SUPPLEMENTAL MATERIAL

for the manuscript

COL4A1 Is Associated With Arterial Stiffness By Genome Wide Association Scan.

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Introduction

Founder population

In population genetics, founder effect refers to the establishment of a new colony by a very small number of individuals from a larger population, often many hundreds or thousands of years ago. When such founder groups expand to large modern populations without substantial in-migration, they facilitate detailed genealogical research and reconstruction of extended multigenerational pedigrees; and the relatedness and relative environmental homogeneity make it easier to determine the alleles that contribute to the values of a complex phenotype or to a disease. [1-3]

Methods

Reproducibility of PWV in SardiNIA

Each measurement of PWV was performed by 2 sonographers, with one recording the waveforms at the carotid site, and the other simultaneously recording the waveforms at the femoral site. Because several sonographers participated in the collection of the PWV data, a reproducibility study was performed on 3 subjects who underwent repeated measurements of

PWV using random combinations of testers (there were 5 testers at the carotid site and 3 testers at the femoral site). Each subject underwent approximately 45 measurements of PWV. The coefficient of variation for PWV ranged from 7.2 to 10.6%. Both general linear models as well as mixed effects models showed that only inter-subject differences significantly contributed to the overall error in the measurement of PWV, whereas carotid testers, femoral testers, and the interaction between carotid and femoral testers did not.

Imputations

For individuals who were genotyped with the 500k chip, the SNP being tested is coded as 0, 1, or 2, depending on the number of copies carried of an arbitrary reference allele. If this SNP was not included in the 10K chip, than for individuals with missing genotype data (i.e. those genotyped only with the 10k chip), the Lander-Green algorithm was used to estimate the number of copies of this allele that are being carried, which was assigned a score ranging between 0 and 2[4]. Importantly, this score represents a probabilistic estimate of the number of copies of the allele, and it incorporates information on allele frequency, the genotype of relatives for the SNP of interest, their degree of relatedness, and data on flanking markers. Thus, this score need not be an ordinal value of 0, 1 or 2, but can be a fractional number that ranges between 0 and 2, which enables uncertain genotypes to be accounted for. For computational efficiency, the Lander-Green algorithm was applied to sub-pedigrees, each including no more than 20-25 individuals, resulting in a dataset where the average analysis unit consisted of a family with 12.3 members and 3.2 generations.

Kinship coefficient:

Kinship coefficient in the SardiNIA study was determined on the basis of family structure, not genotype data. Family structure, in turn, was determined on the basis of self-reported information, which allowed us to construct family genealogy trees that were up to five generations deep. However, analytic constraints required us to ignore any relatedness that was not observed within these 5 generations. All the first and second degree relationships were verified based on the genotype data using GRR software[4], and adjusted if necessary.

Comparison of PWV protocols in SardiNIA and HAPPI Heart:

The device used to measure PWV in HAPI Heart was the Complior® SP device (Artech Medical, Pantin, France), which differs than the custom-designed device that was used in SardiNIA. However, both devices have been validated[5, 6], and PWV measured with either of these devices has been shown to be an independent predictor of mortality in large epidemiological studies[7, 8]. Importantly, since the raw PWV data from the 2 studies were not combined, rather the results were combined in the meta-analysis; and since the adjustment for family structure and the data transformation and the statistical analyses in HAPI Heart were performed in a manner that was analogous to the way they were performed in SardiNIA, we don't feel that the results of the genetic analyses could be an artifact of the minor methodological differences that arise from the use of two different devices. In fact, several meta-analyses have recently been performed for many other quantitative traits [3, 9-11], and the

combined results have consistently pointed to genes that were associated with the trait in most of the cohorts in spite of differences in the devices or methodologies used to perform the measurements.

Discussion

Collagen Type 4

Type 4 Collagen had not previously been considered to be involved in regulating arterial stiffness. Unlike Collagen types 1 and 3, which are constituents of the extracellular matrix that are found in the medial layer of the arterial walls where they impart the tensile strength to the arteries, type 4 Collagen is a structural component of basement membranes. Basement membranes surround vascular smooth muscle cells in the media. During the aging process within arteries, smooth muscle cells manufacture, secrete and activate type 2 matrix metalloprotease (MMPII), a collagenase whose substrate is type 4 Collagen. Degradation of type 4 Collagen in the basement membranes permits these cells to invade the internal elastic membrane and enter the subendothelial space. These cells then proliferate and secrete matrix, resulting in a diffusely thickened intima that interferes with endothelial function and also alters the transduction of mechanical forces imparted by flow and pressure of the blood[12]. The *COL4A1* polymorphism described in the present study could impact on age-associated arterial stiffening by affecting the binding of MMPII to collagen 4, its substrate. Alternatively, this polymorphism could affect the polymerization of the Collagen 4α1 molecules not only rendering basement membranes more susceptible to metalloprotease

digestion but also differentially affecting formation of the advanced glycation end-products which are known to influence the assembly of type 4 Collagen[13] and to modulate arterial stiffness.

