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Supplementary Materials for

A null mutation in *SERPINE1* protects against biological aging in humans

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Supplementary Materials and Methods

Supplemental Experimental Procedures

Ancestral Records

Ancestral Records with vital status, including date of death was available on 221 Berne Amish individuals either enrolled in our study or in direct lineage to an enrolled participant. Among deceased individuals (n=113), genotype status for *SERPINE1* had been previously ascertained by direct genotyping in 18. From review of the extended pedigree, obligate ascertainment of genotype status for *SERPINE1* was possible for an additional 38 deceased participants. We included only those individuals for whom *SERPINE1* genotype status was able to be identified with a high level of confidence. A high level of confidence was indicated for carrier status only for individuals directly related to the presumed founder I-1 or I-2, and where they had 1) grandchild with directly genotyped carrier status, and 2) child with directly genotyped carrier status (generation III) or obligate *SERPINE1* genotype status for parsimonious inheritance (generation II). A high level of confidence was indicated for normal status for individuals directly related to the presumed founder I-1 or I-2, where they had 1) absence of child with directly genotyped carrier status, and 2) absence of a grandchild with directly genotyped carrier status. A high level of confidence was indicated for normal status for individuals not directly related to the presumed founder I-1 or I-2, where they had 1) absence of child with directly genotyped affected status, and 2) absence of a child with directly genotyped carrier status unless definitely explained by marriage, and 3) absence of siblings with directly genotyped carrier or affected status. Given average family size of 8 to 10 children, the chances of a parent being normal status if all of the children are genotyped normal status is greater than 99.96%.

Validation of Composite Aging Scores

We sought to validate the biological aging composite scores in a generalized US population using the Coronary Artery Risk Development in Young Adults (CARDIA) study. The CARDIA study is a multicenter, community-based longitudinal cohort study designed to investigate the risk factors for the development of cardiovascular disease. In 1985-1986, 5115 black and white men and women aged 18 to 30 years were recruited from 4 urban sites across the United States (Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California). Recruitment was balanced within each center by sex, age, race, and education. CARDIA participants have been followed for more than 30 years with collection of detailed demographic and clinical data. All participants provided written informed consent at each examination, and institutional review boards from each field center and the coordinating center approved the study annually. Detailed descriptions of the study design and conduct have been previously published.(*62*) For this analysis, all participants who attended the Year 25 examination and had available data for components of composite score 1 and 2 were included (N=2793). A subset of participants who also had data for composite score 3 (N=892) were also examined separately.

table S1. Clinical characteristics of homozygous participants for the null *SERPINE1* mutation.

	***SERPINE1* +/+** N= 127	***SERPINE1* -/-** N= 7	**p-value***
Age, years	46±20	27±6	0.11
Female, %	55	71	0.29
Hypertension, %	33	0	0.10
Systolic blood pressure, mmHg	130±18	116±7	0.11
Diastolic blood pressure, mmHg	77±10	74±8.14	0.53
Obesity, %	30	0	0.11
Body mass index, kg/m^2	27.7±5.9	22.9±2.7	0.10
Diabetes, %	7	0	0.39
Fasting glucose, mg/dL**	88 (82-98)	84 (79 -85)	0.26
Total cholesterol, mg/dL	188±40	149±36	0.27
HDL cholesterol, mg/dL	60±15	62±7	0.25
Triglyceride, mg/dL**	81 (57-113)	59 (57-66)	0.37
LDL cholesterol, mg/dL	109±32	73±31	0.11
Serum creatinine, mg/dL	0.80±0.16	0.74±0.11	0.13

HDL = high-density lipoprotein; LDL = low-density lipoprotein;

*Polygenic model adjusted for carrier status and incorporation of family structure in SOLAR;

**Non-normally distributed, reported as median (25^{th}-75^{th} percentile);

table S2. Association of null *SERPINE1* mutant allele status with PAI-1, telomere length, and fasting insulin: secondary analyses.

	***SERPINE1* +/+** N= 127	***SERPINE1* +/-, -/-** N= 50	**p-value**
PAI-1, ng/mL*	9.14 (5.45-16.73)	2.25 (1.02-6.63)	<0.0001
Leukocyte telomere length, relative	0.95 ±0.25	1.04 ±0.25	0.020
Fasting insulin, uIU/mL*	4.90 (3.30-6.70)	4.10 (3.05-5.00)	0.026

table S3. Association of cardiometabolic aging composite scores with 5-year CVD morbidity and mortality in the CARDIA cohort (*n* = 2793).

	Mean (SD) or median (25-75th percentile)	**OR (95% CI) per 1 unit**
Fasting insulin, uIU/mL*	8.7 (5.3-14.2)	1.02 (0.97-1.06)
Tissue Doppler e' velocity, cm/s	9.3 (2.4)	0.69 (0.55-0.86)
Brachial pulse pressure, mm Hg*	44 (39-49)	1.05 (1.01-1.09)
Average carotid IMT, mm*	0.69 (0.62-0.78)	7.76 (0.63-96.01)
Score 1	-0.00 (1.99)	1.46 (1.20-1.78)
Score 2	-0.00 (2.40)	1.35 (1.14-1.60)

HDL = high-density lipoprotein; LDL = low-density lipoprotein;

*Non-normally distributed, reported as median (25th-75th percentile)

table S4. Association of biological aging composite scores including telomere length with 5-year CVD morbidity and mortality in the CARDIA cohort (*n* = 872).

