Supplementary Material to

New evidence from plasma ceramides links apoE polymorphism to greater risk of coronary artery disease in Finnish adults

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Supplementary Table S1. Frequencies of different apoE genotypes (ε2/2, ε3/2, ε3/3, ε4/2, ε4/3 and ε4/4) with summary descriptive data in the YFS cohort in the 2007 follow-up. Values are mean (SD) or n (%). ANOVA p-values for continuous and χ^2 p-values for categorical variables across genotypes are also shown.

	All	ε2/2	ε3/2	ε3/3	ε4/2	ε4/3	ε4/4	p
Number of subjects	2160	4 (0.2)	142 (6.6)	1241 (57.5)	41 (1.9)	651 (30.1)	81 (3.8)	-
Number of males	974 (45.1)	2 (0.2)	56 (5.7)	568 (58.3)	17 (1.7)	296 (30.4)	35 (3.6)	0.7809
Number of females	1186 (54.9)	2 (0.2)	86 (7.3)	673 (56.7)	24 (2.0)	355 (29.9)	46 (3.9)	0.7809
Age [years]	37.6 (5.0)	36.0 (7.4)	36.9 (5.1)	37.7 (5.0)	38.6 (4.0)	37.5 (5.1)	38.0 (4.8)	0.2761
Body mass index [kg/m²]	26.0 (4.8)	36.8 (17.2)	25.5 (4.8)	26.0 (4.6)	25.9 (4.5)	26.0 (4.8)	26.3 (5.3)	4.1e-4
Daily smokers	398 (18.4)	1 (25)	21 (14.8)	229 (18.5)	6 (14.6)	127 (19.5)	14 (17.3)	0.7900
Daily alcohol consumption [ethanol g]	11.3 (17.0)	4.7 (6.5)	12.4 (18.2)	11.9 (18.8)	9.8 (13.9)	10.0 (13.1)	11.5 (13.7)	0.2756
Cholesterol lowering medication	45 (2.1)	0 (0.0)	2 (1.4)	23 (1.9)	2 (4.9)	15 (2.3)	3 (3.7)	0.6184
Diabetes mellitus type 2	22 (1.0)	0 (0.0)	2 (1.4)	15 (1.2)	0 (0.0)	5 (0.8)	0 (0.0)	0.8039
Hypertension	123 (5.7)	2 (50.0)	4 (2.8)	67 (5.4)	1 (2.4)	45 (6.9)	4 (4.9)	4.6e-3
Total cholesterol [mmol/L]	5.05 (0.90)	4.12 (0.76)	4.49 (0.82)	5.03 (0.89)	4.68 (0.82)	5.22 (0.89)	5.28 (0.84)	< 2.2e-16
LDL-C [mmol/L]	3.15 (0.81)	1.94 (0.30)	2.48 (0.65)	3.12 (0.79)	2.76 (0.68)	3.36 (0.80)	3.30 (0.76)	< 2.2e-16
HDL-C [mmol/L]	1.34 (0.33)	1.17 (0.17)	1.41 (0.37)	1.35 (0.33)	1.35 (0.37)	1.31 (0.31)	1.35 (0.41)	0.01501
Triglycerides [mmol/L]	1.40 (0.93)	2.22 (0.87)	1.45 (1.00)	1.38 (0.93)	1.36 (0.97)	1.39 (0.82)	1.70 (1.43)	0.02542
apoB [g/L]	1.02 (0.26)	0.79 (0.16)	0.87 (0.26)	1.01 (0.26)	0.91 (0.23)	1.07 (0.26)	1.09 (0.25)	< 2.2e-16
apoA-I [g/L]	1.60 (0.26)	1.60 (0.22)	1.66 (0.30)	1.61 (0.26)	1.59 (0.25)	1.57 (0.24)	1.60 (0.28)	6.3e-4

Abbreviations: LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; apoB, Apolipoprotein B; apoA-I, Apolipoprotein A-I.

Supplementary Table S2: Complete data including all p-values from the cross-sectional association analyses of apoE polymorphism and ceramides in all YFS subjects in 2007. **<u>Annotation:</u>** FDR p/apoE, false discovery rate corrected F-test p-value for apoE; Beta/ε2+ or ε4+, post-hoc β-coefficient; ci.low/ε2+ or ε4+, 95% post-hoc CI lower limit; ci.high/ε2+ or ε4+, 95% post-hoc CI upper limit; FDR p/ε2+ or ε4+, false discovery rate corrected post-hoc p-value.

