SUPPLEMENTAL MATERIAL

Effects of iron homeostasis on epigenetic age acceleration: A Two-sample Mendelian Randomization Study

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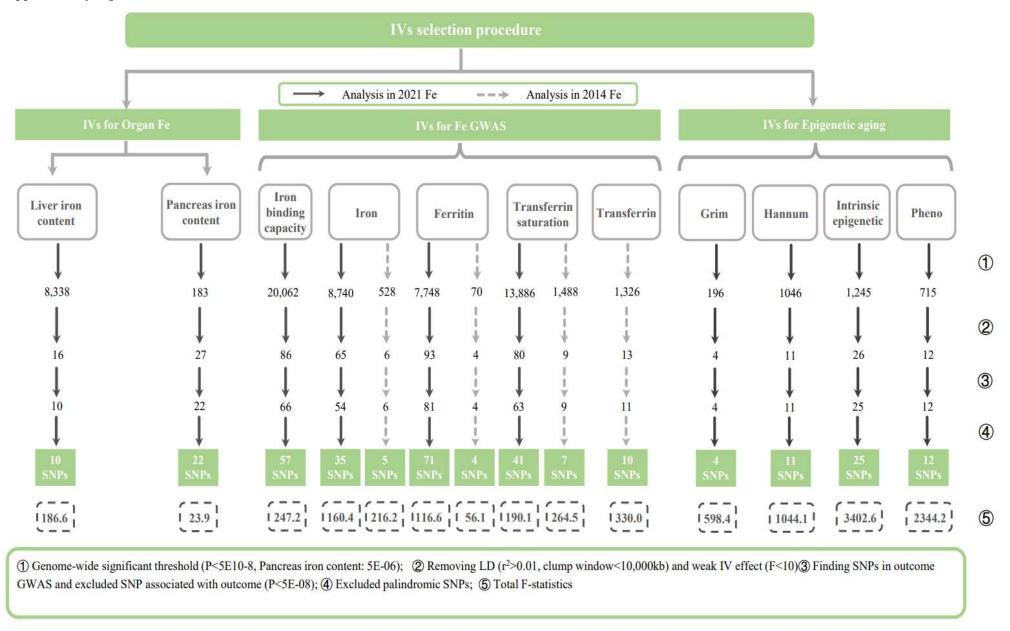
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Supplementary Figure 1. Procedure of IVs selection.



Abbreviations: IVs, instrumental variables; GWAS, genome-wide association study; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.

Supplementary Figure 2. MR analyses of epigenetic aging accelerations with 2014 datasets serum iron biomarkers

Exposure	Outcome	nSNP	Beta (95% CI)		P value
Grim					
	Ferritin	4	0.03 (-0.02, 0.09)	-	2.38E-01
	Iron	4	0.02 (-0.06, 0.09)	-	6.58E-01
	Transferrin saturation	4	0.03 (-0.07, 0.12)	-	5.91E-01
	Transferrin	4	-0.03 (-0.09, 0.04)	-	4.26E-01
Hannum				1	
	Ferritin	5	0.00 (-0.04, 0.03)	+	7.88E-01
	Iron	5	0.00 (-0.04, 0.03)		8.41E-01
	Transferrin saturation	5	0.01 (-0.03, 0.05)	-	6.16E-01
	Transferrin	5	-0.02 (-0.06, 0.02)		2.86E-01
Intrinsic epigenetic	;				
	Ferritin	9	0.00 (-0.03, 0.04)	_	7.85E-01
	Iron	9	0.03 (-0.01, 0.06)	-	1.32E-01
	Transferrin saturation	9	0.02 (-0.02, 0.06)	-	2.42E-01
	Transferrin	9	-0.01 (-0.04, 0.03)		7.49E-01
Pheno					
	Ferritin	5	0.01 (-0.01, 0.04)	-	3.13E-01
	Iron	5	0.02 (-0.02, 0.06)	-	4.25E-01
	Transferrin saturation	5	0.02 (-0.02, 0.06)	-	4.28E-01
	Transferrin	5	-0.02 (-0.05, 0.01)		1.71E-01
			-	-0.1 -0.05 0 0.05 0	.1 0.15

Abbreviations: MR, mendelian randomization; SNP, single nucleotide polymorphism; CI, confidence interval.

