Data Supplement

Supplemental Methods

Apolipoprotein quantification in fractions eluted from gel filtration column for Lp(a) purification

Five microliters from each 0.5 mL fraction eluted from gel filtration column was diluted into 395 microliters of PBS in a 96 well plate. Fifty microliters of each diluted fraction were then coated onto ELISA plates overnight at 4C. Each plate was then washed, blocked with 1% BSA in PBS, then 1 ug/ml of following antibodies against the desired apolipoproteins were added to their respective plate: biotin-LPA4 for apo(a), biotin-goat anti-human apolipoprotein B-100 (Academy BioMed), and biotin-goat anti-human apolipoprotein AI (Academy BioMed). After unbound antibodies were washed off, apolipoproteins were quantified by neutral-avidin conjugated alkaline phosphatase and Lumiphos (Lumigen) generated relative light units (RLUs) as read on a BioTek plate reader.

Total protein from each fraction was quantified by Bradford protein assay (Pierce) using the manufacturer's protocol.

Lipoprotein electrophoresis

Plasma or purified lipoproteins were resolved on an agarose gel and stained for lipids as per the manufacturer's protocol (Helena Laboratories).

Supplemental Figure Legends

Supplemental Figure 1. Quantification of apolipoprotein(a) [apo(a)], apolipoprotein B-100 (apoB100), apolipoprotein AI (apoAI) by ELISA, and total protein in each fraction eluted from the SW-400 gel filtration column as part of the Lp(a) purification process. Relative light units (RLUs) associated with the apoAI ELISA are expressed on a secondary (right) axis due to much lower values compared to apo(a) and apoB100. The fractions delineated by black lines correspond to those pooled for pure Lp(a).

Supplemental Figure 2. Purity of Lp(a) preparation. Agarose gel electrophoresis and lipid staining of plasma, purified LDL, purified HDL and purified Lp(a) from the same donor.

Supplemental Table 1. Patient demographics and lipid parameters in individuals with elevated baseline Lp(a) enrolled in a Lp(a) lowering ASO clinical trial.

Subject ID	Treatment group	Timepoint	Gender	Race	TC (mg/dL)	"LDL-C" (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)	Lp(a) NWRL (nM)	Lp(a) UCSD (mg/dL)	Lp(a)-C (mg/dL)	correct LDL-C (mg/dL)
1	Ionis apo(a)rx	Baseline	F	White	236	153	53	149	334.2	103.2	12.7	140.3
1	Ionis apo(a)rx	Recovery	F	White	230	142	57	153	248.4	71.6	11.5	130.5
1	Ionis apo(a)rx	Trough	F	White	315	222	48	227	80.3	31.6	7.1	214.9
2	Placebo	Baseline	F	White	187	117	55	77	168.1	62.5	8.4	108.6
2	Placebo	Recovery	F	White	183	110	56	87	178.3	73.9	8.8	101.2
2	Placebo	Trough	F	White	167	99	55	63	174.2	57.1	8.5	90.5
3	Ionis apo(a)rx	Baseline	M	White	162	103	36	114	200.0	81.5	5.6	97.4
3	Ionis apo(a)rx	Recovery	M	White	184	122	31	155	189.7	60.1	6.3	115.7
3	Ionis apo(a)rx	Trough	M	White	155	91	29	176	117.9	55.4	5.3	85.7
4	Ionis apo(a)rx	Baseline	M	White	145	77	55	64	234.0	78.4	7.7	69.3
4	Ionis apo(a)rx	Recovery	M	White	166	83	58	125	183.6	65.2	5.5	77.5
4	Ionis apo(a)rx	Trough	M	White	143	72	58	65	50.6	37.9	2.2	69.8
5	Ionis apo(a)rx	Baseline	F	White	157	74	49	171		55.3	13.0	61.0
5	Ionis apo(a)rx	Recovery	F	White	164	84	51	146	193.1	81.3	12.9	71.1
5	Ionis apo(a)rx	Trough	F	White	151	75	47	145	26.5	5.3	2.4	72.6
6	Ionis apo(a)rx	Baseline	F	White	179	103	50	130	387.2	157.8	24.6	78.4
6	Ionis apo(a)rx	Trough	F	White	162	78	61	116	75.5	24.5	5.0	73.0
7	Ionis apo(a)rx	Baseline	M	White	217	155	47	76	374.8	123.1	14.2	140.8
7	Ionis apo(a)rx	Recovery	M	White	240	173	44	116	439.9	98.2	18.0	155.0
7	Ionis apo(a)rx	Trough	M	White	207	144	41	108	243.6	81.1	11.7	132.3
8	Ionis apo(a)rx	Baseline	M	White	214	147	35	160	175.3	72.7	8.7	138.3
8	Ionis apo(a)rx	Recovery	M	White	197	137	30	151	227.6	72.3	9.0	128.0
8	Ionis apo(a)rx	Trough	M	White	189	137	31	103	121.5	54.1	5.7	131.3
9	Ionis apo(a)rx	Baseline	M	White	204	127	58	93	338.5	114.8	12.6	114.4
9	Ionis apo(a)rx	Recovery	M	White	198	106	69	115	209.9	101.2	12.8	93.2
9	Ionis apo(a)rx	Trough	M	White	211	113	69	145	112.1	44.4	9.2	103.8
10	Placebo	Baseline	M	White	146	97	29	101	223.6	94.2	9.0	88.0
10	Placebo	Trough	M	White	130	76	30	118	189.1	99.3	10.9	65.1
11	Ionis apo(a)rx	Baseline	F	Other	250	134	52	319	350.7	111.4	18.7	115.4
11	Ionis apo(a)rx	Recovery	F	Other	248	134	60	271	326.6	95.7	15.3	118.7
11	Ionis apo(a)rx	Trough	F	Other	244	125	58	305	115.2	55.1	9.9	115.1

