	A	0.066	0.000	0.001	0.800	9.490E-05	0.017
rs6837324	A	G	A	G	-0.086	0.000	0.001	0.800	3.160E-07	0.017
rs6911131	A	G	A	G	-0.146	0.004	0.003	0.092	3.090E-05	0.035
rs6990534	A	G	A	G	-0.107	-0.001	0.001	0.670	3.600E-09	0.018
rs701006	G	A	G	A	0.114	0.002	0.001	0.040	1.350E-11	0.017
rs719316	T	C	T	C	0.065	0.002	0.001	0.160	7.050E-05	0.016
rs7260482	A	C	A	C	-0.089	0.000	0.001	0.940	1.840E-06	0.019
rs72922276	G	A	G	A	0.132	-0.002	0.002	0.390	6.880E-06	0.029
rs72928038	G	A	G	A	-0.161	0.000	0.002	0.890	9.010E-11	0.025
rs72989863	G	A	G	A	0.068	-0.001	0.001	0.500	7.010E-05	0.017
rs73414214	C	A	C	A	0.103	-0.002	0.002	0.310	3.744E-04	0.029
rs760517	C	T	C	T	0.084	0.000	0.001	0.990	6.620E-06	0.019
rs7731626	G	A	G	A	0.093	0.003	0.001	0.010	2.850E-06	0.020
rs7855251	T	C	T	C	0.110	-0.001	0.001	0.660	4.230E-08	0.020
rs7975763	C	T	C	T	-0.121	-0.001	0.001	0.650	7.800E-09	0.021
rs8062446	T	C	T	C	0.085	-0.002	0.001	0.210	8.410E-07	0.017
rs9308424	A	G	A	G	-0.091	0.001	0.001	0.390	1.340E-06	0.019
rs9591325	T	C	T	C	0.212	0.004	0.002	0.097	4.160E-10	0.034
rs9610458	T	C	T	C	0.114	-0.001	0.001	0.220	4.570E-12	0.017
rs962052	C	T	C	T	0.068	0.001	0.001	0.640	2.103E-04	0.018
rs9808753	G	A	G	A	0.081	0.004	0.002	0.015	4.805E-04	0.023
rs9843355	G	A	G	A	0.134	0.003	0.002	0.053	4.730E-10	0.021
rs9878602	T	G	T	G	0.084	0.003	0.001	0.004	2.600E-07	0.016

rs9955954	G	A	G	A	-0.110	0.000	0.004	0.980	1.540E-08	0.019
rs9992763	T	G	T	G	-0.090	-0.002	0.001	0.150	4.510E-08	0.016

Supplementary Table 5. Single SNP effects between MS and falls

SNP	b	se	p
rs9878602	0.0407191	0.0141779	0.0040787
rs7731626	0.0338696	0.0131349	0.0099204
rs9808753	0.0511996	0.0210284	0.0149006
rs10191360	-0.0272116	0.0123231	0.0272323
rs11852059	0.0351912	0.0160442	0.0282794
rs1026916	0.0203119	0.0095607	0.0336275
rs701006	0.0217184	0.0105721	0.0399447
rs140522	0.0233565	0.0113911	0.040325
rs3737798	0.0275048	0.0135835	0.0428819
rs11578655	0.0390692	0.0201127	0.0520753
rs9843355	0.0217962	0.0112696	0.053104
rs12925972	0.0243653	0.0126489	0.0540708
rs6496663	-0.0232281	0.0128492	0.0706451
rs3923387	0.030114	0.0169865	0.0762588
rs2590438	-0.0404945	0.023024	0.0786132
rs35486093	0.0201356	0.011525	0.0806157
rs11749040	0.0151535	0.0089607	0.0908165
rs6911131	-0.0305713	0.0181346	0.0918342
rs9591325	0.0183933	0.0110904	0.0972171
rs11809700	0.0151788	0.0092558	0.1010203
rs17741873	0.0264107	0.0171457	0.1234703
rs6032662	0.0152553	0.0102821	0.1378953
rs9992763	0.0192153	0.0132762	0.1477994
rs11231749	0.0246731	0.0173263	0.1544393
rs1014486	-0.0161516	0.0114098	0.156896
rs719316	0.0255046	0.0182991	0.1633876
rs62420820	-0.0139511	0.0101161	0.1678627
rs10063294	0.0164289	0.0119746	0.1700688
rs61863928	-0.0155462	0.0114531	0.1746628
rs13327021	0.0146036	0.0109511	0.182358
rs2269434	0.0182347	0.0144311	0.2063849
rs8062446	-0.0180684	0.0144619	0.2115275
rs9610458	-0.01286	0.0104104	0.2167198
rs55858457	0.0134996	0.0112654	0.2307913
rs2836438	-0.0207132	0.0174904	0.2363094
rs10245867	-0.0223827	0.0201067	0.2656256
rs405343	0.0144707	0.0132975	0.2764943
rs35540610	0.010959	0.0103501	0.2896765
rs61708525	0.0171172	0.0161819	0.2901466
rs4796224	-0.0144993	0.0139002	0.2969029
rs73414214	-0.0205641	0.0202415	0.309659
rs10271373	0.0168963	0.0166966	0.3115565
rs1077667	-0.0094716	0.0095782	0.322727
rs12365699	-0.0107606	0.0110755	0.3312631