	F-test	Post-hoc comparison							
Ceramide	FDR p apoE	Beta ε2+	ci.low ε2+	ci.high ε2+	FDR p ε2+	Beta ε4+	ci.low ε4+	ci.high ε4+	FDR p ε4+
Cer(d16:1/16:0)	3.78E-03	-0.2618	-0.4288	-0.0947	3.55E-02	0.0845	-0.0033	0.1724	2.06E-01
Cer(d16:1/18:0)	1.13E-02	-0.2636	-0.4207	-0.1065	2.62E-02	0.0238	-0.0588	0.1065	7.71E-01
Cer(d16:1/20:0)	2.46E-01	-0.1241	-0.2879	0.0397	3.36E-01	0.0484	-0.0378	0.1345	5.42E-01
Cer(d16:1/22:0)	4.37E-01	-0.1250	-0.2886	0.0387	3.78E-01	0.0117	-0.0743	0.0978	9.14E-01
Cer(d16:1/23:0)	6.47E-01	-0.0794	-0.2483	0.0894	6.53E-01	-0.0421	-0.1309	0.0467	6.53E-01
Cer(d16:1/24:0)	7.03E-01	-0.0663	-0.2310	0.0985	6.84E-01	-0.0348	-0.1214	0.0518	6.84E-01
Cer(d16:1/24:1)	3.19E-01	-0.1556	-0.3173	0.0062	2.06E-01	-0.0067	-0.0917	0.0784	9.50E-01
Cer(d18:1/14:0)	6.18E-02	-0.2510	-0.4225	-0.0795	3.99E-02	0.0033	-0.0866	0.0932	9.67E-01
Cer(d18:1/16:0)	1.87E-04	-0.2784	-0.4415	-0.1153	2.62E-02	0.1252	0.0395	0.2110	3.99E-02
Cer(d18:1/18:0)	7.31E-02	-0.0944	-0.2482	0.0593	5.03E-01	0.0920	0.0111	0.1728	1.42E-01
Cer(d18:1/20:0)	7.31E-02	-0.0975	-0.2595	0.0645	5.07E-01	0.0989	0.0137	0.1841	1.37E-01
Cer(d18:1/22:0)	8.38E-02	-0.0513	-0.2092	0.1066	7.21E-01	0.1021	0.0191	0.1852	1.12E-01
Cer(d18:1/23:0)	7.69E-01	-0.0313	-0.1977	0.1351	8.73E-01	0.0292	-0.0583	0.1167	7.21E-01
Cer(d18:1/24:0)	7.69E-01	-0.0274	-0.1953	0.1404	8.98E-01	0.0318	-0.0565	0.1200	7.21E-01
Cer(d18:1/24:1)	2.12E-01	-0.0237	-0.1819	0.1346	9.07E-01	0.0878	0.0046	0.1710	1.88E-01
Cer(d18:1/26:0)	8.56E-01	-0.0429	-0.2156	0.1299	8.02E-01	-0.0170	-0.1078	0.0738	8.73E-01
Cer(d18:1/26:1)	6.47E-01	-0.0052	-0.1750	0.1646	9.67E-01	0.0527	-0.0366	0.1420	5.09E-01
Cer(d18:2/16:0)	3.78E-03	-0.2524	-0.4202	-0.0847	3.99E-02	0.0922	0.0040	0.1804	1.88E-01
Cer(d18:2/18:0)	8.38E-02	-0.1305	-0.2914	0.0305	3.36E-01	0.0754	-0.0092	0.1601	2.54E-01
Cer(d18:2/20:0)	9.89E-03	-0.1740	-0.3422	-0.0057	1.88E-01	0.1153	0.0269	0.2038	8.78E-02
Cer(d18:2/22:0)	9.89E-03	-0.0761	-0.2429	0.0908	6.62E-01	0.1452	0.0574	0.2329	2.62E-02
Cer(d18:2/23:0)	4.94E-01	-0.0071	-0.1749	0.1606	9.67E-01	0.0648	-0.0234	0.1530	3.95E-01
Cer(d18:2/24:0)	4.37E-01	-0.0403	-0.2054	0.1248	8.02E-01	0.0619	-0.0249	0.1487	3.97E-01
Cer(d18:2/24:1)	8.38E-02	-0.1179	-0.2833	0.0475	3.97E-01	0.0861	-0.0009	0.1731	2.05E-01
Cer(d18:2/26:0)	7.69E-01	-0.0649	-0.2374	0.1076	7.07E-01	0.0079	-0.0828	0.0986	9.50E-01
Cer(d18:2/26:1)	2.12E-01	0.1695	-0.0020	0.3410	2.05E-01	0.0610	-0.0286	0.1506	4.29E-01
Cer(d20:1/22:0)	1.37E-01	0.0090	-0.1546	0.1726	9.67E-01	0.1048	0.0187	0.1908	1.12E-01
Cer(d20:1/23:0)	6.47E-01	-0.0676	-0.2377	0.1024	6.84E-01	0.0369	-0.0526	0.1263	6.84E-01
Cer(d20:1/24:0)	6.72E-01	-0.0155	-0.1834	0.1525	9.50E-01	0.0462	-0.0421	0.1345	5.92E-01
Cer(d20:1/24:1)	3.39E-01	0.0010	-0.1624	0.1643	9.91E-01	0.0789	-0.0069	0.1648	2.36E-01
Ceramide ratios									
Cer(d18:1/16:0)/Cer(d18:1/24:0)	4.37E-01	-0.1150	-0.2865	0.0565	4.30E-01	0.0308	-0.0594	0.1209	7.21E-01
Cer(d18:1/20:0)/Cer(d18:1/24:0)	6.72E-01	-0.0431	-0.2143	0.1281	8.02E-01	0.0393	-0.0507	0.1293	6.80E-01
Cer(d18:1/24:1)/Cer(d18:1/24:0)	8.30E-01	0.0172	-0.1559	0.1903	9.50E-01	0.0305	-0.0606	0.1215	7.21E-01

Supplementary Table S3: Complete data including all p-values from the cross-sectional association analyses of apoE polymorphism and ceramides in YFS subjects without ε4/2 subgroup in 2007. **Annotation:** FDR p/apoE, false discovery rate corrected F-test p-value for apoE; Beta/ε2+ or ε4+, post-hoc β-coefficient; ci.low/ε2+ or ε4+, 95% post-hoc CI lower limit; ci.high/ε2+ or ε4+, 95% post-hoc CI upper limit; FDR p/ε2+ or ε4+, false discovery rate corrected post-hoc p-value.

	F-test	Post-hoc comparison							
Ceramide	FDR p apoE	Beta ε2+	ci.low ε2+	ci.high ε2+	FDR p ε2+	Beta ε4+	ci.low ε4+	ci.high ε4+	FDR p ε4+
Cer(d16:1/16:0)	1.74E-03	-0.2623	-0.4293	-0.0953	2.77E-02	0.1052	0.0158	0.1945	1.22E-01
Cer(d16:1/18:0)	1.10E-02	-0.2630	-0.4204	-0.1055	2.77E-02	0.0274	-0.0569	0.1116	7.39E-01
Cer(d16:1/20:0)	2.04E-01	-0.1240	-0.2880	0.0399	3.80E-01	0.0607	-0.0270	0.1484	4.17E-01
Cer(d16:1/22:0)	4.60E-01	-0.1249	-0.2887	0.0390	3.80E-01	0.0152	-0.0724	0.1029	8.75E-01
Cer(d16:1/23:0)	7.36E-01	-0.0798	-0.2489	0.0892	6.50E-01	-0.0349	-0.1254	0.0555	7.01E-01
Cer(d16:1/24:0)	7.36E-01	-0.0667	-0.2316	0.0982	6.89E-01	-0.0370	-0.1252	0.0513	6.89E-01
Cer(d16:1/24:1)	2.94E-01	-0.1560	-0.3180	0.0060	2.02E-01	-0.0031	-0.0898	0.0835	9.78E-01
Cer(d18:1/14:0)	5.23E-02	-0.2506	-0.4220	-0.0792	3.94E-02	0.0078	-0.0837	0.0992	9.54E-01
Cer(d18:1/16:0)	8.81E-05	-0.2783	-0.4413	-0.1152	2.77E-02	0.1370	0.0498	0.2243	2.77E-02
Cer(d18:1/18:0)	5.23E-02	-0.0945	-0.2482	0.0593	4.71E-01	0.0992	0.0169	0.1814	1.20E-01
Cer(d18:1/20:0)	5.23E-02	-0.0971	-0.2593	0.0651	4.81E-01	0.1068	0.0200	0.1935	1.17E-01
Cer(d18:1/22:0)	1.08E-01	-0.0516	-0.2096	0.1065	7.39E-01	0.0986	0.0141	0.1832	1.22E-01
Cer(d18:1/23:0)	8.11E-01	-0.0323	-0.1989	0.1343	8.75E-01	0.0224	-0.0668	0.1115	8.18E-01
Cer(d18:1/24:0)	8.63E-01	-0.0282	-0.1961	0.1397	8.75E-01	0.0164	-0.0735	0.1062	8.75E-01
Cer(d18:1/24:1)	2.44E-01	-0.0242	-0.1825	0.1341	8.86E-01	0.0853	0.0006	0.1700	1.88E-01
Cer(d18:1/26:0)	8.11E-01	-0.0438	-0.2165	0.1290	8.18E-01	-0.0299	-0.1223	0.0625	7.39E-01
Cer(d18:1/26:1)	8.11E-01	-0.0056	-0.1754	0.1642	9.78E-01	0.0379	-0.0529	0.1288	6.89E-01
Cer(d18:2/16:0)	4.23E-03	-0.2519	-0.4195	-0.0843	3.56E-02	0.0925	0.0028	0.1822	1.85E-01
Cer(d18:2/18:0)	5.74E-02	-0.1297	-0.2906	0.0313	3.59E-01	0.0881	0.0020	0.1743	1.85E-01
Cer(d18:2/20:0)	4.92E-03	-0.1740	-0.3422	-0.0058	1.85E-01	0.1291	0.0391	0.2191	4.08E-02
Cer(d18:2/22:0)	1.10E-02	-0.0755	-0.2424	0.0915	6.70E-01	0.1451	0.0558	0.2344	2.77E-02
Cer(d18:2/23:0)	4.73E-01	-0.0072	-0.1750	0.1606	9.78E-01	0.0692	-0.0206	0.1590	3.80E-01
Cer(d18:2/24:0)	5.09E-01	-0.0403	-0.2055	0.1248	8.18E-01	0.0571	-0.0313	0.1454	4.38E-01
Cer(d18:2/24:1)	1.03E-01	-0.1175	-0.2831	0.0480	4.17E-01	0.0845	-0.0040	0.1731	2.02E-01
Cer(d18:2/26:0)	8.11E-01	-0.0655	-0.2380	0.1070	7.01E-01	-0.0008	-0.0931	0.0915	1.00E+00
Cer(d18:2/26:1)	2.31E-01	0.1696	-0.0020	0.3411	1.93E-01	0.0610	-0.0302	0.1522	4.17E-01
Cer(d20:1/22:0)	2.52E-01	0.0081	-0.1557	0.1719	9.78E-01	0.0906	0.0029	0.1782	1.85E-01
Cer(d20:1/23:0)	7.36E-01	-0.0689	-0.2391	0.1013	6.89E-01	0.0209	-0.0702	0.1119	8.29E-01
Cer(d20:1/24:0)	8.11E-01	-0.0164	-0.1844	0.1516	9.49E-01	0.0317	-0.0582	0.1215	7.35E-01
Cer(d20:1/24:1)	5.43E-01	0.0000	-0.1635	0.1635	1.00E+00	0.0609	-0.0266	0.1484	4.17E-01
Ceramide ratios									
Cer(d18:1/16:0)/Cer(d18:1/24:0)	2.90E-01	-0.1148	-0.2862	0.0567	4.17E-01	0.0538	-0.0379	0.1456	4.85E-01
Cer(d18:1/20:0)/Cer(d18:1/24:0)	4.73E-01	-0.0424	-0.2135	0.1287	8.18E-01	0.0628	-0.0287	0.1544	4.17E-01
Cer(d18:1/24:1)/Cer(d18:1/24:0)	7.36E-01	0.0178	-0.1553	0.1909	9.49E-01	0.0493	-0.0433	0.1419	5.59E-01