Beyond its role as a structural component of the basement membrane, type 4 Collagen also provides structural support and anchorage for cells, serves as a ligand for cell surface receptors, modulates endothelial cell proliferation, and regulates angiogenesis and tumor growth. Thus, allelic variants of *COL4A1* could differentially affect the permeability of the basement membrane, or the cell-matrix and/or cell-basement membrane interactions of the endothelial or smooth muscle cells in the arterial wall. In this context, it is noteworthy that the *MAGI1* gene product also plays a role in cell-cell interactions, as a scaffolding protein at cell-cell junctions and as a mediator of vascular endothelial cell adhesions[14].

Finally, allelic variants could differentially influence the type or quantity of growth factors secreted by these cells (e.g. TGF-β), which, in turn, would influence signaling cascades e.g. SMAD signaling, initiated by TGF-β receptor activation, which leads to increased production of collagen I and III matrix proteins known to directly affect arterial stiffness.

We should note that at the present time there is no functional evidence implicating *COL4A1* as a determinant of PWV. Therefore the foregoing discussion of its putative role in influencing arterial stiffness remains speculative. Furthermore, by using standard prediction algorithms we could not find any evidence that the Glycine to Histidine substitution at position 1334 that is associated with the rs3742207 SNP leads to any significant changes in the 3-dimensional views of protein structure or domain interactions, even though this replacement could lead to changes in the local charge.

Supplementary Table S1.

The top 100 SNPs associated with PWV in the initial GWAS in the SardiNIA cohort.

SNP	CHR	POSITION	MARKER	ALLELE1	ALLELE2	FREQ1	EFFECT	SE	H2	LOD	PVALUE	gc_p-value*	GENE
SNP_A-1945628	1	11063525	rs1194820	G	T	0.771	-0.108	0.021	1.151	5.638	3.48E-07	2.33E-06	SRM, U88966, FRAP1, EXOSC10
SNP_A-2146504	1	11139324	rs1057079	T	C	0.764	-0.103	0.021	1.07	5.172	1.06E-06	6.09E-06	SRM, U88966, FRAP1, EXOSC10
SNP_A-2248327	1	11064909	rs910660	G	A	0.771	-0.102	0.022	1.025	4.898	2.04E-06	1.07E-05	SRM, U88966, FRAP1, EXOSC10
SNP_A-1958507	20	46472188	rs732791	A	T	0.763	0.099	0.021	0.983	4.82	2.46E-06	1.26E-05	-
SNP_A-4226147	1	95627748	rs12032935	G	T	0.621	-0.084	0.018	0.933	4.722	3.11E-06	1.55E-05	-
SNP_A-1810120	13	109616599	rs3742207	T	G	0.559	-0.085	0.018	0.994	4.682	3.43E-06	1.68E-05	COL4A1
SNP_A-4277712	4	151475987	rs10031796	G	A	0.91	0.141	0.03	0.9	4.638	3.81E-06	1.84E-05	DCAMKL2, AB209181
SNP_A-1905780	20	46485633	rs16993776	A	G	0.765	0.097	0.021	0.932	4.632	3.86E-06	1.86E-05	-
SNP_A-1871247	14	91106545	rs1704693	C	G	0.722	-0.089	0.019	0.888	4.6	4.18E-06	1.99E-05	C14orf161
SNP_A-1934075	1	95632284	rs12568065	C	T	0.619	-0.083	0.018	0.897	4.573	4.45E-06	2.11E-05	-
SNP_A-2138220	1	95627844	rs12040149	C	A	0.62	-0.083	0.018	0.896	4.57	4.49E-06	2.12E-05	-
SNP_A-4226109	18	4384577	rs7244876	A	G	0.933	-0.164	0.036	0.924	4.555	4.65E-06	2.19E-05	-
SNP_A-2019391	21	39497183	rs7281540	T	C	0.834	-0.107	0.023	0.883	4.546	4.76E-06	2.23E-05	AB080587, BRWD1, AJ238214, DSCR2
SNP_A-1811989	14	91101729	rs1743074	T	C	0.723	-0.089	0.019	0.875	4.543	4.79E-06	2.24E-05	C14orf161
SNP_A-2304434	17	29772173	rs756882	G	A	0.805	-0.102	0.022	0.906	4.538	4.85E-06	2.27E-05	-
SNP_A-4301686	1	95618898	rs4949963	T	A	0.619	-0.082	0.018	0.877	4.495	5.38E-06	2.48E-05	-
SNP_A-2282847	14	91089550	rs17799683	G	A	0.892	-0.124	0.028	0.824	4.413	6.55E-06	2.94E-05	C14orf161