12	Ionis apo(a)rx	Baseline	M	White	299	196	58	226	322.3	80.3	33.4	162.6
12	Ionis apo(a)rx	Recovery	M	White	263	186	54	117	240.5	54.4	17.1	169.0
12	Ionis apo(a)rx	Trough	M	White	212	134	49	143	45.2	12.9	5.6	128.5
13	Ionis apo(a)rx	Baseline	F	White	213	163	34	81	525.8	138.6	24.4	138.6
13	Ionis apo(a)rx	Recovery	F	White	211	147	38	128	329.5	109.4	23.8	123.2
13	Ionis apo(a)rx	Trough	F	White	202	139	43	101	33.7	19.2	5.2	133.8
14	Ionis apo(a)rx	Baseline	M	White	130	66	43	105	456.1	134.4	28.0	38.0
14	Ionis apo(a)rx	Recovery	M	White	138	66	42	149	363.2	114.1	18.5	47.5
14	Ionis apo(a)rx	Trough	M	White	102	42	36	120	147.3	60.6	8.0	34.0
15	Ionis apo(a)rx	Baseline	F	White	209	130	61	89	469.8	127.3	25.3	104.7
15	Ionis apo(a)rx	Recovery	F	White	206	128	62	80	407.7	98.0	22.7	105.3
15	Ionis apo(a)rx	Trough	F	White	194	122	53	97	167.0	67.0	17.2	104.8
16	Ionis apo(a)rx	Baseline	F	White	204	115	59	148	497.8	129.7	19.8	95.2
16	Ionis apo(a)rx	Trough	F	White	149	75	46	142	158.1	56.3	5.0	70.0
17	Ionis apo(a)rx	Baseline	F	White	176	99	54	114	396.5	155.8	24.2	74.8
17	Ionis apo(a)rx	Recovery	F	White	187	110	53	120	392.3	132.9	20.8	89.2
17	Ionis apo(a)rx	Trough	F	White	138	65	57	80	98.6	55.9	9.5	55.5
18	Ionis apo(a)rx	Baseline	M	White	198	85	91	108	239.3	78.0	9.6	75.4
18	Ionis apo(a)rx	Recovery	M	White	189	78	99	61	201.3	76.9	11.6	66.4
18	Ionis apo(a)rx	Trough	M	White	200	95	90	74	146.9	55.4	7.7	87.3
19	Placebo	Baseline	M	White	247	173	49	123	228.9	77.2	17.1	155.9
19	Placebo	Recovery	M	White	215	147	47	104	248.8	65.7	15.5	131.5
19	Placebo	Trough	M	White					243.8	91.6	17.9	
20	Ionis apo(a)rx	Baseline	F	White	192	101	73	91	149.3	62.2	11.3	89.8
20	Ionis apo(a)rx	Recovery	F	White	179	88	64	135	67.2	27.3	8.3	79.7
20	Ionis apo(a)rx	Trough	F	White	188	94	71	114	9.0	2.8	0.6	93.4
21	Placebo	Baseline	M	White	218	125	40	264	161.8	65.4	26.5	98.5
21	Placebo	Recovery	M	White	260	123	36	436	182.5	53.8	30.8	92.2
21	Placebo	Trough	M	White	270	139	38	411	131.9	57.6	21.8	117.2
22	Placebo	Baseline	F	White	259	106	124	146	542.4	150.4	26.2	79.8
22	Placebo	Recovery	F	White	264	123	122	95	465.6	133.6	27.9	95.1
22	Placebo	Trough	F	White	238	100	120	88	527.0	166.8	22.4	77.6
23	Ionis apo(a)rx	Baseline	M	White	149	62	59	138	156.5	67.2	13.2	48.8
23	Ionis apo(a)rx	Recovery	M	White	157	63	55	196	145.0	58.8	11.0	52.0