Supplementary Table S4: Complete data including all p-values from the apoB-adjusted cross-sectional association analyses of apoE polymorphism and ceramides in all YFS subjects in 2007. **<u>Annotation:</u>** FDR p/apoE, false discovery rate corrected F-test p-value for apoE; FDR p/apoB, false discovery rate corrected F-test p-value for apoB; Beta/apoB, linear regression β-coefficient; Beta/ε2+ or ε4+, post-hoc β-coefficient; ci.low/ε2+ or ε4+, 95% post-hoc CI lower limit; ci.high/ε2+ or ε4+, 95% post-hoc CI upper limit; FDR p/ε2+ or ε4+, false discovery rate corrected post-hoc p-value.

	F-test			Post-hoc comparison between apoE genotypes							
Ceramide	FDR p apoE	FDR p apoB	Beta apoB	Beta ε2+	ci.low ε2+	ci.high ε2+	FDR p ε2+	Beta ε4+	ci.low ε4+	ci.high ε4+	FDR p ε4+
Cer(d16:1/16:0)	7.11E-01	1.19E-103	0.5109	0.0286	-0.1230	0.1802	8.39E-01	-0.0298	-0.1090	0.0494	6.21E-01
Cer(d16:1/18:0)	2.08E-01	7.20E-93	0.4570	-0.0038	-0.1481	0.1404	9.73E-01	-0.0793	-0.1548	-0.0039	1.30E-01
Cer(d16:1/20:0)	1.27E-02	1.67E-118	0.5324	0.1785	0.0322	0.3247	6.92E-02	-0.0708	-0.1472	0.0057	1.70E-01
Cer(d16:1/22:0)	9.16E-04	3.72E-124	0.5430	0.1836	0.0385	0.3287	5.80E-02	-0.1098	-0.1856	-0.0339	2.33E-02
Cer(d16:1/23:0)	6.49E-05	1.17E-96	0.5000	0.2047	0.0503	0.3591	4.42E-02	-0.1540	-0.2347	-0.0733	2.47E-03
Cer(d16:1/24:0)	3.49E-05	5.26E-107	0.5112	0.2242	0.0753	0.3731	1.75E-02	-0.1492	-0.2271	-0.0714	2.47E-03
Cer(d16:1/24:1)	1.14E-03	1.21E-114	0.5177	0.1386	-0.0063	0.2836	1.61E-01	-0.1225	-0.1983	-0.0467	1.13E-02
Cer(d18:1/14:0)	2.25E-01	6.50E-60	0.4049	-0.0263	-0.1894	0.1368	8.46E-01	-0.0866	-0.1716	-0.0015	1.45E-01
Cer(d18:1/16:0)	5.18E-01	1.82E-179	0.6347	0.0822	-0.0539	0.2183	4.45E-01	-0.0168	-0.0879	0.0544	8.17E-01
Cer(d18:1/18:0)	1.27E-02	1.87E-135	0.5303	0.2069	0.0723	0.3416	1.73E-02	-0.0267	-0.0971	0.0437	6.21E-01
Cer(d18:1/20:0)	1.55E-02	2.40E-131	0.5512	0.2157	0.0731	0.3582	1.75E-02	-0.0245	-0.0990	0.0501	6.86E-01
Cer(d18:1/22:0)	4.76E-04	1.33E-159	0.5847	0.2810	0.1463	0.4156	1.45E-03	-0.0287	-0.0991	0.0417	5.95E-01
Cer(d18:1/23:0)	1.47E-04	7.90E-117	0.5374	0.2741	0.1253	0.4229	2.94E-03	-0.0911	-0.1689	-0.0133	7.98E-02
Cer(d18:1/24:0)	3.56E-02	2.84E-56	0.3846	0.1911	0.0307	0.3515	7.59E-02	-0.0543	-0.1382	0.0295	4.26E-01
Cer(d18:1/24:1)	1.33E-04	2.19E-152	0.5743	0.3027	0.1666	0.4387	8.87E-04	-0.0407	-0.1118	0.0304	4.81E-01
Cer(d18:1/26:0)	2.28E-01	3.14E-19	0.2289	0.0872	-0.0846	0.2590	5.22E-01	-0.0682	-0.1580	0.0216	3.11E-01
Cer(d18:1/26:1)	2.17E-01	9.17E-33	0.2972	0.1637	-0.0027	0.3301	1.48E-01	-0.0138	-0.1008	0.0732	8.46E-01
Cer(d18:2/16:0)	9.33E-01	1.26E-59	0.3955	-0.0277	-0.1874	0.1320	8.46E-01	0.0037	-0.0798	0.0872	9.73E-01
Cer(d18:2/18:0)	6.59E-01	1.93E-56	0.3695	0.0798	-0.0740	0.2336	5.22E-01	-0.0070	-0.0874	0.0734	9.21E-01
Cer(d18:2/20:0)	6.82E-01	5.47E-76	0.4454	0.0791	-0.0782	0.2364	5.22E-01	0.0157	-0.0666	0.0979	8.39E-01
Cer(d18:2/22:0)	1.86E-01	1.92E-65	0.4114	0.1577	-0.0001	0.3155	1.48E-01	0.0531	-0.0294	0.1356	4.26E-01
Cer(d18:2/23:0)	9.16E-04	3.75E-103	0.5118	0.2837	0.1314	0.4360	2.92E-03	-0.0497	-0.1293	0.0299	4.32E-01
Cer(d18:2/24:0)	2.40E-03	2.60E-106	0.5108	0.2499	0.1006	0.3993	8.65E-03	-0.0524	-0.1305	0.0257	4.14E-01
Cer(d18:2/24:1)	3.13E-01	2.31E-75	0.4362	0.1299	-0.0249	0.2847	2.35E-01	-0.0115	-0.0924	0.0694	8.58E-01
Cer(d18:2/26:0)	5.01E-01	5.93E-21	0.2394	0.0711	-0.1001	0.2424	5.95E-01	-0.0457	-0.1352	0.0439	5.22E-01
Cer(d18:2/26:1)	9.16E-04	1.31E-35	0.3128	0.3414	0.1740	0.5088	1.45E-03	-0.0099	-0.0970	0.0771	8.90E-01
Cer(d20:1/22:0)	1.86E-01	6.76E-33	0.2871	0.1722	0.0118	0.3325	1.23E-01	0.0405	-0.0433	0.1244	5.39E-01
Cer(d20:1/23:0)	6.82E-01	5.02E-22	0.2424	0.0701	-0.0986	0.2388	5.95E-01	-0.0174	-0.1055	0.0708	8.39E-01
Cer(d20:1/24:0)	5.86E-01	2.49E-17	0.2104	0.1041	-0.0633	0.2715	4.32E-01	-0.0009	-0.0884	0.0866	9.84E-01
Cer(d20:1/24:1)	2.33E-01	1.25E-30	0.2762	0.1579	-0.0026	0.3184	1.48E-01	0.0171	-0.0668	0.1010	8.39E-01
Ceramide ratios											
Cer(d18:1/16:0)/Cer(d18:1/24:0)	1.42E-01	8.33E-04	-0.0857	-0.1637	-0.3371	0.0097	1.63E-01	0.0500	-0.0407	0.1406	5.00E-01
Cer(d18:1/20:0)/Cer(d18:1/24:0)	6.27E-01	6.85E-01	-0.0103	-0.0489	-0.2225	0.1246	7.51E-01	0.0416	-0.0491	0.1324	5.65E-01
Cer(d18:1/24:1)/Cer(d18:1/24:0)	7.11E-01	1.35E-01	-0.0389	-0.0049	-0.1804	0.1705	9.73E-01	0.0392	-0.0525	0.1309	5.95E-01