	CARDIA N=892	**OR (95% CI)**
Leukocyte telomere length, relative	0.71 (0.12)	0.11 (0.00-352.24)
Absolute telomere length	4979 (285)	1.00 (1.00-1.00)
Fasting insulin, uIU/mL*	8.5 (5.3-14.0)	1.03 (0.96-1.11)
Tissue Doppler e' velocity, cm/s	9.2 (2.3)	0.59 (0.34-1.01)
Brachial pulse pressure, mm Hg*	44 (39-49)	1.06 (1.01-1.12)
Average carotid IMT, mm*	0.67 (0.61-0.75)	162.57 (2.48-10645.21)
Score 1	-0.07 (1.93)	1.98 (1.33-2.95)
Score 2	-0.07 (2.19)	1.87 (1.28-2.73)
Score 3	-0.08 (2.61)	1.61 (1.18-2.22)

HDL = high-density lipoprotein; LDL = low-density lipoprotein;

*Non-normally distributed, reported as median (25^{th}-75^{th} percentile)

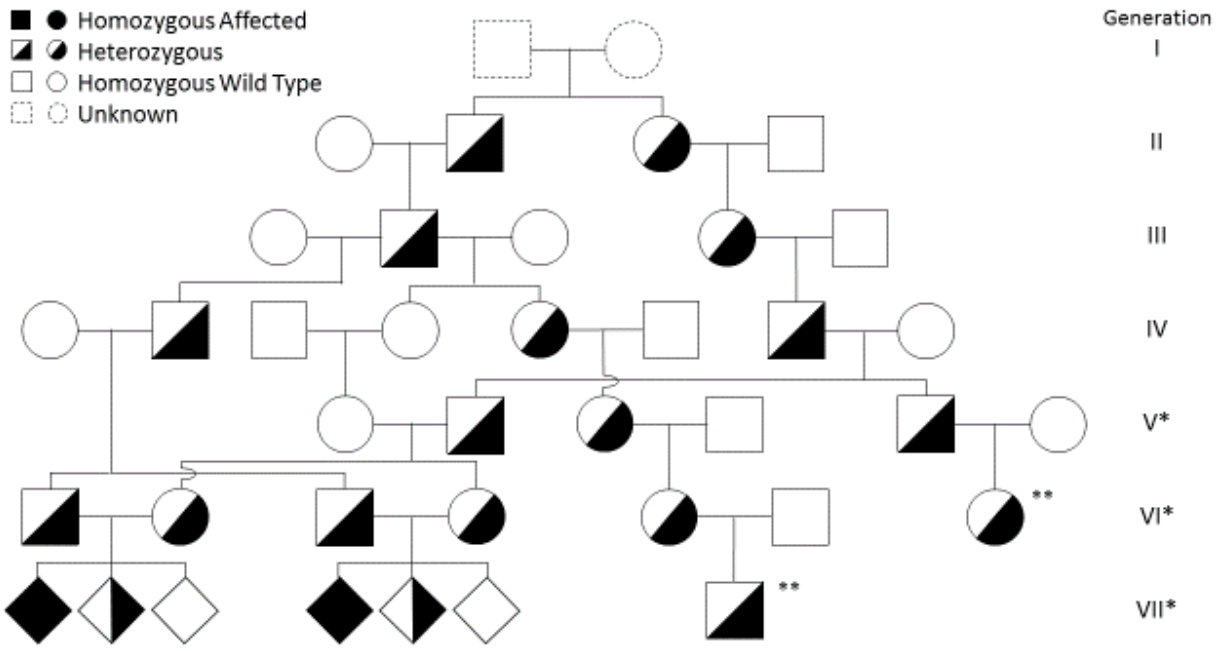

fig. S1. Abbreviated pedigree of the Berne Amish kindred. Genotype status is indicated by the symbols shown in the key, with squares indicating male sex and circles indicating female sex. Solid circles and squares represent individuals who are homozygous for the null *SERPINE1* gene and half-solid circles and squares represent individuals who are heterozygous. In generation I, either the husband or the wife are an obligate carrier of the null *SERPINE1* gene, given that they are the ancestors of all the enrolled participants with one or two copies of the null *SERPINE1* gene. *Of the participants enrolled and included in the primary analysis (heterozygous and unaffected), 40 individuals were from generation V (born between 1926-1950, including V_3, V_4), 54 individuals were from generation VI (born between 1951-1975, including VI_1, VI_2, VI_5, VI_6), and 76 individuals were from generation VII (born between 1976-1997). **Individual VI_7 and VIII_7 are only related by the ancestors in generation I and share a kinship coefficient of 0.05%.