

SUPPLEMENTAL MATERIAL

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

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Supplementary Figure 1. Procedure of IVs selection.

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

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

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

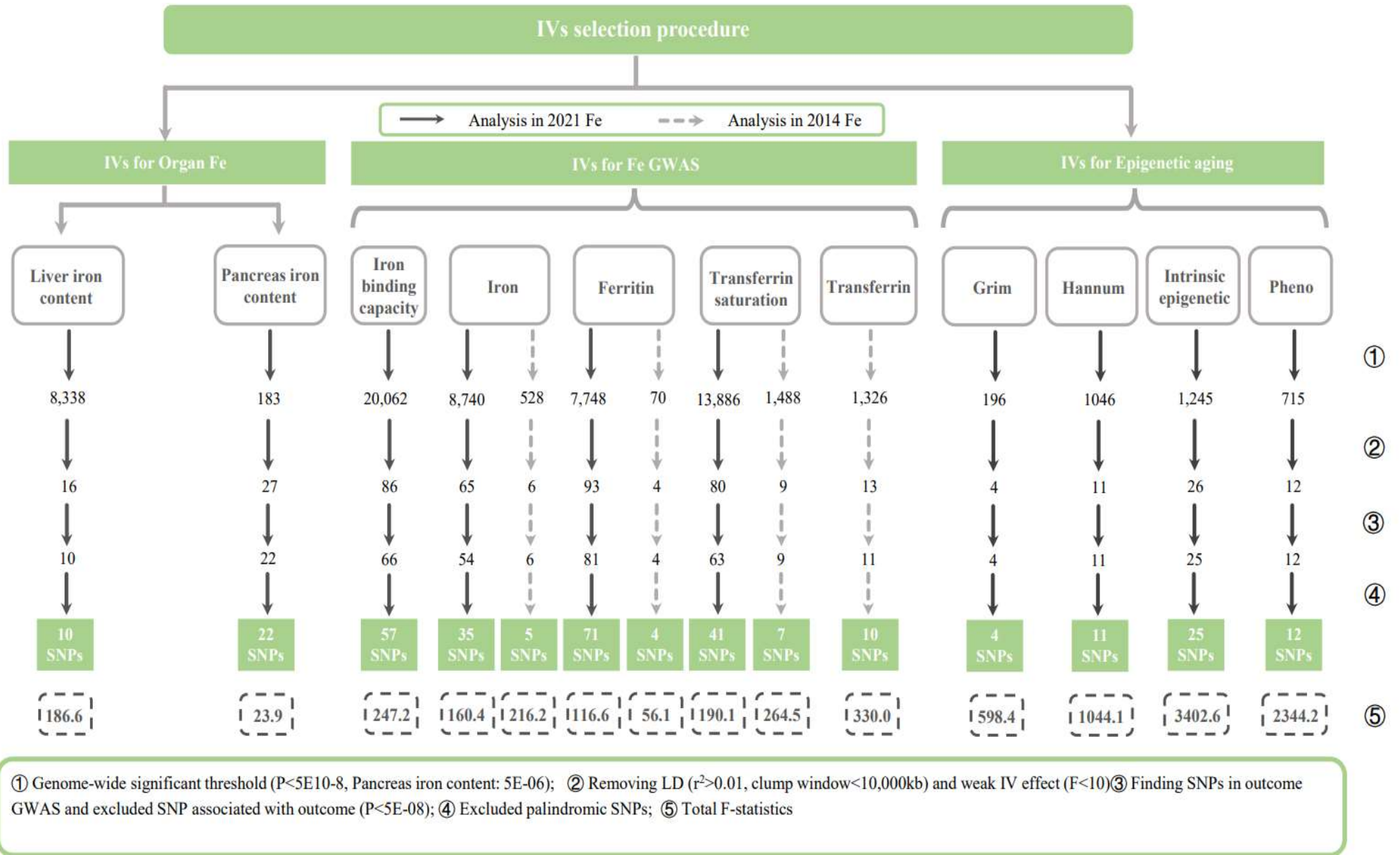
Supplementary Table 1. Sensitivity analyses of the epigenetic aging acceleration with the serum iron biomarkers in 2014 datasets.