Supplementary Table S5: Complete data including all p-values from the LDL-C-adjusted cross-sectional association analyses of apoE polymorphism and ceramides in all YFS subjects in 2007. **Annotation:** FDR p/apoE, false discovery rate corrected F-test p-value for apoE; FDR p/LDL-C, false discovery rate corrected F-test p-value for LDL-C; Beta/LDL-C, linear regression β-coefficient; Beta/ε2+ or ε4+, post-hoc β-coefficient; ci.low/ε2+ or ε4+, 95% post-hoc CI lower limit; ci.high/ε2+ or ε4+, 95% post-hoc CI upper limit; FDR p/ε2+ or ε4+, false discovery rate corrected post-hoc p-value.

	F-test			Post-hoc comparison between apoE genotypes							
	FDR p	FDR p	Beta	Beta	ci.low	ci.high	FDR p	Beta	ci.low	ci.high	FDR p
Ceramide	apoE	LDL-C	LDL-C	ε2+	ε2+	ε2+	ε2+	ε4+	ε4+	ε4+	ε4+
Cer(d16:1/16:0)	6.42E-01	4.04E-72	0.3994	0.0717	-0.0869	0.2302	5.63E-01	-0.0102	-0.0922	0.0717	9.03E-01
Cer(d16:1/18:0)	5.87E-01	1.64E-41	0.2868	-0.0241	-0.1783	0.1302	8.68E-01	-0.0453	-0.1251	0.0344	4.70E-01
Cer(d16:1/20:0)	2.12E-01	1.06E-46	0.3164	0.1400	-0.0199	0.2999	2.15E-01	-0.0267	-0.1093	0.0560	6.95E-01
Cer(d16:1/22:0)	8.28E-02	3.17E-50	0.3277	0.1486	-0.0105	0.3077	2.03E-01	-0.0660	-0.1482	0.0162	2.56E-01
Cer(d16:1/23:0)	4.77E-03	6.62E-44	0.3163	0.1846	0.0193	0.3499	1.26E-01	-0.1172	-0.2026	-0.0317	5.15E-02
Cer(d16:1/24:0)	1.58E-03	1.40E-53	0.3407	0.2181	0.0585	0.3777	5.15E-02	-0.1156	-0.1981	-0.0332	5.15E-02
Cer(d16:1/24:1)	8.28E-02	2.45E-49	0.3210	0.1124	-0.0450	0.2698	3.44E-01	-0.0828	-0.1642	-0.0015	1.69E-01
Cer(d18:1/14:0)	2.08E-01	1.86E-53	0.3545	0.0386	-0.1274	0.2045	8.08E-01	-0.0798	-0.1655	0.0058	2.03E-01
Cer(d18:1/16:0)	1.77E-01	1.19E-133	0.5166	0.1529	0.0082	0.2975	1.49E-01	0.0027	-0.0721	0.0775	9.99E-01
Cer(d18:1/18:0)	9.78E-02	7.91E-61	0.3382	0.1879	0.0402	0.3356	6.45E-02	0.0118	-0.0646	0.0881	8.68E-01
Cer(d18:1/20:0)	8.59E-02	2.38E-63	0.3634	0.2059	0.0507	0.3611	5.15E-02	0.0127	-0.0676	0.0929	8.68E-01
Cer(d18:1/22:0)	2.56E-03	7.23E-90	0.4185	0.2982	0.1512	0.4451	2.35E-03	0.0028	-0.0731	0.0788	9.99E-01
Cer(d18:1/23:0)	1.52E-03	2.68E-72	0.3986	0.3015	0.1436	0.4593	3.55E-03	-0.0654	-0.1470	0.0162	2.56E-01
Cer(d18:1/24:0)	4.21E-02	4.12E-40	0.3004	0.2234	0.0584	0.3884	5.15E-02	-0.0395	-0.1248	0.0458	5.58E-01
Cer(d18:1/24:1)	4.77E-03	9.23E-69	0.3696	0.2849	0.1342	0.4356	3.55E-03	0.0002	-0.0777	0.0780	1.00E+00
Cer(d18:1/26:0)	9.12E-02	6.01E-23	0.2318	0.1507	-0.0224	0.3237	2.15E-01	-0.0720	-0.0720	0.0174	2.56E-01
Cer(d18:1/26:1)	1.44E-01	1.85E-24	0.2357	0.1916	0.0218	0.3613	1.26E-01	-0.0032	-0.0909	0.0846	9.99E-01
Cer(d18:2/16:0)	7.40E-01	6.83E-64	0.3781	0.0632	-0.0974	0.2239	6.46E-01	0.0025	-0.0806	0.0855	9.99E-01
Cer(d18:2/18:0)	6.42E-01	1.13E-30	0.2513	0.0795	-0.0805	0.2394	5.31E-01	0.0159	-0.0668	0.0985	8.63E-01
Cer(d18:2/20:0)	5.33E-01	2.38E-35	0.2825	0.0619	-0.1043	0.2282	6.67E-01	0.0483	-0.0376	0.1342	4.70E-01
Cer(d18:2/22:0)	1.21E-01	1.54E-31	0.2643	0.1445	-0.0210	0.3101	2.15E-01	0.0825	-0.0031	0.1680	2.02E-01
Cer(d18:2/23:0)	4.89E-03	8.36E-57	0.3569	0.2909	0.1289	0.4528	5.76E-03	-0.0199	-0.1035	0.0638	8.08E-01
Cer(d18:2/24:0)	4.89E-03	1.05E-66	0.3800	0.2769	0.1193	0.4346	6.40E-03	-0.0282	-0.1097	0.0532	6.72E-01
Cer(d18:2/24:1)	4.94E-01	2.26E-33	0.2698	0.1073	-0.0565	0.2712	3.98E-01	0.0221	-0.0626	0.1067	7.89E-01
Cer(d18:2/26:0)	9.86E-02	1.31E-31	0.2736	0.1635	-0.0076	0.3347	2.02E-01	-0.0570	-0.1455	0.0314	4.01E-01
Cer(d18:2/26:1)	1.52E-03	2.30E-26	0.2473	0.3715	0.2006	0.5425	1.40E-03	0.0011	-0.0868	0.0890	1.00E+00
Cer(d20:1/22:0)	8.28E-02	4.35E-29	0.2485	0.2165	0.0537	0.3793	5.15E-02	0.0458	-0.0383	0.1300	4.83E-01
Cer(d20:1/23:0)	4.21E-01	1.55E-21	0.2207	0.1166	-0.0540	0.2873	3.72E-01	-0.0155	-0.1037	0.0727	8.68E-01
Cer(d20:1/24:0)	3.07E-01	1.74E-17	0.1949	0.1472	-0.0220	0.3164	2.15E-01	0.0000	-0.0875	0.0874	1.00E+00
Cer(d20:1/24:1)	1.71E-01	4.17E-21	0.2096	0.1760	0.0120	0.3399	1.46E-01	0.0292	-0.0555	0.1139	6.72E-01
Ceramide ratios											
Cer(d18:1/16:0)/Cer(d18:1/24:0)	1.50E-01	1.71E-02	-0.0562	-0.1619	-0.3375	0.0137	2.03E-01	0.0441	-0.0467	0.1348	5.36E-01
Cer(d18:1/20:0)/Cer(d18:1/24:0)	3.14E-01	1.31E-02	-0.0586	-0.0920	-0.2672	0.0833	5.01E-01	0.0532	-0.0374	0.1438	4.64E-01
Cer(d18:1/24:1)/Cer(d18:1/24:0)	4.21E-01	5.37E-05	-0.0962	-0.0631	-0.2399	0.1137	6.72E-01	0.0533	-0.0381	0.1446	4.64E-01