rs32658	0.0164936	0.0174488	0.344528
rs6072343	0.0206489	0.0223627	0.3558182
rs12147246	-0.0112269	0.0124605	0.3675915
rs2286974	0.0098623	0.0110089	0.3703322
rs438613	0.0076605	0.0085731	0.3715631
rs72922276	-0.0124256	0.0144092	0.3885047
rs9308424	-0.011971	0.0139371	0.3903771
rs6589706	-0.0097618	0.0114241	0.3928332
rs2469434	0.0168397	0.0198006	0.3950656
rs1800693	-0.0080636	0.0094994	0.3959633
rs1399180	0.0159448	0.0197924	0.4204724
rs10801908	0.0066588	0.0084803	0.4323279
rs1177228	-0.0095585	0.0123948	0.4406057
rs10951154	0.0131933	0.0175495	0.4521864
rs1365120	-0.0112627	0.0151367	0.4568357
rs2084007	0.0107723	0.0149218	0.470347
rs1465697	-0.0075709	0.0110098	0.491673
rs6564681	-0.0094856	0.0138073	0.4920835
rs4325907	-0.0083772	0.0124474	0.5009406
rs72989863	-0.0121365	0.0181512	0.5037285
rs4728142	0.0129851	0.0194285	0.5039083
rs2150879	-0.0075462	0.0114748	0.510771
rs6670198	0.0054049	0.0085061	0.5251585
rs354033	0.0078898	0.0125432	0.5293446
rs2986736	0.0082583	0.0139762	0.5546006
rs12971909	0.0081218	0.0143668	0.5718568
rs60600003	0.0080571	0.0148044	0.5862783
rs2546890	0.0053172	0.0101037	0.5987036
rs1112718	0.0056825	0.0114032	0.6182571
rs11899404	0.0055055	0.011627	0.6358497
rs962052	0.0094029	0.020049	0.6390706
rs34695601	0.0057077	0.0124768	0.6473358
rs7975763	0.0054972	0.0121186	0.6501057
rs57116599	-0.0052659	0.011844	0.6566038
rs7855251	-0.0055304	0.0124631	0.6572313
rs6990534	0.0052145	0.0121211	0.6670503
rs67111717	0.005601	0.0131391	0.6699017
rs12622670	-0.0047231	0.0111082	0.670696
rs28703878	0.003924	0.0100644	0.6966175
rs2585447	-0.0046263	0.0127719	0.7171839
rs1738074	-0.003785	0.0104698	0.7177121
rs13066789	-0.0061236	0.0172619	0.7227792
rs2289746	0.0050858	0.0147762	0.7307024
rs2317231	0.0040645	0.0118721	0.7320803
rs11919880	0.0055962	0.0169259	0.7409229
rs11256593	-0.0019886	0.0063854	0.7554758
rs1076928	0.0042264	0.0155881	0.7862916
rs1250551	-0.0027636	0.0106066	0.7944393
rs3184504	-0.0044597	0.0172604	0.7961172
rs6837324	0.0035874	0.0139927	0.7976582