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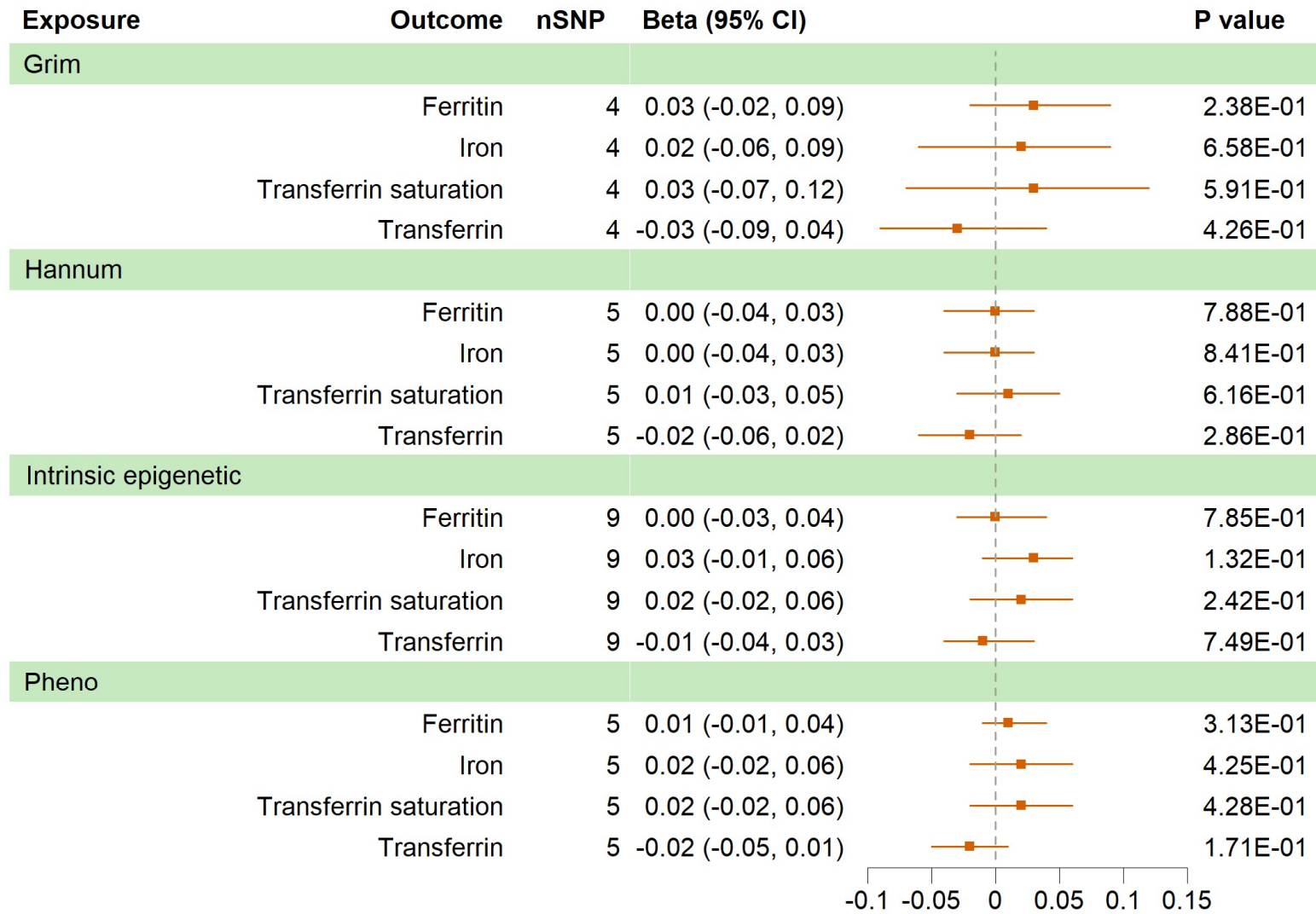
Supplementary table 4-83. Instrumental SNPs for serum iron biomarkers, organic iron content and epigenetic aging acceleration in the univariable mendelian randomization analysis.