Supplementary Figure 3. MR analyses of epigenetic aging accelerations with 2021 datasets serum iron biomarkers

Exposure	Outcome	nSNP	Beta (95% CI)		P value
Grim					
	Ferritin	4	0.01 (-0.04, 0.06)	-	7.48E-01
	Iron	4	0.02 (-0.01, 0.05)	-	1.62E-01
	Iron binding capacity	4	-0.03 (-0.08, 0.02)	-	2.65E-01
	Transferrin saturation	4	0.03 (-0.01, 0.06)	-	1.17E-01
Hannum					
	Ferritin	11	0.00 (-0.01, 0.02)	+	3.47E-01
	Iron	11	0.00 (-0.01, 0.02)	-	6.89E-01
	Iron binding capacity	11	-0.01 (-0.02, 0.00)	-	1.27E-01
	Transferrin saturation	11	0.01 (-0.01, 0.02)		5.00E-01
Intrinsic epigenetic					
	Ferritin	26	0.00 (-0.01, 0.01)	+	4.54E-01
	Iron	25	-0.01 (-0.01, 0.00)	•	9.67E-02
	Iron binding capacity	24	0.00 (-0.01, 0.01)	-	8.21E-01
	Transferrin saturation	23	-0.01 (-0.02, 0.00)	-	1.17E-01
Pheno					
	Ferritin	11	0.00 (-0.01, 0.01)	-	8.05E-01
	Iron	12	0.00 (-0.01, 0.01)	-	7.81E-01
	Iron binding capacity	12	0.00 (-0.01, 0.01)	+	8.88E-01
	Transferrin saturation	11	0.00 (-0.01, 0.01)	+	6.98E-01
			-0.	1 -0.05 0 0.05	0.1

Abbreviations: MR, mendelian randomization; SNP, single nucleotide polymorphism; CI, confidence interval.

Supplementary Figure 4. MR analyses of epigenetic aging accelerations with organic iron content

Exposure	Outcome	nSNP	Beta (95% CI)		P value
Grim					
	Liver iron content	4	0.00 (-0.04, 0.05)	+	8.82E-01
	Pancreas iron content	4	0.04 (-0.01, 0.08)	-	1.21E-01
Hannum				 	
	Liver iron content	11	-0.02 (-0.04, 0.01)	-	1.88E-01
	Pancreas iron content	11	0.00 (-0.03, 0.03)	+	8.32E-01
Intrinsic epigenetic					
	Liver iron content	25	0.00 (-0.02, 0.02)	_	7.57E-01
	Pancreas iron content	26	0.00 (-0.01, 0.02)	-	7.50E-01
Pheno					
	Liver iron content	12	0.01 (-0.01, 0.02)	-	3.70E-01
	Pancreas iron content	12	0.00 (-0.02, 0.02)	-	9.01E-01
			-0	.05 0 0.05	0.1

Abbreviations: MR, mendelian randomization; SNP, single nucleotide polymorphism; CI, confidence interval.

Supplementary Table 1. Sensitivity analyses of the epigenetic aging acceleration with the serum iron biomarkers in 2014 datasets.