Supplementary Table S6: Complete data including all p-values from the HDL-C-adjusted cross-sectional association analyses of apoE polymorphism and ceramides in all YFS subjects in 2007. **Annotation:** FDR p/apoE, false discovery rate corrected F-test p-value for apoE; FDR p/HDL-C, false discovery rate corrected F-test p-value for HDL-C; Beta/HDL-C, linear regression β-coefficient; Beta/ε2+ or ε4+, post-hoc β-coefficient; ci.low/ε2+ or ε4+, 95% post-hoc CI lower limit; ci.high/ε2+ or ε4+, 95% post-hoc CI upper limit; FDR p/ε2+ or ε4+, false discovery rate corrected post-hoc p-value.

	F-test			Post-hoc comparison between apoE genotypes							
	FDR p	FDR p	Beta	Beta	ci.low	ci.high	FDR p	Beta	ci.low	ci.high	FDR p
Ceramide	apoE	HDL-C	HDL-C	ε2+	ε2+	ε2+	ε2+	ε4+	ε4+	ε4+	ε4+
Cer(d16:1/16:0)	1.01E-03	6.21E-05	0.1109	-0.2740	-0.4403	-0.1076	2.13E-02	0.0967	0.0091	0.1842	1.53E-01
Cer(d16:1/18:0)	1.22E-02	8.67E-01	0.0058	-0.2642	-0.4215	-0.1070	2.13E-02	0.0244	-0.0584	0.1072	7.73E-01
Cer(d16:1/20:0)	2.33E-01	7.76E-01	0.0104	-0.1253	-0.2892	0.0387	3.41E-01	0.0495	-0.0368	0.1358	5.21E-01
Cer(d16:1/22:0)	4.48E-01	8.67E-01	0.0046	-0.1255	-0.2892	0.0383	3.41E-01	0.0122	-0.0740	0.0984	8.89E-01
Cer(d16:1/23:0)	6.58E-01	1.54E-01	0.0433	-0.0842	-0.2531	0.0846	6.19E-01	-0.0374	-0.1263	0.0515	6.59E-01
Cer(d16:1/24:0)	6.97E-01	7.81E-04	0.0900	-0.0762	-0.2405	0.0881	6.59E-01	-0.0250	-0.1115	0.0615	7.73E-01
Cer(d16:1/24:1)	2.74E-01	1.14E-01	0.0453	-0.1606	-0.3223	0.0011	1.89E-01	-0.0017	-0.0869	0.0834	9.83E-01
Cer(d18:1/14:0)	4.01E-02	6.23E-03	0.0781	-0.2589	-0.4301	-0.0877	2.93E-02	0.0118	-0.0781	0.1017	8.92E-01
Cer(d18:1/16:0)	1.09E-04	1.68E-01	0.0402	-0.2828	-0.4460	-0.1197	2.13E-02	0.1296	0.0438	0.2155	2.93E-02
Cer(d18:1/18:0)	9.01E-02	1.12E-01	-0.0439	-0.0896	-0.2433	0.0641	5.21E-01	0.0872	0.0063	0.1681	1.53E-01
Cer(d18:1/20:0)	8.18E-02	3.43E-01	-0.0281	-0.0944	-0.2565	0.0676	5.21E-01	0.0958	0.0105	0.1811	1.53E-01
Cer(d18:1/22:0)	9.99E-02	3.43E-01	-0.0283	-0.0482	-0.2061	0.1098	7.73E-01	0.0990	0.0159	0.1822	1.20E-01
Cer(d18:1/23:0)	7.17E-01	3.17E-01	0.0319	-0.0348	-0.2013	0.1317	8.50E-01	0.0327	-0.0550	0.1203	7.14E-01
Cer(d18:1/24:0)	6.92E-01	1.59E-02	0.0673	-0.0348	-0.2025	0.1328	8.50E-01	0.0391	-0.0492	0.1274	6.59E-01
Cer(d18:1/24:1)	1.86E-01	5.03E-01	0.0206	-0.0259	-0.1842	0.1324	8.82E-01	0.0901	0.0067	0.1734	1.53E-01
Cer(d18:1/26:0)	8.73E-01	5.24E-02	0.0582	-0.0493	-0.2219	0.1233	7.73E-01	-0.0106	-0.1015	0.0803	8.97E-01
Cer(d18:1/26:1)	4.72E-01	9.55E-05	0.1084	-0.0171	-0.1862	0.1520	8.97E-01	0.0646	-0.0245	0.1536	3.53E-01
Cer(d18:2/16:0)	7.00E-04	4.91E-10	0.1621	-0.2703	-0.4364	-0.1042	2.13E-02	0.1099	0.0225	0.1974	1.01E-01
Cer(d18:2/18:0)	8.18E-02	3.43E-01	0.0284	-0.1336	-0.2946	0.0274	3.12E-01	0.0785	-0.0063	0.1633	2.19E-01
Cer(d18:2/20:0)	1.08E-02	8.67E-01	0.0040	-0.1744	-0.3428	-0.0061	1.75E-01	0.1158	0.0271	0.2044	8.66E-02
Cer(d18:2/22:0)	1.22E-02	2.82E-01	-0.0342	-0.0723	-0.2392	0.0946	6.59E-01	0.1414	0.0536	0.2293	2.13E-02
Cer(d18:2/23:0)	4.72E-01	8.67E-01	0.0041	-0.0076	-0.1755	0.1603	9.58E-01	0.0653	-0.0231	0.1537	3.53E-01
Cer(d18:2/24:0)	3.38E-01	1.24E-02	0.0688	-0.0479	-0.2128	0.1170	7.73E-01	0.0694	-0.0174	0.1563	3.22E-01
Cer(d18:2/24:1)	8.18E-02	5.24E-01	0.0202	-0.1201	-0.2856	0.0454	3.53E-01	0.0883	0.0011	0.1754	1.83E-01
Cer(d18:2/26:0)	6.92E-01	8.10E-04	0.0931	-0.0752	-0.2472	0.0969	6.59E-01	0.0181	-0.0725	0.1086	8.50E-01
Cer(d18:2/26:1)	1.86E-01	3.78E-04	0.0997	0.1611	-0.0098	0.3320	2.19E-01	0.0721	-0.0174	0.1615	3.22E-01
Cer(d20:1/22:0)	1.60E-01	5.01E-01	-0.0220	0.0114	-0.1522	0.1751	9.33E-01	0.1024	0.0162	0.1886	1.20E-01
Cer(d20:1/23:0)	5.95E-01	6.99E-01	0.0138	-0.0692	-0.2394	0.1010	6.69E-01	0.0384	-0.0512	0.1280	6.59E-01
Cer(d20:1/24:0)	6.58E-01	6.18E-01	0.0168	-0.0173	-0.1853	0.1507	8.97E-01	0.0480	-0.0404	0.1365	5.57E-01
Cer(d20:1/24:1)	3.26E-01	8.02E-01	0.0089	0.0000	-0.1635	0.1634	1.00E+00	0.0799	-0.0061	0.1660	2.19E-01
Ceramide ratios											
Cer(d18:1/16:0)/Cer(d18:1/24:0)	4.72E-01	9.09E-02	-0.0518	-0.1093	-0.2807	0.0622	4.65E-01	0.0251	-0.0652	0.1154	7.73E-01
Cer(d18:1/20:0)/Cer(d18:1/24:0)	7.66E-01	6.79E-04	-0.0955	-0.0325	-0.2033	0.1382	8.50E-01	0.0289	-0.0610	0.1188	7.73E-01
Cer(d18:1/24:1)/Cer(d18:1/24:0)	8.73E-01	1.18E-02	-0.0733	0.0253	-0.1476	0.1982	8.89E-01	0.0224	-0.0686	0.1135	8.14E-01