Supplementary Figure 1. Procedure of IVs selection.



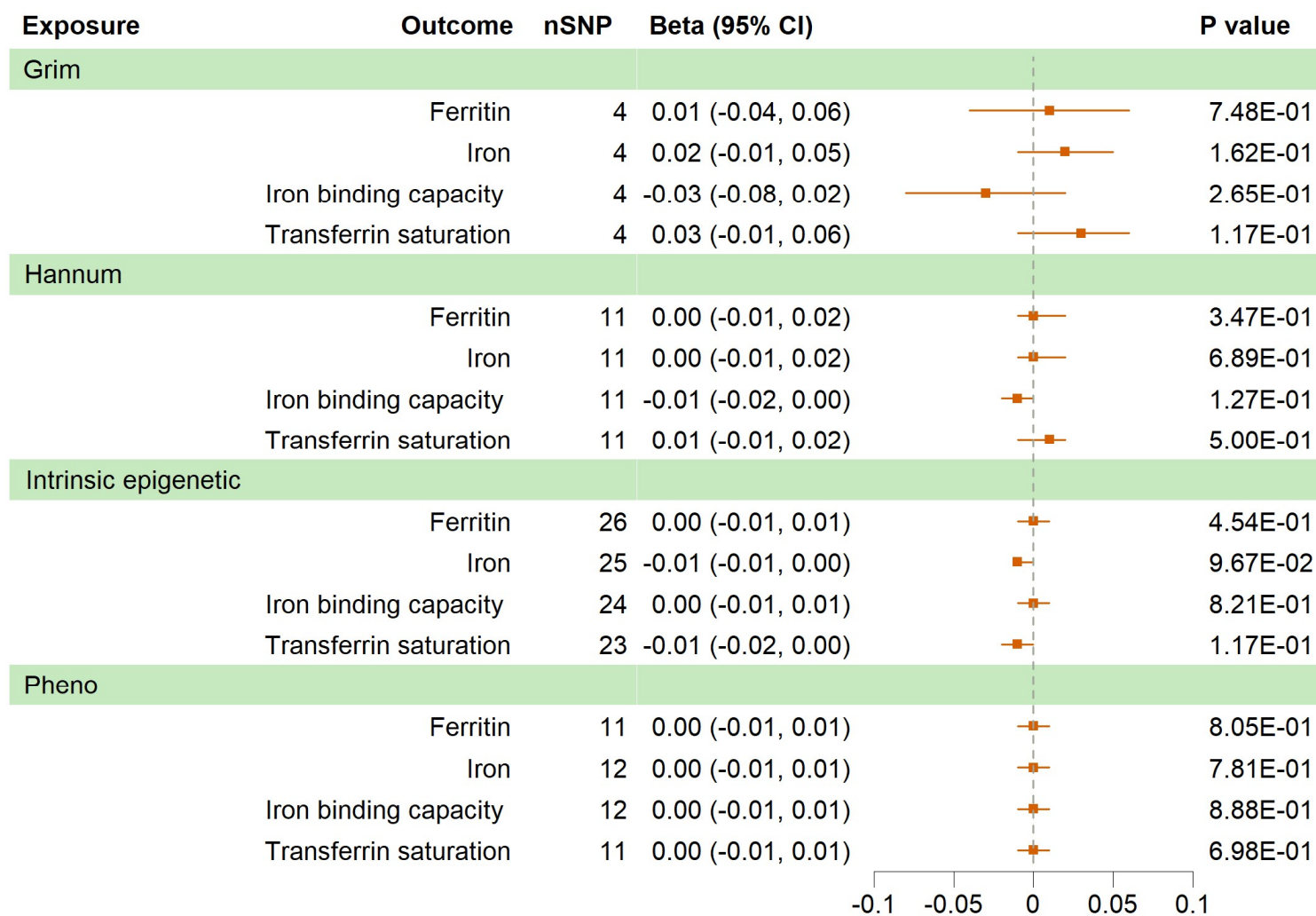
Abbreviations: IVs, instrumental variables; GWAS, genome-wide association study; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.