Exposure Outcome		Weighted median		MR-Egger regression		- Heterogeneity ^a	MR-PRESSO outlier detect ^b		Pleiotropy ^c
Exposure	Outcome	Beta (95% CI)	P Value	Beta (95% CI)	P Value	Heterogenetry	Beta (95% CI)	P Value	F lelotropy
Grim	Ferritin	0.04 (-0.03, 0.1)	2.56E-01	0.30 (-0.75, 1.35)	6.31E-01	I2 = 14.5%; Cochrane Q = 4; P = 0.32	No significat	nt outliers	Intercept = -0.05 ; P = 0.667
Grim	Iron	0.01 (-0.06, 0.08)	8.01E-01	0.28 (-1.16, 1.71)	7.42E-01	12 = 45.3%; Cochrane Q = 5; P = 0.139	No significa	nt outliers	Intercept = -0.048 ; P = 0.756
Grim	Transferrin Saturation	0.04 (-0.04, 0.11)	3.22E-01	0.05 (-1.82, 1.92)	9.62E-01	I2 = 65.5%; Cochrane Q = 9; P = 0.034	No significar	nt outliers	Intercept = -0.005 ; P = 0.981
Grim	Transferrin	-0.05 (-0.12, 0.02)	1.72E-01	0.31 (-0.95, 1.58)	6.73E-01	I2 = 30.2%; Cochrane $Q = 4$; $P = 0.231$	No significar	nt outliers	Intercept = -0.064 ; P = 0.648
Hannum	Ferritin	0.00 (-0.04, 0.04)	9.72E-01	-0.01 (-0.17, 0.14)	8.85E-01	I2 = 0%; Cochrane $Q = 1$; $P = 0.955$	No significar	nt outliers	Intercept = 0.002 ; P = 0.929
Hannum	Iron	0.01 (-0.04, 0.05)	7.84E-01	0.08 (-0.08, 0.24)	4.05E-01	I2 = 0%; Cochrane $Q = 1$; $P = 0.840$	No significar	nt outliers	Intercept = -0.023 ; P = 0.374
Hannum	Transferrin Saturation	0.02 (-0.03, 0.07)	4.42E-01	0.08 (-0.08, 0.25)	3.93E-01	I2 = 0%; Cochrane $Q = 1$; $P = 0.881$	No significar	nt outliers	Intercept = -0.02 ; P = 0.433
Hannum	Transferrin	-0.02 (-0.07, 0.03)	4.38E-01	-0.05 (-0.21, 0.12)	6.28E-01	I2 = 0%; Cochrane $Q = 1$; $P = 0.899$	No significa	nt outliers	Intercept = 0.007 ; P = 0.787
IE	Ferritin	0.01 (-0.04, 0.05)	8.07E-01	0.02 (-0.30, 0.34)	8.92E-01	I2 = 16.5%; Cochrane Q =10; P = 0.295	No significat	nt outliers	Intercept = -0.004 ; P = 0.913
ΙΕ	Iron	0.02 (-0.02, 0.07)	3.51E-01	0.20 (-0.13, 0.52)	2.68E-01	I2 = 22%; Cochrane $Q = 10$; $P = 0.248$	No significat	nt outliers	Intercept = -0.038 ; P = 0.332
IE	Transferrin Saturation	0.02 (-0.02, 0.07)	3.70E-01	0.14 (-0.22, 0.49)	4.73E-01	I2 = 26.8%; Cochrane Q =11; P = 0.206	No significat	nt outliers	Intercept = -0.025 ; P = 0.544
IE	Transferrin	0.00 (-0.05, 0.04)	9.44E-01	0.10 (-0.19, 0.40)	5.18E-01	I2 = 0%; Cochrane $Q = 7$; $P = 0.524$	No significa	nt outliers	Intercept = -0.024 ; P = 0.494
Pheno	Ferritin	0.01 (-0.03, 0.04)	6.81E-01	-0.03 (-0.11, 0.04)	4.55E-01	I2 = 1.1%; Cochrane $Q = 4$; $P = 0.400$	No significa	nt outliers	Intercept = 0.021 ; P = 0.268
Pheno	Iron	0.01 (-0.03, 0.05)	5.81E-01	-0.08 (-0.16, 0.00)	1.53E-01	I2 = 39.9%; Cochrane Q = 7; P = 0.155	No significa	nt outliers	Intercept = 0.041 ; P = 0.088
Pheno	Transferrin Saturation	0.02 (-0.02, 0.06)	2.62E-01	-0.07 (-0.15, 0.01)	2.04E-01	I2 = 41.1%; Cochrane Q = 7; P = 0.148	No significat	nt outliers	Intercept = 0.036 ; P = 0.116
Pheno	Transferrin	-0.01 (-0.05, 0.03)	5.37E-01	-0.02 (-0.11, 0.06)	5.95E-01	I2 = 0%; Cochrane Q = 3;	No significar	nt outliers	Intercept = 0.001 ; P = 0.948