Supplementary Table S7: Complete data from the cross-sectional association analyses of high-sensitivity CRP and ceramides in all YFS subjects in 2007, adjusted for BMI, age, sex and apoE genotype. **Annotation:** FDR p, false discovery rate corrected p-value for CRP; Beta, linear regression β-coefficient for CRP; ci.low, 95% CI lower limit; ci.high, 95% CI upper limit.

Ceramide	Beta	ci.low	ci.high	FDP p
Cer(d16:1/16:0)	0.0983	0.0509	0.1457	1.61E-04
Cer(d16:1/18:0)	0.1533	0.1091	0.1976	1.57E-10
Cer(d16:1/20:0)	0.1066	0.0601	0.1530	3.36E-05
Cer(d16:1/22:0)	0.0273	-0.0192	0.0739	3.44E-01
Cer(d16:1/23:0)	0.0058	-0.0422	0.0539	8.64E-01
Cer(d16:1/24:0)	-0.0131	-0.0600	0.0338	6.66E-01
Cer(d16:1/24:1)	0.0917	0.0458	0.1376	2.76E-04
Cer(d18:1/14:0)	0.0489	0.0002	0.0976	9.00E-02
Cer(d18:1/16:0)	0.1446	0.0985	0.1906	7.23E-09
Cer(d18:1/18:0)	0.2188	0.1760	0.2615	1.20E-21
Cer(d18:1/20:0)	0.1312	0.0854	0.1769	1.45E-07
Cer(d18:1/22:0)	0.0984	0.0536	0.1431	6.26E-05
Cer(d18:1/23:0)	0.0730	0.0257	0.1203	6.34E-03
Cer(d18:1/24:0)	0.0682	0.0205	0.1159	1.20E-02
Cer(d18:1/24:1)	0.1769	0.1324	0.2213	1.50E-13
Cer(d18:1/26:0)	0.0612	0.0121	0.1103	3.17E-02
Cer(d18:1/26:1)	0.1082	0.0601	0.1563	4.51E-05
Cer(d18:2/16:0)	0.0590	0.0113	0.1067	3.17E-02
Cer(d18:2/18:0)	0.1233	0.0778	0.1689	6.72E-07
Cer(d18:2/20:0)	0.0230	-0.0249	0.0709	4.42E-01
Cer(d18:2/22:0)	-0.0092	-0.0567	0.0383	7.74E-01
Cer(d18:2/23:0)	-0.0147	-0.0625	0.0331	6.44E-01
Cer(d18:2/24:0)	-0.0407	-0.0877	0.0063	1.35E-01
Cer(d18:2/24:1)	0.0417	-0.0054	0.0887	1.35E-01
Cer(d18:2/26:0)	-0.0017	-0.0509	0.0474	9.54E-01
Cer(d18:2/26:1)	0.0174	-0.0312	0.0661	5.89E-01
Cer(d20:1/22:0)	0.0442	-0.0024	0.0907	1.09E-01
Cer(d20:1/23:0)	0.0418	-0.0066	0.0902	1.35E-01
Cer(d20:1/24:0)	0.0400	-0.0078	0.0878	1.44E-01
Cer(d20:1/24:1)	0.0747	0.0283	0.1211	4.42E-03
Ceramide ratios				
Cer(d18:1/16:0)/Cer(d18:1/24:0)	0.0014	-0.0474	0.0503	9.54E-01
Cer(d18:1/20:0)/Cer(d18:1/24:0)	0.0233	-0.0254	0.0721	4.42E-01
Cer(d18:1/24:1)/Cer(d18:1/24:0)	0.0556	0.0063	0.1048	5.24E-02

Supplementary Table S8: Complete data including all p-values from the sex-stratified cross-sectional association analyses of apoE polymorphism and ceramides in YFS subjects in 2007. **Annotation:** FDR p/apoE, false discovery rate corrected F-test p-value for apoE; Beta/ε2+ or ε4+, post-hoc β-coefficient; ci.low/ε2+ or ε4+, 95% post-hoc CI lower limit; ci.high/ε2+ or ε4+, 95% post-hoc CI upper limit; FDR p/ε2+ or ε4+, false discovery rate corrected post-hoc p-value.