rs6738544	-0.0045821	0.0184752	0.8041226
rs72928038	-0.0013234	0.009619	0.8905741
rs17051321	-0.0014401	0.0145783	0.9213117
rs34681760	-0.0015113	0.0157856	0.9237252
rs10951042	-0.0013181	0.0152402	0.9310771
rs7260482	-0.0010929	0.0151282	0.9424094
rs62013236	0.0005368	0.0137395	0.9688372
rs59655222	0.0003574	0.0105651	0.9730155
rs17724508	-0.0004114	0.0122572	0.9732216
rs11125803	0.0007544	0.022494	0.9732466
rs9955954	-0.0011987	0.0408933	0.9766141
rs35703946	0.0002427	0.0101079	0.9808429
rs12211604	0.0001683	0.0132516	0.9898663
rs760517	0.0001253	0.0157757	0.9936624
rs3809627	3.90E-05	0.0124542	0.9975024
All - Inverse variance weighted	0.0036925	0.0013232	0.0052616
All - MR Egger	0.0015514	0.0048781	0.7510841

Supplementary Table 6. MVMR between MS and fracture adjusting for risk factors

Unadjusted model (MR between MS and fracture)	0.002	0.001	0.001	1.002	1.000-1.004
Adjusted by					
frailty	0.002	0.001	0.006	1.002	1.000-1.004
fall	0.002	0.001	0.007	1.002	1.000-1.004
Appendicular fat free mass	0.001	0.001	0.025	1.001	0.999-1.003
whole body fat free mass	0.002	0.001	0.01	1.002	1.000-1.004
heel BMD	0.002	0.001	0.013	1.002	1.000-1.004
vitamin D	0.002	0.001	0.002	1.002	1.000-1.004
right grip strength	0.002	0.001	0.002	1.002	1.000-1.004
left grip strength	0.002	0.001	0.001	1.002	1.000-1.004
frailty+ fall	0.002	0.001	0.012	1.002	1.000-1.004
frailty+ fall+ heel BMD+whole body fat free mass + appendicular fat free mass**	0.002	0.001	0.024	1.002	1.000-1.004
heel BMD+whole body fat free mass + appendicular fat free mass+ vitd+gripR+gripL	0.002	0.001	0.004	1.002	1.000-1.004
frailty+ fall+ heel BMD+whole body fat free mass + appendicular fat free mass+vitd+gripR+gripL	0.002	0.001	0.033	1.002	1.000-1.004

Supplementary Table 7. MVMR between MS and frailty adjusting for musculskeletal risk factors

	MR effects of MS to frailty				
	Beta	**Se**	**pval**	**OR**	**95% CI**
Unadjusted model	0.012	0.004	0.007	1.012	1.004-1.020
Adjusted by fall	0.002	0.001	0.058	1.002	1.000-1.004
Adjusted by appendicular fat free mass	0.013	0.004	0.003	1.013	1.005-1.021
Adjusted by whole body fat free mass	0.013	0.004	0.004	1.013	1.005-1.021
Adjusted by right grip strength	0.011	0.005	0.02	1.011	1.001-1.021
Adjusted by left grip strength	0.011	0.004	0.015	1.011	1.003-1.019

Adjusted by all except fall*	0.013	0.005	0.004	1.013	1.003-1.023
Adjusted by all	0.01	0.004	0.027	1.01	1.002-1.018

To account for the directional effects among the 3 musculoskeletal outcomes; fracture, fall and frailty, we conducted bidirectional MR. As expected, Fall causally increased fracture risk (OR: 1.215, 95% CI: 1.098-1.345, p=1.63E-04) and frailty risk (OR: 4.870, 95% CI: 2.412-9.832, p=1.01E-05).