Supplementary Table 2. Sensitivity analyses of the epigenetic aging acceleration with the serum iron biomarkers in 2021 datasets.

Evenorura		Weighted median		MR-Egger regression			MR-PRESSO outlier detect ^b		Pleiotropy ^c
Exposure	Outcome	Beta (95% CI)	P Value	Beta (95% CI)	P Value	- Heterogeneity ^a	Beta (95% CI)	P Value	Pleiotropy
Grim	Ferritin	0.00 (-0.03, 0.02)	8.15E-01	0.24 (-0.80, 1.28)	6.96E-01	12 = 87.6%; Cochrane Q = 24; P = 0.001	No significant outliers		Intercept = -0.043; P = 0.705
Grim	Iron	0.02 (-0.01, 0.04)	2.30E-01	0.07 (-0.49, 0.63)	8.30E-01	I2 = 37.7%; Cochrane Q = 5; P = 0.186	No significant outliers		Intercept = -0.009 ; P = 0.877
Grim	Iron binding capacity	-0.03 (-0.06, 0.00)	7.78E-02	-0.54 (-1.23, 0.15)	2.64E-01	12 = 74.2%; Cochrane $Q = 12$; $P = 0.009$	-0.03 (-0.07, 0.01)	0.45	Intercept = 0.095 ; P = 0.282
Grim	Transferrin saturation	0.02 (-0.01, 0.05)	2.45E-01	0.36 (-0.20, 0.91)	3.34E-01	I2 = 51.2%; Cochrane Q = 6; P = 0.104	No significant	outliers	Intercept = -0.061 ; P = 0.364
Hannum	Ferritin	0.01 (-0.01, 0.02)	2.90E-01	0.00 (-0.03, 0.03)	8.62E-01	I2 = 0%; Cochrane $Q = 6$; $P = 0.791$	No significant	outliers	Intercept = 0.002 ; P = 0.601
Hannum	Iron	0.01 (-0.01, 0.03)	2.78E-01	-0.01 (-0.05, 0.04)	7.77E-01	I2 = 45.2%; Cochrane $Q = 18$; $P = 0.051$	No significant	outliers	Intercept = 0.003 ; P = 0.66
Hannum	Iron binding capacity	-0.01 (-0.03, 0.01)	2.33E-01	-0.02 (-0.06, 0.02)	4.27E-01	I2 = 0%; Cochrane $Q = 5$; $P = 0.916$	No significant outliers		Intercept = 0.002 ; P = 0.76
Hannum	Transferrin saturation	0.01 (-0.01, 0.03)	3.39E-01	0.00 (-0.05, 0.05)	1.00E+00	12 = 38.4%; Cochrane $Q = 16$; $P = 0.093$	No significant	outliers	Intercept = 0.002 ; P = 0.815
ΙE	Ferritin	0.00 (-0.01, 0.01)	4.23E-01	0.00 (-0.02, 0.02)	8.72E-01	12 = 53.5%; Cochrane $Q = 54$; $P = 0.001$	0.00 (0.00, 0.00)	1.00	Intercept = 0.001 ; P = 0.896
ΙE	Iron	-0.01 (-0.02, 0.00)	9.27E-02	0.00 (-0.02, 0.02)	7.61E-01	12 = 15.1%; Cochrane $Q = 28$; $P = 0.249$	No significant	outliers	Intercept = -0.001 ; P = 0.712
ΙΕ	Iron binding capacity	0.00 (-0.02, 0.01)	6.96E-01	-0.01 (-0.03, 0.02)	5.80E-01	I2 = 33%; Cochrane Q = 34; P = 0.061	No significant outliers		Intercept = 0.002 ; P = 0.612
ΙE	Transferrin saturation	-0.01 (-0.02, 0.00)	2.02E-01	0.00 (-0.02, 0.03)	8.15E-01	I2 = 47%; Cochrane Q = 42; P = 0.007	-0.02 (-0.02, -0.02)	0.00	Intercept = -0.004 ; P = 0.345
Pheno	Ferritin	0.00 (-0.01, 0.01)	6.60E-01	0.00 (-0.03, 0.02)	8.32E-01	I2 = 39.2%; Cochrane Q = 16; P = 0.087	No significant	outliers	Intercept = 0.001 ; P = 0.888