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



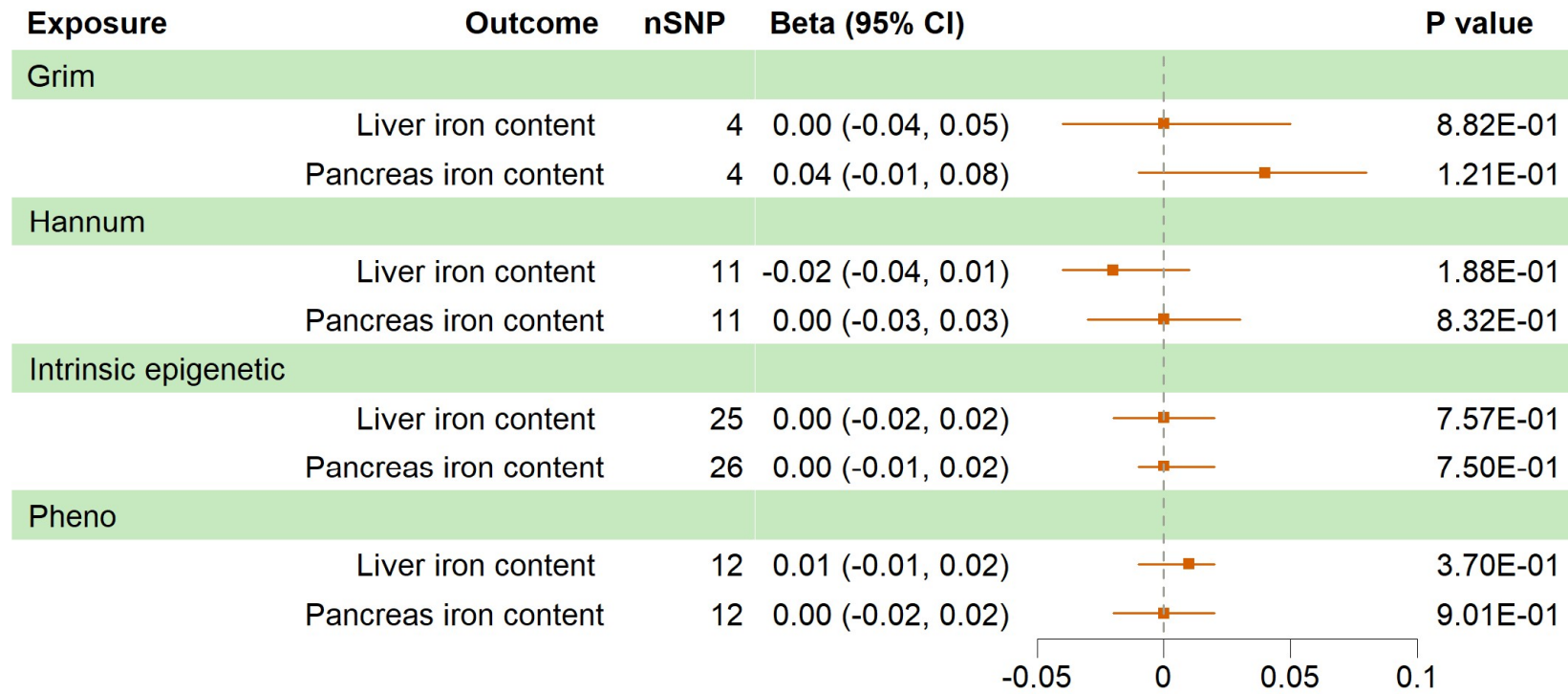
Abbreviations: MR, mendelian randomization; SNP, single nucleotide polymorphism; CI, confidence interval.