Discussion

In this study, we found genetic correlation with shared pleotropic effects between MS and fall proven by LDSR and MR with minimal effect size. However, we don't find meaningful causal relation to support epidemiological observation with increased musculoskeletal traits in MS patients. The MS doesn't causally increase risks of musculoskeletal traits. Based on the univariable MR between MS and musculoskeletal risk factors, none of the well-known muscle and bone-related risk factors (whole-body and appendicular fat-free masses, both grip strengths, heel BMD and vitamin D) were causally affected by MS. In bidirectional MR among the three musculoskeletal outcomes, fall causally increased fracture and frailty risk.

Taking into all these findings together, we didn't provide evidence to support that MS causally increased risks of neither musculoskeletal traits nor other musculoskeletal risk factors. The widely observed musculoskeletal disorders in MS patients in clinical studies might be more likely due to secondary factors that are associated with MS disease progression and treatment.

When we further explored SNPs commonly found in MS and fall with the criteria of GWAS/suggestive GWA significant variants, genetic variants mapped to a gene associated with iron biomarkers was found. Therefore, we could hypothesize MS and falls share pleiotropic genetic effects in iron biomarker pathways. It is well known that there is a correlation between anemia and falls, and specifically patients with lower hemoglobin levels are more likely to fall [36]. MRI and histological studies also suggest that iron levels are dysregulated in MS: iron accumulates in grey matter and is depleted in normal-appearing white matter [37].

Most of the previous observational studies predominantly reported high fracture risk in MS in comparison to age-matched controls. Dyn one study, Bazelier et al, 2011 [38] reported 3-fold increased hip fracture risk in patients with MS during 5 years of follow-up. However, their baseline fractures were higher in MS than age, and sex-matched controls, furthermore the baseline falls history was higher in MS. Therefore, increased fractures observed in MS patients in this study might have been via increased fall risk in this population.

Falls are common in people with MS, with a large international data set demonstrating that 56% MS individuals fall at least once within 3 months' period with 37% categorized as frequent fallers [4]. Notably, MS patients fall more frequently and experience more injurious falls, and are more likely to attribute their falls to tripping and distraction [39].

Medication use is a well-established risk factor for falls, either directly (affecting balance, attention, or muscle tone) or indirectly (as proxies of underlying conditions influencing the risk of falling; joint pain, arthrosis, and cardiovascular diseases among many others). A recent falls GWAS study examined the genetic correlation of falls with medication use and found medications such as opioids, anti-inflammatory and anti-rheumatic drugs, anilides, and drugs for peptic ulcer and gastro-esophageal reflux disease were in positive genetic correlations with falls [40]. Medication use associated with MS treatments and complications should be one of the strong risk factors for falls in this population. However, we are not able to evaluate such hypothesis based on our current study design.

Based on our study, genetic factors of MS might predispose MS patients to increased risk of falls by shared genetic pleiotropy but with minimal effects. Therefore, taking into account the secondary environmental or treatment-related factors such as careful medication use and incorporating stringent fall prevention strategy in patient care will mitigate the undesirable muscle and bone effects in MS patients.

We have a few novelties and strengths to mention. Given the highly increased availability of public GWAS data and identification of thousands of trait-associated loci in each trait, we could utilize LDSR and a multifaceted MR approach together and could elucidate the stepwise causal relations, and draw new perspectives on musculoskeletal traits in MS patients. We also illuminated genetically predisposed iron biomarker alteration might have a role in fall risk in MS patients. However, we have some limitations. Due to the low heritability of musculoskeletal traits and risk factors **Table 1**, incorporating MR analysis might induce limited implications as well as GWAS data from these phenotypes might not highly reliable to elucidate any causal inference.

In conclusion, genetic association between MS and falls still stands, suggesting there are shared pleiotropic genetic factors between these two phenotypes. However, we didn't establish a causal relationship between MS and multiple musculoskeletal traits which were reported by observational studies.

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