^a Heterogeneity in the random effect IVW methods was reported. ^b MR-PRESSO (NbDistribution = 10,000, P < 0.05). ^c MR-Egger was used to detect Pleiotropy. There is no pleiotropy was observed among all analyses (P>0.05). Abbreviation: CI confidence interval, MR-PRESSO Mendelian Randomization Pleiotropy RESidual Sum and Outlier, IE Intrinsic epigenetic.

Pheno	Iron	0.00 (-0.01, 0.01)	7.78E-01	0.00 (-0.03, 0.03)	9.79E-01	I2 = 37.3%; Cochrane $Q = 18$; $P = 0.093$	No significant outliers	Intercept = 0.001 ; $P = 0.898$
Pheno	Iron binding capacity	0.00 (-0.01, 0.01)	9.53E-01	0.01 (-0.02, 0.03)	7.01E-01	12 = 0%; Cochrane Q = 7; P = 0.837	No significant outliers	Intercept = -0.002 ; P = 0.72
Pheno	Transferrin saturation	-0.01 (-0.02, 0.00)	1.56E-01	0.00 (-0.04, 0.03)	7.53E-01	I2 = 16.3%; Cochrane $Q = 12$; $P = 0.289$	No significant outliers	Intercept = 0.001 ; P = 0.844

^a Heterogeneity in the random effect IVW methods was reported. ^b MR-PRESSO (NbDistribution = 10,000, P < 0.05). ^c MR-Egger was used to detect Pleiotropy. There is no pleiotropy was observed among all analyses (P>0.05). Abbreviation: CI confidence interval, MR-PRESSO Mendelian Randomization Pleiotropy RESidual Sum and Outlier, IE Intrinsic epigenetic.

Supplementary Table 3. Sensitivity analyses of the epigenetic aging acceleration with the organic iron content.