23	Ionis apo(a)rx	Trough	M	White	141	52	53	178	79.5	38.9	9.6	42.4
24	Ionis apo(a)rx	Baseline	F	White	202	125	52	123	822.8	115.8	35.0	90.0
24	Ionis apo(a)rx	Recovery	F	White	178	118	44	78	697.0	102.7	25.7	92.3
24	Ionis apo(a)rx	Trough	F	White	181	107	44	148	505.8	101.0	19.7	87.3
25	Ionis apo(a)rx	Baseline	M	White	235	155	63	85	176.6	69.2	10.2	144.8
25	Ionis apo(a)rx	Recovery	M	White	212	136	61	76	166.1	53.4	8.2	127.8
25	Ionis apo(a)rx	Trough	M	White	188	117	57	68	90.3	27.5	5.9	111.1
26	Placebo	Baseline	M	White	212	151	49	60	270.0	98.4	15.9	135.1
26	Placebo	Recovery	M	White	200	128	60	61	220.2	79.3	14.4	113.6
26	Placebo	Trough	M	White	208	138	52	88	240.1	91.7	15.8	122.2
27	Placebo	Baseline	M	White	155	100	42	66	204.9	73.2	13.2	86.8
27	Placebo	Recovery	M	White	135	82	44	46	190.0	73.2	10.8	71.2
27	Placebo	Trough	M	White	150	99	39	61	239.2	78.0	9.7	89.3
28	Placebo	Baseline	F	White	248	151	67	148	209.0	70.3	12.6	138.4
28	Placebo	Recovery	F	White	277	180	64	166	165.1	52.1	14.6	165.4
28	Placebo	Trough	F	White					170.4	54.2	16.4	
29	Ionis apo(a)rx	Baseline	F	White	166	72	61	164	522.4	123.8	21.5	50.5
29	Ionis apo(a)rx	Recovery	F	White	238	143	58	186	490.3	92.8	22.5	120.5
29	Ionis apo(a)rx	Trough	F	White	135	47	51	186	218.8	84.6	11.8	35.2

Supplemental Table 2. Spearman correlation coefficients (p-values) between Lp(a)-C and various Lp(a) and plasma lipoprotein parameters in individuals with elevated baseline Lp(a) enrolled in a Lp(a) lowering ASO clinical trial.