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



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



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
		Beta (95% CI)	P Value	Beta (95% CI)	P Value		Beta (95% CI)	P Value	
Grim	Ferritin	0.04 (-0.03, 0.1)	2.56E-01	0.30 (-0.75, 1.35)	6.31E-01	I ² = 14.5%; Cochrane Q = 4; P = 0.32	No significant 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	I ² = 45.3%; Cochrane Q = 5; P = 0.139	No significant 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	I ² = 65.5%; Cochrane Q = 9; P = 0.034	No significant 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	I ² = 30.2%; Cochrane Q = 4; P = 0.231	No significant 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	I ² = 0%; Cochrane Q = 1; P = 0.955	No significant 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	I ² = 0%; Cochrane Q = 1; P = 0.840	No significant 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	I ² = 0%; Cochrane Q = 1; P = 0.881	No significant 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	I ² = 0%; Cochrane Q = 1; P = 0.899	No significant 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	I ² = 16.5%; Cochrane Q = 10; P = 0.295	No significant outliers	Intercept = -0.004; P = 0.913	
IE	Iron	0.02 (-0.02, 0.07)	3.51E-01	0.20 (-0.13, 0.52)	2.68E-01	I ² = 22%; Cochrane Q = 10; P = 0.248	No significant 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	I ² = 26.8%; Cochrane Q = 11; P = 0.206	No significant 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	I ² = 0%; Cochrane Q = 7; P = 0.524	No significant 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	I ² = 1.1%; Cochrane Q = 4; P = 0.400	No significant 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	I ² = 39.9%; Cochrane Q = 7; P = 0.155	No significant 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	I ² = 41.1%; Cochrane Q = 7; P = 0.148	No significant 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	I ² = 0%; Cochrane Q = 3;	No significant outliers	Intercept = 0.001; P = 0.948	

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

Exposure	Outcome	Weighted median		MR-Egger regression		Heterogeneity ^a	MR-PRESSO outlier detect ^b		Pleiotropy ^c
		Beta (95% CI)	P Value	Beta (95% CI)	P Value		Beta (95% CI)	P Value	
Grim	Ferritin	0.00 (-0.03, 0.02)	8.15E-01	0.24 (-0.80, 1.28)	6.96E-01	I2 = 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	I2 = 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	I2 = 38.4%; Cochrane Q = 16; P = 0.093	No significant outliers		Intercept = 0.002; P = 0.815
IE	Ferritin	0.00 (-0.01, 0.01)	4.23E-01	0.00 (-0.02, 0.02)	8.72E-01	I2 = 53.5%; Cochrane Q = 54; P = 0.001	0.00 (0.00, 0.00)	1.00	Intercept = 0.001; P = 0.896
IE	Iron	-0.01 (-0.02, 0.00)	9.27E-02	0.00 (-0.02, 0.02)	7.61E-01	I2 = 15.1%; Cochrane Q = 28; P = 0.249	No significant outliers		Intercept = -0.001; P = 0.712
IE	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
IE	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

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	I2 = 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.

Exposure	Outcome	Weighted median		MR-Egger regression		Heterogeneity ^a	MR-PRESSO outlier detect ^b		Pleiotropy ^c
		Beta (95% CI)	P Value	Beta (95% CI)	P Value		Beta (95% CI)	P Value	
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; P = 0.370	No significant outliers	Intercept = 0.048; P = 0.586	
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; P = 0.888	No significant outliers	Intercept = 0.056; P = 0.530	
Hannum	Liver iron content	-0.02 (-0.05, 0.01)	2.67E-01	0.02 (-0.06, 0.09)	6.80E-01	I2 = 34.8%; Cochrane Q = 15; P = 0.120	No significant outliers	Intercept = -0.010; P = 0.368	
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; P = 0.149	No significant outliers	Intercept = -0.006; P = 0.656	
IE	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; P = 0.002	0.01 (-0.01, 0.03)	2.21E-01	Intercept = -0.004; P = 0.530
IE	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; P = 0.146	No significant outliers	Intercept = -0.001; P = 0.841	
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; P = 0.385	No significant outliers	Intercept = 0.007; P = 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; P = 0.564	No significant outliers	Intercept = 0.007; P = 0.454	

^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