	FEMALE								
	F-test	Post-hoc comparison							
Ceramide	FDR p apoE	Beta ε2+	ci.low ε2+	ci.high ε2+	FDR p ε2+	Beta ε4+	ci.low ε4+	ci.high ε4+	FDR p ε4+
Cer(d16:1/16:0)	4.64E-02	-0.2958	-0.5146	-0.0770	1.07E-01	0.0696	-0.0509	0.1901	6.06E-01
Cer(d16:1/18:0)	3.49E-02	-0.3454	-0.5519	-0.1389	7.01E-02	0.0096	-0.1041	0.1233	9.23E-01
Cer(d16:1/20:0)	5.33E-01	-0.0993	-0.3166	0.1181	6.74E-01	0.0706	-0.0491	0.1903	6.06E-01
Cer(d16:1/22:0)	7.99E-01	-0.0951	-0.3153	0.1251	6.89E-01	0.0034	-0.1178	0.1247	9.70E-01
Cer(d16:1/23:0)	7.04E-01	-0.0169	-0.2396	0.2057	9.23E-01	-0.0762	-0.1988	0.0464	6.06E-01
Cer(d16:1/24:0)	7.04E-01	-0.0299	-0.2523	0.1925	8.94E-01	-0.0710	-0.1935	0.0515	6.06E-01
Cer(d16:1/24:1)	7.04E-01	-0.0877	-0.3057	0.1303	7.10E-01	-0.0580	-0.1780	0.0621	6.74E-01
Cer(d18:1/14:0)	1.51E-01	-0.2697	-0.4926	-0.0468	1.47E-01	-0.0845	-0.2066	0.0377	6.06E-01
Cer(d18:1/16:0)	3.49E-02	-0.3069	-0.5222	-0.0916	1.05E-01	0.1023	-0.0162	0.2208	4.72E-01
Cer(d18:1/18:0)	4.64E-02	-0.1325	-0.3360	0.0711	6.06E-01	0.1454	0.0333	0.2575	1.21E-01
Cer(d18:1/20:0)	5.57E-02	-0.1240	-0.3380	0.0900	6.06E-01	0.1426	0.0247	0.2604	1.47E-01
Cer(d18:1/22:0)	3.61E-01	-0.0985	-0.3132	0.1162	6.74E-01	0.0976	-0.0206	0.2158	4.98E-01
Cer(d18:1/23:0)	9.09E-01	-0.0453	-0.2643	0.1737	8.54E-01	0.0042	-0.1164	0.1247	9.70E-01
Cer(d18:1/24:0)	9.09E-01	-0.0408	-0.2631	0.1814	8.62E-01	0.0159	-0.1065	0.1383	8.94E-01
Cer(d18:1/24:1)	4.54E-01	-0.0222	-0.2372	0.1928	9.17E-01	0.1015	-0.0169	0.2199	4.72E-01
Cer(d18:1/26:0)	7.99E-01	-0.0927	-0.3177	0.1322	7.09E-01	-0.0413	-0.1651	0.0826	7.83E-01
Cer(d18:1/26:1)	8.98E-01	-0.0476	-0.2699	0.1747	8.54E-01	0.0190	-0.1034	0.1414	8.81E-01
Cer(d18:2/16:0)	7.24E-02	-0.3035	-0.5212	-0.0858	1.05E-01	0.0117	-0.1082	0.1316	9.17E-01
Cer(d18:2/18:0)	3.77E-01	-0.1406	-0.3501	0.0690	6.06E-01	0.0696	-0.0458	0.1850	6.06E-01
Cer(d18:2/20:0)	3.49E-02	-0.2406	-0.4591	-0.0221	2.04E-01	0.1333	0.0129	0.2536	2.04E-01
Cer(d18:2/22:0)	5.57E-02	-0.0639	-0.2834	0.1557	7.93E-01	0.1720	0.0511	0.2929	1.05E-01
Cer(d18:2/23:0)	8.55E-01	0.0015	-0.2165	0.2195	9.89E-01	0.0454	-0.0747	0.1654	7.38E-01
Cer(d18:2/24:0)	8.74E-01	-0.0509	-0.2700	0.1682	8.54E-01	0.0242	-0.0964	0.1448	8.54E-01
Cer(d18:2/24:1)	4.48E-01	-0.1512	-0.3708	0.0685	6.06E-01	0.0595	-0.0615	0.1804	6.74E-01
Cer(d18:2/26:0)	6.09E-01	-0.1628	-0.3863	0.0607	6.06E-01	-0.0377	-0.1608	0.0853	7.93E-01
Cer(d18:2/26:1)	6.09E-01	0.1112	-0.1116	0.3341	6.74E-01	0.0782	-0.0443	0.2007	6.06E-01
Cer(d20:1/22:0)	4.54E-01	-0.0649	-0.2842	0.1544	7.93E-01	0.0941	-0.0267	0.2148	5.57E-01
Cer(d20:1/23:0)	7.04E-01	-0.1106	-0.3340	0.1128	6.74E-01	0.0243	-0.0987	0.1473	8.54E-01
Cer(d20:1/24:0)	7.04E-01	-0.0783	-0.3010	0.1445	7.71E-01	0.0562	-0.0665	0.1789	6.74E-01
Cer(d20:1/24:1)	3.61E-01	-0.0719	-0.2920	0.1483	7.83E-01	0.1097	-0.0115	0.2309	4.56E-01
Ceramide ratios									
Cer(d18:1/16:0)/Cer(d18:1/24:0)	7.04E-01	-0.1122	-0.3358	0.1113	6.74E-01	0.0350	-0.0881	0.1581	7.93E-01
Cer(d18:1/20:0)/Cer(d18:1/24:0)	5.90E-01	-0.0450	-0.2671	0.1770	8.54E-01	0.0855	-0.0368	0.2078	6.06E-01
Cer(d18:1/24:1)/Cer(d18:1/24:0)	7.99E-01	0.0377	-0.1870	0.2625	8.74E-01	0.0556	-0.0681	0.1794	6.74E-01