F	Outcome	Weighted n	nedian	MR-Egger reg	ression	II.4	MR-PRESSO outlier detect ^b		DI : 4 C		
Exposure	Outcome	Beta (95% CI)	P Value	Beta (95% CI)	P Value	Heterogeneity ^a	Beta (95% CI)	P Value	- Pleiotropy ^c		
Grim	Liver iron content	0.02 (-0.03, 0.07)	5.03E-01	-0.26 (-1.05, 0.54)	5.91E-01	I2 = 4.5%; Cochrane $Q = 3$;	No significan	t outliers	Intercept = 0.048 ; P = 0.586		
Grilli	Liver from content	0.02 (-0.03, 0.07)	3.03E-01	-0.20 (-1.03, 0.34)	3.91E-01	P = 0.370	No significan	outhers	mercept – 0.048; P – 0.380		
Grim	Pancreas iron content	0.04 (-0.02, 0.09)	1.65E-01	-0.26 (-1.05, 0.52)	5.78E-01	I2 = 0%; Cochrane $Q = 1$;	No significan	outliers	Intercent = 0.056: D = 0.530		
Ollii	Fancieas non content	0.04 (-0.02, 0.09)	1.03E-01	-0.20 (-1.03, 0.32)	3.76E-01	P = 0.888	No significan	outhers	Intercept = 0.056 ; P = 0.530		
Hannum	Liver iron content	-0.02 (-0.05, 0.01)	2.67E-01	2.67E-01 0.02 (-0.06, 0.09)	6.80E-01	I2 = 34.8%; Cochrane $Q = 15$;	No significant outliers		Nii6441		Intercept = -0.010 ; P = 0.368
Haimum	Liver from content	-0.02 (-0.03, 0.01)	2.07E-01	0.02 (-0.00, 0.09)	0.80E-01	P = 0.120			тистеерт 0.010, 1 0.300		
Hannum	Pancreas iron content	-0.01 (-0.05, 0.03)	6.06E-01	0.02 (-0.06, 0.11)	6.27E-01	I2 = 31.3%; Cochrane $Q = 15$;	No significant outliers		Intercept = -0.006 ; P = 0.656		
Haimum	1 ancreas from content	-0.01 (-0.03, 0.03)	0.00L-01	0.02 (-0.00, 0.11)	0.27L-01	P = 0.149					
ΙE	Liver iron content	0.01 (-0.01, 0.02)	6.06E-01	0.02 (-0.03, 0.07)	4.87E-01	I2 = 50.1%; Cochrane $Q = 48$;	0.01 (-0.01, 0.03) 2.21E-01		Intercept = -0.004 ; P = 0.530		
IL.	Liver non content	0.01 (0.01, 0.02)	0.00L 01	0.02 (0.03, 0.07)	4.07L 01	P = 0.002	0.01 (0.01, 0.03)	2.212 01	пистеерт 0.004, г 0.330		
ΙE	Pancreas iron content	0.00 (-0.02, 0.03)	6.64E-01	0.01 (-0.04, 0.05)	7.62E-01	I2 = 22.9%; Cochrane $Q = 32$;	No significant outliers		Intercept = -0.001 ; P = 0.841		
IL.	i diferens from content	0.00 (0.02, 0.03)	0.04E 01	0.01 (0.04, 0.05)	7.02E 01	P = 0.146			тистеерт 0.001,1 0.041		
Pheno	Liver iron content	0.01 (-0.01, 0.03)	2.53E-01	-0.01 (-0.06, 0.04)	6.81E-01	I2 = 6.1%; Cochrane $Q = 12$;	No significant outliers		Intercept = 0.007 ; P = 0.449		
Theno	Liver non content	0.01 (0.01, 0.03)	2.33E 01	0.01 (0.00, 0.04)	0.012 01	P = 0.385			тегеерг 0.007,1 0.449		
Pheno	Pancreas iron content	0.00 (-0.03, 0.02)	8.37E-01	-0.02 (-0.07, 0.03)	5.10E-01	I2 = 0%; Cochrane $Q = 10$;	No significant outliers		Intercept = 0.007 ; P = 0.454		
1 110110	i ancicas non content	0.00 (0.03, 0.02)	0.5712-01	0.02 (0.07, 0.03)	3.10L-01	P = 0.564			тегеері 0.007,1 0.434		

^a Heterogeneity in the random effect IVW methods was reported. ^b MR-PRESSO (NbDistribution = 10,000, P < 0.05). ^c MR-Egger was used to detect Pleiotropy. There is no pleiotropy was observed among all analyses (P>0.05). Abbreviation: CI confidence interval, MR-PRESSO Mendelian Randomization Pleiotropy RESidual Sum and Outlier, IE Intrinsic epigenetic.

Supplementary table 4-83: See supplementary file: Supplementary table 4-83.xlsx