SNP_A-2019381	21	39437639	rs4816613	A	G	0.82	-0.104	0.023	0.876	4.353	7.56E-06	3.33E-05	AB080587, BRWD1, AJ238214, DSCR2
SNP_A-2019382	21	39437774	rs4817994	C	G	0.82	-0.104	0.023	0.876	4.353	7.57E-06	3.33E-05	AB080587, BRWD1, AJ238214, DSCR2
SNP_A-2201277	13	109617587	rs589985	A	G	0.563	-0.082	0.018	0.915	4.337	7.85E-06	3.44E-05	COL4A1
SNP_A-4201480	5	97239524	rs11135532	C	G	0.84	-0.111	0.025	0.928	4.317	8.25E-06	3.59E-05	-
SNP_A-2016988	21	26186455	rs454017	C	A	0.928	0.148	0.033	0.812	4.303	8.53E-06	3.69E-05	APP
SNP_A-2301509	13	109617144	rs694225	G	A	0.563	-0.081	0.018	0.903	4.292	8.76E-06	3.78E-05	COL4A1
SNP_A-4247337	4	151475498	rs7659254	C	T	0.913	0.135	0.03	0.805	4.244	9.84E-06	4.18E-05	DCAMKL2, AB209181
SNP_A-1789811	13	109617363	rs1213026	G	A	0.562	-0.081	0.018	0.887	4.211	1.07E-05	4.47E-05	COL4A1
SNP_A-2019405	21	39588141	rs2836978	T	C	0.849	-0.109	0.025	0.838	4.207	1.08E-05	4.51E-05	AB080587, BRWD1, AJ238214, DSCR2
SNP_A-4233332	8	109653323	rs13257259	T	A	0.62	0.081	0.018	0.85	4.197	1.10E-05	4.61E-05	KIAA0103
SNP_A-1903882	20	46492084	rs993425	G	C	0.728	0.088	0.02	0.859	4.168	1.18E-05	4.89E-05	-
SNP_A-2166248	20	46471877	rs732790	T	A	0.782	0.095	0.022	0.851	4.16	1.20E-05	4.98E-05	-
SNP_A-2019388	21	39479848	rs2297255	G	C	0.818	-0.1	0.023	0.823	4.145	1.25E-05	5.14E-05	AB080587, BRWD1, AJ238214, DSCR2
SNP_A-1893621	1	177878471	rs10797677	A	G	0.638	-0.08	0.018	0.818	4.138	1.27E-05	5.21E-05	-
SNP_A-2117728	1	95602998	rs4950054	C	T	0.618	-0.079	0.018	0.808	4.111	1.36E-05	5.51E-05	-
SNP_A-4205602	4	151471517	rs1459751	T	G	0.91	0.132	0.03	0.795	4.098	1.40E-05	5.66E-05	DCAMKL2, AB209181
SNP_A-4284308	17	23127161	rs4796052	C	T	0.603	0.078	0.018	0.806	4.061	1.53E-05	6.12E-05	NOS2A
SNP_A-2019385	21	39452857	rs6517516	G	A	0.822	-0.1	0.023	0.814	4.057	1.54E-05	6.17E-05	AB080587, BRWD1, AJ238214, DSCR2
SNP_A-2080047	4	151470110	rs6535720	G	A	0.913	0.122	0.028	0.661	4.055	1.55E-05	6.20E-05	DCAMKL2, AB209181
SNP_A-1846050	17	23148826	rs16949	T	C	0.624	0.076	0.018	0.757	4.013	1.72E-05	6.77E-05	NOS2A
SNP_A-4248604	17	17194880	rs242247	G	A	0.935	0.156	0.036	0.821	3.988	1.83E-05	7.13E-05	NT5M
SNP_A-1997660	9	110671392	rs544828	G	A	0.788	0.09	0.021	0.745	3.979	1.86E-05	7.26E-05	-
SNP_A-2099062	14	96989660	rs1994179	G	C	0.629	0.075	0.018	0.73	3.975	1.88E-05	7.33E-05	-
SNP_A-2156877	9	110673454	rs498102	A	G	0.787	0.089	0.021	0.734	3.927	2.12E-05	8.10E-05	-
SNP_A-	11	74919329	rs661928	T	C	0.696	-0.084	0.02	0.836	3.924	2.13E-	8.15E	AL833941