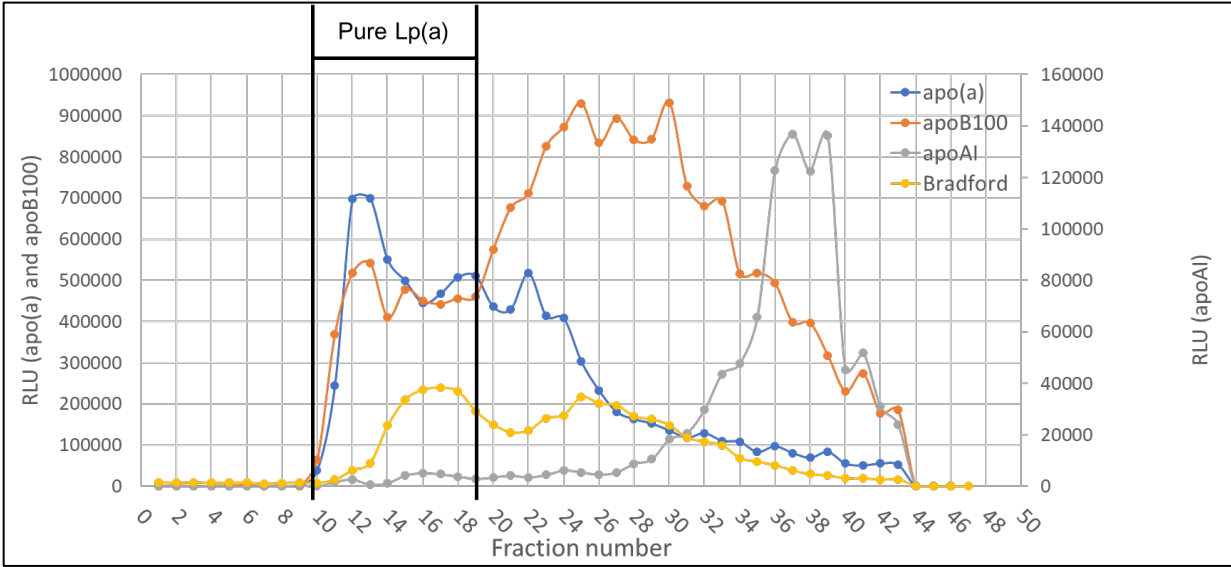|	Placebo ASO (n = 8)			apo(a)$_{rx}$ ASO (n = 21)		
	Lp(a) molar (nM)	Lp(a)-C (mg/dL)	% Lp(a)-C	Lp(a) molar (nM)	Lp(a)-C (mg/dL)	% Lp(a)-C
Total cholesterol (mg/dL)	0.04 (p = 0.9)	**0.74 (p <0.001)**	**0.75 (p < 0.001)**	0.2 (p = 0.08)	0.25 (p = 0.52)	0.18 (p = 0.16)
"LDL-C" (mg/dL)	-0.02 (p = 0.95)	0.4 (p = 0.058)	**0.68 (p = 0.001)**	**0.28 (p = 0.03)**	0.23 (p = 0.08)	0.13 (p = 0.3)
LDL-C$_{corr}$ (mg/dL)	-0.12 (p = 0.6)	0.12 (p = 0.6)	**0.50 (p = 0.02)**	0.12 (p = 0.4)	0.06 (p = 0.6)	0.89 (p = 0.5)
HDL-C (mg/dL)	0.36 (p = 0.1)	0.13 (p = 0.6)	0.04 (p = 0.9)	-0.12 (p = 0.4)	0.009 (p = .95)	0.12 (p = 0.4)
TG (mg/dL)	-0.27 (p = 0.24)	**0.54 (p = 0.01)**	**0.58 (p = 0.006)**	0.01 (p = 0.9)	0.08 (p = 0.5)	0.15 (p = 0.2)
Lp(a) mass (mg/dL)	**0.8 (p < 0.001)**	0.14 (p = 0.5)	**-0.54 (p = 0.009)**	**0.92 (p < 0.001)**	**0.82 (p < 0.001)**	-0.2 (p = 0.1)
Lp(a) molar (nM)	1	0.27 (p = 0.2)	-0.26 (p = 0.2)	1	**0.86 (p < 0.001)**	-0.04 (p = 0.7)
apo(a) major isoform size	-0.3 (p = 0.18)	**-0.46 (p = 0.03)**	**-0.42 (p = 0.049)**	na	na	na
apo(a) minor isoform size	-0.2 (p = 0.37)	0.26 (p = 0.2)	0.3 (p = 0.2)	na	na	na
OxPL-apoB (nM)	**0.69 (p < 0.001)**	0.2 (p = 0.4)	-0.37 (p = 0.095)	**0.95 (p < 0.001)**	**0.76 (p < 0.001)**	-0.15 (p = 0.3)
Lp(a)-C (mg/dL)	0.27 (p = 0.2)	1	**0.69 (p < 0.001)**	**0.86 (p < 0.001)**	1	0.3 (p = 0.17)
% Lp(a)-C	-0.26 (p = 0.2)	**0.69 (p < 0.001)**	1	-0.04 (p = 0.7)	0.3 (p = 0.17)	1