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SNP_A-1954971	10	123764372	rs7919547	G	A	0.582	-0.076	0.018	0.772	3.873	2.41E-05	9.07E-05	TACC2
SNP_A-4261235	6	114017197	rs4555958	G	A	0.669	-0.08	0.019	0.79	3.873	2.41E-05	9.07E-05	-
SNP_A-2148911	11	42404449	rs4755605	A	G	0.875	0.105	0.025	0.672	3.856	2.51E-05	9.40E-05	-
SNP_A-4288247	2	218565238	rs1424917	C	G	0.713	0.082	0.02	0.769	3.851	2.54E-05	9.50E-05	TNS1
SNP_A-1894443	10	123764416	rs7919851	A	G	0.582	-0.075	0.018	0.767	3.844	2.59E-05	9.64E-05	TACC2
SNP_A-4275345	4	151495409	rs4532220	C	A	0.909	0.127	0.03	0.744	3.839	2.62E-05	9.74E-05	DCAMKL2, AB209181
SNP_A-2126969	12	107402604	rs4388939	A	G	0.909	0.124	0.03	0.712	3.835	2.64E-05	9.82E-05	-
SNP_A-4288324	9	110673051	rs2767006	C	A	0.788	0.088	0.021	0.716	3.832	2.66E-05	9.88E-05	-
SNP_A-2175210	14	91116273	rs722081	T	A	0.718	-0.081	0.019	0.737	3.816	2.76E-05	1.02E-04	C14orf161
SNP_A-1941908	10	123762573	rs10788229	A	G	0.581	-0.075	0.018	0.761	3.809	2.81E-05	1.04E-04	TACC2
SNP_A-4279107	7	32100547	rs11768207	G	C	0.658	0.076	0.018	0.718	3.805	2.84E-05	1.05E-04	AK091734
SNP_A-2237883	6	114006654	rs4341037	C	G	0.67	-0.079	0.019	0.758	3.793	2.93E-05	1.07E-04	-
SNP_A-2116582	20	46471709	rs4810788	C	A	0.745	0.086	0.021	0.782	3.785	2.98E-05	1.09E-04	-
SNP_A-2313081	2	59136116	rs1024766	G	A	0.652	-0.076	0.018	0.73	3.775	3.05E-05	1.11E-04	-
SNP_A-1808597	18	43889962	rs11664553	G	T	0.843	-0.099	0.024	0.725	3.746	3.28E-05	1.18E-04	-
SNP_A-4256844	10	123761293	rs12571870	A	C	0.582	-0.074	0.018	0.745	3.74	3.32E-05	1.20E-04	TACC2
SNP_A-4280537	14	91113496	rs1704609	G	C	0.719	-0.08	0.019	0.722	3.739	3.33E-05	1.20E-04	C14orf161
SNP_A-1805190	7	122863069	rs4731112	C	G	0.676	0.077	0.019	0.73	3.732	3.39E-05	1.22E-04	CR749438, WASL, ASB15
SNP_A-2223873	10	77343635	rs11816174	A	G	0.676	0.079	0.019	0.767	3.725	3.44E-05	1.24E-04	C10orf11
SNP_A-2276112	11	127376187	rs11221126	C	A	0.543	0.072	0.017	0.705	3.725	3.45E-05	1.24E-04	-
SNP_A-1838872	5	79411480	rs2288395	G	C	0.723	0.08	0.019	0.708	3.712	3.56E-05	1.27E-04	THBS4
SNP_A-2202767	3	140326401	rs661149	G	A	0.767	0.084	0.02	0.7	3.683	3.82E-05	1.35E-04	BPESC1
SNP_A-1895268	12	94498374	rs10859907	T	C	0.865	-0.104	0.025	0.696	3.676	3.89E-05	1.37E-04	-
SNP_A-2194277	10	38131154	rs3739992	G	A	0.772	0.086	0.021	0.73	3.668	3.96E-05	1.39E-04	AK027057, ZNF25, ZNF33A, BC036038
SNP_A-1958937	5	110218300	rs13357752	A	G	0.778	0.085	0.021	0.699	3.63	4.34E-05	1.51E-04	-
SNP_A-2279558	1	119907084	rs454510	G	A	0.505	0.072	0.018	0.727	3.609	4.57E-05	1.58E-04	-

SNP_A-2271920	5	97244183	rs6859385	T	G	0.841	-0.098	0.024	0.711	3.602	4.64E-05	1.60E-04	-
SNP_A-2232006	20	46495329	rs6095120	G	C	0.781	0.088	0.022	0.734	3.57	5.02E-05	1.71E-04	-
SNP_A-1836879	5	110189680	rs12517265	C	T	0.775	0.085	0.021	0.704	3.569	5.03E-05	1.72E-04	-
SNP_A-4267149	8	109621322	rs10505124	A	C	0.602	0.073	0.018	0.71	3.566	5.07E-05	1.73E-04	KIAA0103
SNP_A-4267548	9	83119573	rs1359169	G	A	0.868	0.105	0.026	0.708	3.565	5.08E-05	1.73E-04	BC037253, FRMD3, AY137774, BC023560, AK223597, AK094281
SNP_A-2170138	12	117295164	rs17440956	G	A	0.865	-0.105	0.026	0.724	3.542	5.38E-05	1.82E-04	SDS3, BC002756, SUDS3, TAOK3
SNP_A-2050003	6	12999287	rs7750679	T	C	0.505	-0.072	0.018	0.718	3.54	5.41E-05	1.82E-04	PHACTR1, BC047159
SNP_A-2006708	2	132991575	rs2315380	T	C	0.723	-0.081	0.02	0.727	3.535	5.47E-05	1.84E-04	-
SNP_A-2233461	6	7506660	rs2757632	A	G	0.583	-0.071	0.018	0.673	3.533	5.49E-05	1.85E-04	DSP
SNP_A-4244296	9	83132937	rs1323780	C	A	0.871	0.106	0.026	0.704	3.516	5.72E-05	1.92E-04	BC037253, FRMD3, AY137774, BC023560, AK223597, AK094281
SNP_A-2312625	3	195801179	rs1706016	C	T	0.54	0.072	0.018	0.709	3.512	5.78E-05	1.93E-04	AY597813, BC034353, AY303778, BC007772, TMEM44, AK126914
SNP_A-1892007	7	32103576	rs10271037	G	T	0.654	0.073	0.018	0.662	3.511	5.79E-05	1.94E-04	AK091734
SNP_A-1880815	3	195800787	rs1706017	C	T	0.541	0.072	0.018	0.708	3.511	5.80E-05	1.94E-04	AY597813, BC034353, AY303778, BC007772, TMEM44, AK126914
SNP_A-2039539	19	17656360	rs8106359	G	C	0.516	0.074	0.018	0.751	3.498	5.97E-05	1.99E-04	-
SNP_A-2077776	1	40874545	rs4660449	G	T	0.726	0.079	0.02	0.693	3.485	6.18E-05	2.05E-04	AK094323, AK127677, RIMS3, NFYC
SNP_A-2176681	6	13002890	rs9296512	G	C	0.51	-0.071	0.018	0.706	3.481	6.23E-05	2.07E-04	PHACTR1, BC047159
SNP_A-2169005	9	16844839	rs1339550	C	T	0.902	-0.113	0.028	0.63	3.471	6.39E-05	2.11E-04	BNC2
SNP_A-4194655	4	171572150	rs7698213	G	A	0.563	0.073	0.018	0.728	3.468	6.44E-05	2.12E-04	-
SNP_A-2060462	8	125011048	rs7838453	C	A	0.682	-0.073	0.018	0.643	3.454	6.66E-05	2.19E-04	C8ORFK23
SNP_A-1914252	1	100883020	rs11578560	C	T	0.546	0.07	0.018	0.676	3.449	6.73E-05	2.21E-04	VCAM1
SNP_A-2249896	5	110225865	rs6888588	A	G	0.777	0.084	0.021	0.687	3.443	6.83E-05	2.24E-04	-

SNP_A-2190693	17	23130802	rs4795067	A	G	0.55	0.069	0.017	0.656	3.432	7.02E-05	2.29E-04	NOS2A
SNP_A-2160094	1	40847036	rs2744808	C	T	0.724	0.079	0.02	0.686	3.428	7.09E-05	2.31E-04	AK094323, AK127677, RIMS3, NFYC
SNP_A-2283289	5	23488681	rs2914263	G	A	0.911	0.121	0.031	0.662	3.422	7.19E-05	2.34E-04	-
SNP_A-2136756	7	28267685	rs6977204	G	A	0.616	-0.071	0.018	0.662	3.422	7.20E-05	2.34E-04	CREB5
SNP_A-1818349	11	65339642	rs3903072	T	G	0.516	-0.07	0.018	0.688	3.402	7.55E-05	2.44E-04	FLJ30934, MUS81, BC040981, EFEMP2
SNP_A-1783528	7	32084966	rs1450870	C	T	0.58	0.07	0.018	0.659	3.392	7.74E-05	2.49E-04	AK091734
SNP_A-2234780	17	23121258	rs9797244	T	C	0.616	0.072	0.018	0.672	3.369	8.18E-05	2.62E-04	NOS2A
SNP_A-1906570	5	154598503	rs6580149	G	A	0.808	-0.086	0.022	0.632	3.355	8.47E-05	2.69E-04	-