Data from all three timepoints (Baseline, Trough, and Recovery) included. OxPL-apoB = oxidized phospholipid on apolipoprotein B-100; TC = total cholesterol; LDL-C = low density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; TG = triglycerides; %Lp(a)-C = percent Lp(a)-C / Lp(a) mass. The natural relationship between apo(a) isoform size and Lp(a) levels is preserved only in the placebo group, and therefore correlations with apo(a) isoform size were only reported for this group. Statistically significant correlations in bold.

Supplemental Table 3. Spearman correlation coefficients (p-values) between Lp(a)-C and various Lp(a) and plasma lipoprotein parameters in baseline samples.

	Baseline samples (n = 29)		
	Lp(a) molar (nM)	Lp(a)-C (mg/dL)	% Lp(a)-C
Total cholesterol (mg/dL)	0.12 (p = 0.6)	0.29 (p = 0.12)	0.39 (p = 0.05)
"LDL-C" (mg/dL)	0.05 (p = 0.79)	0.17 (p = 0.37)	0.25 (p = 0.19)
LDL-C$_{corr}$ (mg/dL)	-0.13 (p = 0.53)	-0.06 (p = 0.74)	0.09 (p = 0.65)
HDL-C (mg/dL)	0.07 (p = 0.72)	0.11 (p = 0.59)	-0.01 (p = 0.97)
TG (mg/dL)	0.09 (p = 0.66)	0.33 (p = 0.07)	**0.52 (p = 0.004)**
Lp(a) mass (mg/dL)	**0.92 (p < 0.001)**	**0.57 (p = 0.001)**	-0.15 (p = 0.45)
Lp(a) molar (nM)	1	**0.65 (p < 0.001)**	0.18 (p = 0.35)
apo(a) major isoform size	**-0.38 (p = 0.046)**	**-0.39 (p = 0.04)**	-0.23 (p = 0.24)
apo(a) minor isoform size	-0.29 (p = 0.14)	-0.07 (p = 0.74)	-0.04 (p = 0.86)
OxPL-apoB (nM)	**0.90 (p < 0.001)**	**0.58 (p = 0.002)**	-0.02 (p = 0.93)
Lp(a)-C (mg/dL)	**0.65 (p < 0.001)**	1	**0.71 (p < 0.001)**
% Lp(a)-C	0.12 (p = 0.56)	**0.71 (p < 0.001)**	1

OxPL-apoB = oxidized phospholipid on apolipoprotein B-100; TC = total cholesterol; LDL-C = low density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; TG = triglycerides; %Lp(a)-C = percent Lp(a)-C / Lp(a) mass. The natural relationship between apo(a) isoform size and Lp(a) levels is preserved only in the placebo group, and therefore correlations with apo(a) isoform size were only reported for this group. Statistically significant correlations in bold.

Supplemental Figure 1.

Supplemental Figure 2.