*- adjusted p-values according to the genomic control method (Devlin B, Roeder K. Genomic control for association studies. *Biometrics.* 1999;55:997-1004

SUPPLEMENTAL FIGURES

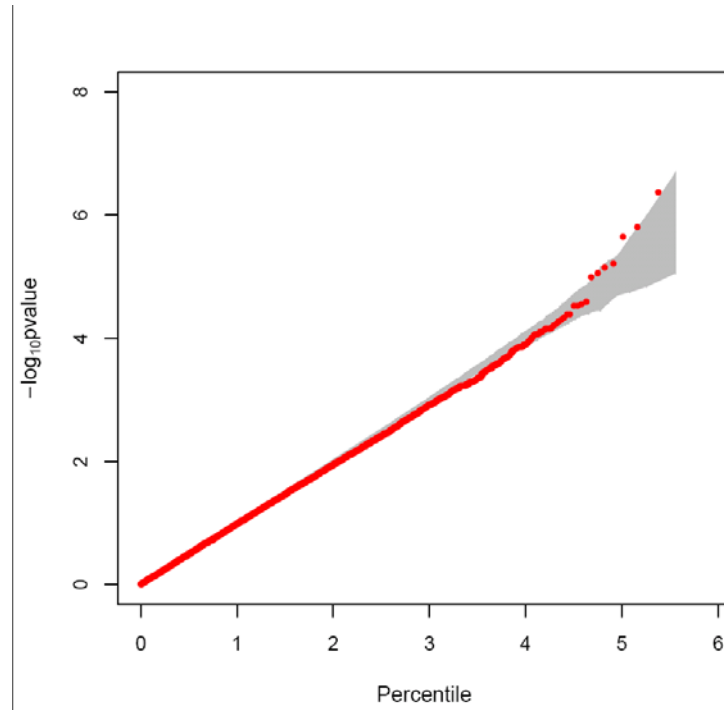## Original Phenotype
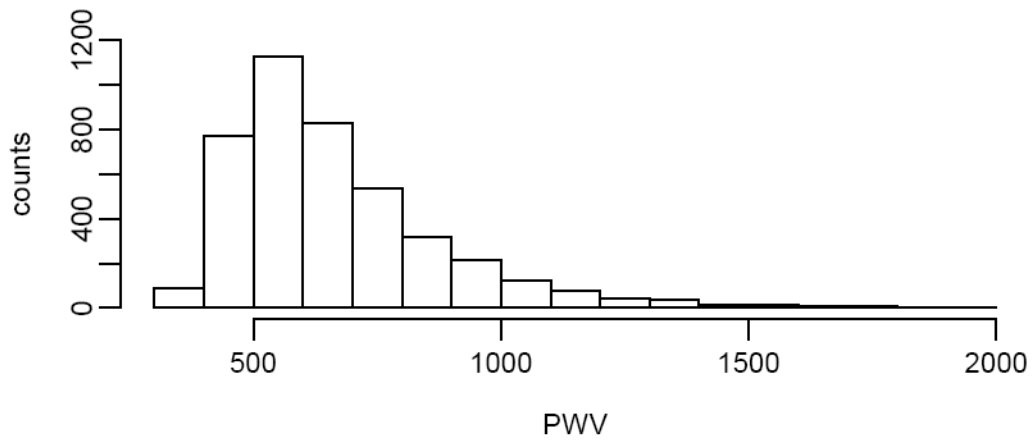
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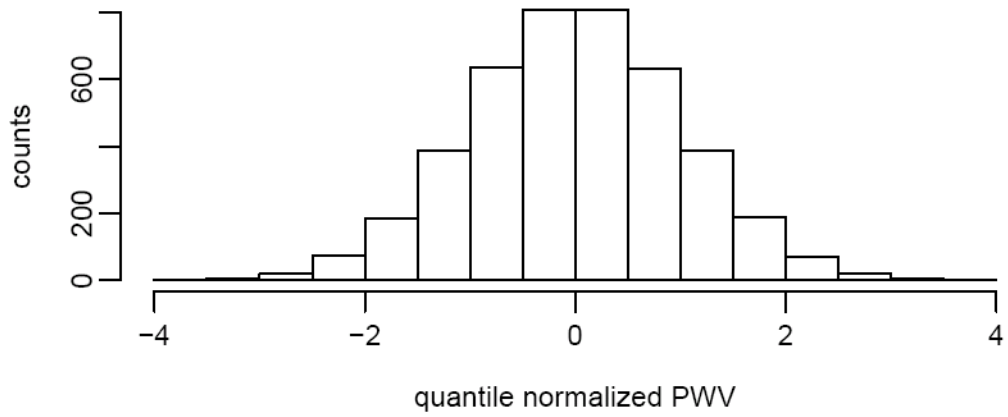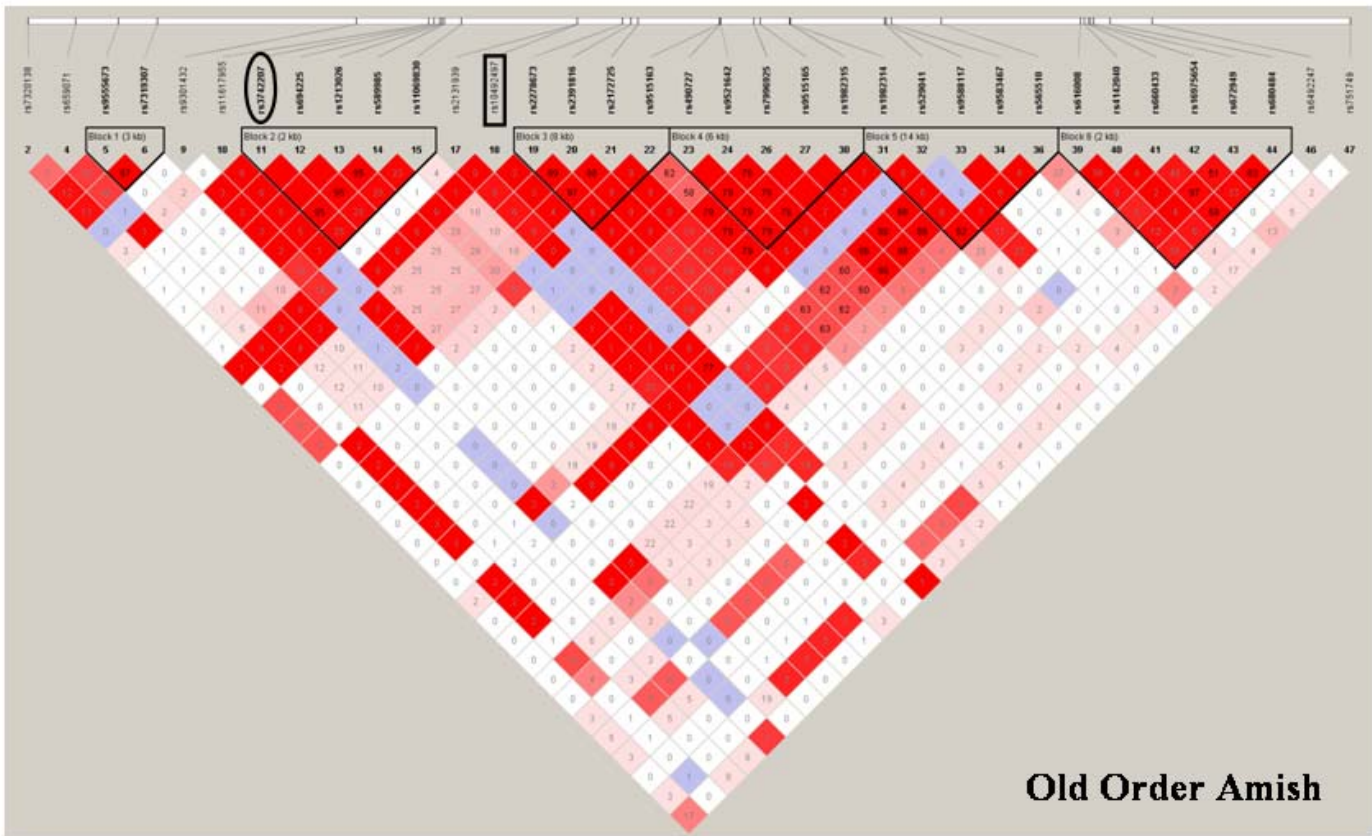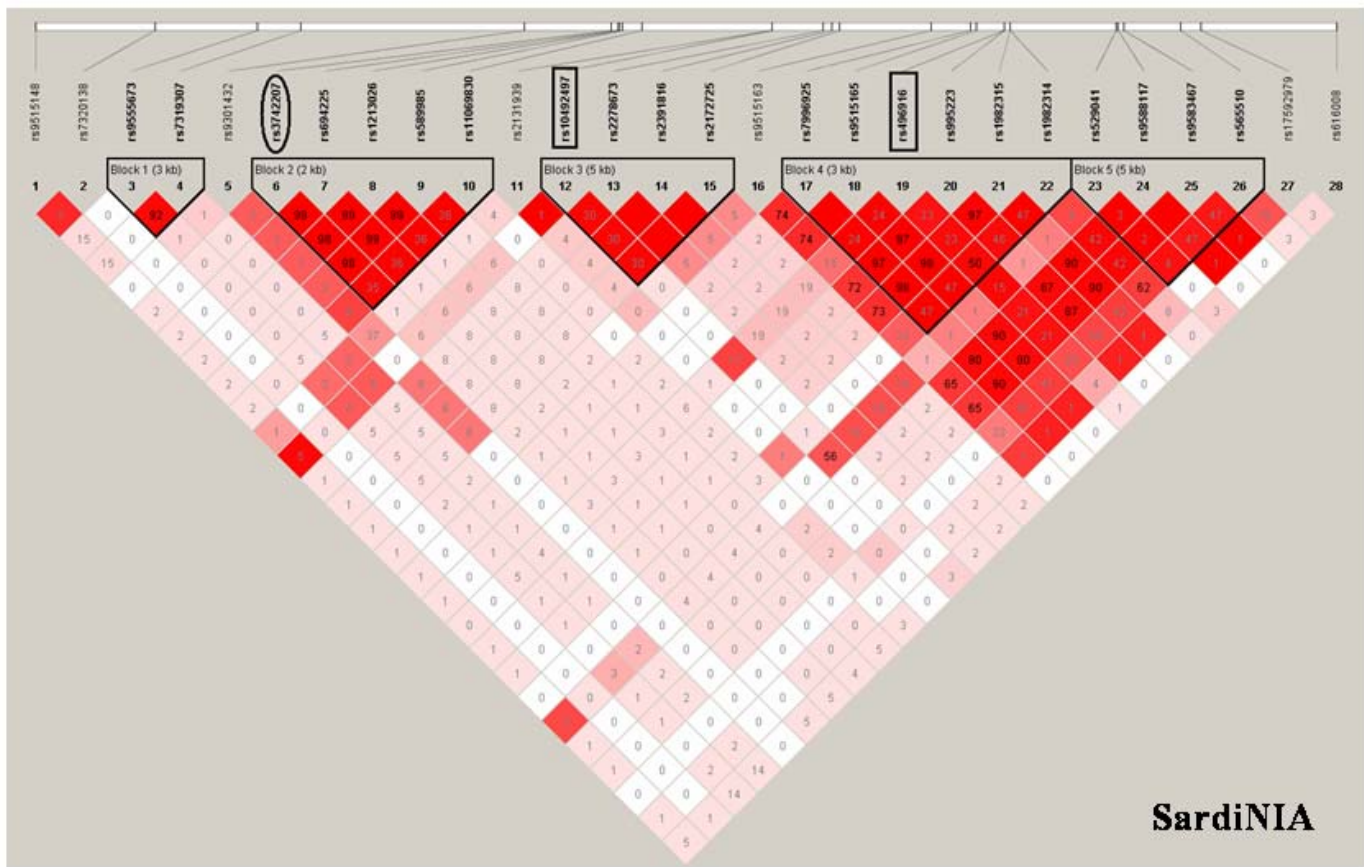

Figure S1

Figure S2

Figure S3

Figure Legends

Figure S1: Frequency distribution of PWV values before and after inverse normal transformation. The raw values have a skewed distribution. The transformed values appear normally distributed.

Figure S2: Quantile-quantile plot of SNPs associated with PWV in the SardiNIA study after adjusting the p-values according to the genomic control method[15]. Red symbols represent all tested SNPs (N= 362,129) in the GWA scan. The gray area corresponds to the 90% confidence region from a null distribution of p-values (generated from 100 simulations).

Figure S3. High resolution linkage disequilibrium plot and LD blocks in the Sardinian (top panel) and Old Order Amish (bottom panel) populations around SNP rs3742207 (highlighted with a circle) from Haploview[16]. LD coloring scheme is the standard D'/LOD scheme, while numbers shown within squares are r^2 values. Genotyped SNPs in the region slightly differ between the two cohorts, but clearly define the length of the second haplotype block, delimited by SNPs rs3742207 and rs11069830, and of identical length in the HapMap CEU population. Squared SNPs are those analyzed by the Framingham study. No other SNP analyzed by Framingham falls in this region.

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