	MALE								
	F-test	Post-hoc comparison							
Ceramide	FDR p apoE	Beta ε2+	ci.low ε2+	ci.high ε2+	FDR p ε2+	Beta ε4+	ci.low ε4+	ci.high ε4+	FDR p ε4+
Cer(d16:1/16:0)	3.65E-01	-0.2003	-0.4625	0.0619	5.21E-01	0.1159	-0.0135	0.2454	4.75E-01
Cer(d16:1/18:0)	5.87E-01	-0.1603	-0.4076	0.0871	6.41E-01	0.0507	-0.0715	0.1729	7.23E-01
Cer(d16:1/20:0)	6.19E-01	-0.1385	-0.3919	0.1150	7.21E-01	0.0466	-0.0785	0.1717	7.86E-01
Cer(d16:1/22:0)	6.19E-01	-0.1580	-0.4150	0.0990	6.74E-01	0.0411	-0.0858	0.1679	8.15E-01
Cer(d16:1/23:0)	7.24E-01	-0.1600	-0.4243	0.1043	6.74E-01	0.0097	-0.1208	0.1402	9.69E-01
Cer(d16:1/24:0)	7.25E-01	-0.1097	-0.3730	0.1536	7.23E-01	0.0228	-0.1072	0.1528	9.69E-01
Cer(d16:1/24:1)	4.36E-01	-0.2471	-0.5042	0.0100	4.75E-01	0.0625	-0.0644	0.1894	7.23E-01
Cer(d18:1/14:0)	3.65E-01	-0.2290	-0.5011	0.0432	5.21E-01	0.1042	-0.0302	0.2386	5.21E-01
Cer(d18:1/16:0)	4.37E-02	-0.2376	-0.5005	0.0253	4.75E-01	0.1697	0.0398	0.2995	3.46E-01
Cer(d18:1/18:0)	7.25E-01	-0.0350	-0.2778	0.2077	9.69E-01	0.0516	-0.0683	0.1714	7.23E-01
Cer(d18:1/20:0)	7.24E-01	-0.0353	-0.2901	0.2195	9.69E-01	0.0731	-0.0527	0.1989	7.00E-01
Cer(d18:1/22:0)	4.36E-01	0.0224	-0.2309	0.2757	9.69E-01	0.1308	0.0058	0.2559	4.75E-01
Cer(d18:1/23:0)	7.25E-01	-0.0092	-0.2719	0.2534	9.75E-01	0.0723	-0.0574	0.2019	7.21E-01
Cer(d18:1/24:0)	7.25E-01	0.0012	-0.2668	0.2693	9.93E-01	0.0658	-0.0665	0.1982	7.23E-01
Cer(d18:1/24:1)	6.03E-01	-0.0172	-0.2752	0.2408	9.69E-01	0.0979	-0.0295	0.2253	5.21E-01
Cer(d18:1/26:0)	9.92E-01	0.0327	-0.2396	0.3049	9.69E-01	0.0205	-0.1139	0.1549	9.69E-01
Cer(d18:1/26:1)	6.03E-01	0.0628	-0.2056	0.3312	9.08E-01	0.1066	-0.0259	0.2391	5.21E-01
Cer(d18:2/16:0)	4.37E-02	-0.1773	-0.4428	0.0882	6.28E-01	0.1968	0.0657	0.3279	2.17E-01
Cer(d18:2/18:0)	5.34E-01	-0.1085	-0.3636	0.1465	7.23E-01	0.0906	-0.0353	0.2166	5.50E-01
Cer(d18:2/20:0)	5.34E-01	-0.0584	-0.3241	0.2074	9.16E-01	0.1068	-0.0244	0.2380	5.21E-01
Cer(d18:2/22:0)	4.36E-01	-0.0803	-0.3442	0.1836	8.26E-01	0.1285	-0.0018	0.2588	4.75E-01
Cer(d18:2/23:0)	6.19E-01	-0.0100	-0.2754	0.2554	9.75E-01	0.0953	-0.0357	0.2263	5.50E-01
Cer(d18:2/24:0)	5.34E-01	-0.0169	-0.2836	0.2497	9.69E-01	0.1181	-0.0136	0.2497	4.75E-01
Cer(d18:2/24:1)	4.36E-01	-0.0653	-0.3292	0.1987	9.01E-01	0.1296	-0.0007	0.2599	4.75E-01
Cer(d18:2/26:0)	7.25E-01	0.0874	-0.1860	0.3608	8.15E-01	0.0686	-0.0664	0.2035	7.23E-01
Cer(d18:2/26:1)	5.34E-01	0.2575	-0.0159	0.5309	4.75E-01	0.0483	-0.0848	0.1815	7.86E-01
Cer(d20:1/22:0)	4.36E-01	0.1112	-0.1499	0.3723	7.23E-01	0.1329	0.0040	0.2618	4.75E-01
Cer(d20:1/23:0)	7.25E-01	-0.0154	-0.2833	0.2525	9.69E-01	0.0609	-0.0714	0.1932	7.23E-01
Cer(d20:1/24:0)	7.99E-01	0.0759	-0.1921	0.3439	8.48E-01	0.0454	-0.0869	0.1777	8.07E-01
Cer(d20:1/24:1)	7.25E-01	0.1154	-0.1470	0.3778	7.23E-01	0.0631	-0.0665	0.1926	7.23E-01
Ceramide ratios									
Cer(d18:1/16:0)/Cer(d18:1/24:0)	7.25E-01	-0.1252	-0.3978	0.1473	7.23E-01	0.0168	-0.1178	0.1513	9.69E-01
Cer(d18:1/20:0)/Cer(d18:1/24:0)	9.92E-01	-0.0289	-0.3024	0.2447	9.69E-01	-0.0154	-0.1504	0.1197	9.69E-01
Cer(d18:1/24:1)/Cer(d18:1/24:0)	9.93E-01	-0.0161	-0.2899	0.2577	9.69E-01	-0.0033	-0.1385	0.1319	9.77E-01

Supplementary Table S9: Complete data including all p-values from the cross-sectional association analyses of apoE polymorphism and sphingomyelins in all YFS subjects in 2007. **Annotation:** FDR p/apoE, false discovery rate corrected F-test p-value for apoE; Beta/ε2+ or ε4+, post-hoc β-coefficient; ci.low/ε2+ or ε4+, 95% post-hoc CI lower limit; ci.high/ε2+ or ε4+, 95% post-hoc CI upper limit; FDR p/ε2+ or ε4+, false discovery rate corrected post-hoc p-value. Sphingomyelin indexes a and b separate lipids with same mass but different retention time.

	F-test	Post-hoc comparison between apoE genotypes							
Spingomyelin	FDR p apoE	Beta ε2+	ci.low ε2+	ci.high ε2+	FDR p ε2+	Beta ε4+	ci.low ε4+	ci.high ε4+	FDR p ε4+
SM(30:2)	6.41E-01	-0.0638	-0.2218	0.0942	6.17E-01	-0.0352	-0.1183	0.0479	5.94E-01
SM(31:0)	5.29E-01	-0.0584	-0.2283	0.1116	6.84E-01	-0.0569	-0.1464	0.0327	4.16E-01
SM(31:1)	2.30E-01	-0.1534	-0.3200	0.0133	1.72E-01	-0.0445	-0.1321	0.0431	4.94E-01
SM(31:2)	9.23E-01	-0.0421	-0.1939	0.1098	7.64E-01	-0.0098	-0.0902	0.0705	9.23E-01
SM(32:0)	9.15E-02	-0.2065	-0.3750	-0.0381	7.02E-02	-0.0346	-0.1232	0.0540	6.27E-01
SM(32:1)	1.72E-02	-0.2712	-0.4399	-0.1026	1.34E-02	-0.0415	-0.1302	0.0471	5.44E-01
SM(32:2)	4.71E-01	-0.1036	-0.2472	0.0400	3.31E-01	-0.0016	-0.0771	0.0739	9.91E-01
SM(33:0)	2.12E-01	-0.1695	-0.3372	-0.0017	1.35E-01	-0.0123	-0.1005	0.0759	9.23E-01
SM(33:1)	1.72E-02	-0.2761	-0.4467	-0.1056	1.34E-02	-0.0009	-0.0906	0.0888	9.97E-01
SM(33:2)	3.07E-01	-0.0810	-0.2353	0.0733	4.78E-01	0.0476	-0.0335	0.1287	4.56E-01
SM(34:0)	1.14E-03	-0.3004	-0.4686	-0.1322	1.22E-02	0.0813	-0.0071	0.1698	1.72E-01
SM(34:1)	6.66E-07	-0.4275	-0.5953	-0.2596	5.26E-05	0.1022	0.0139	0.1904	7.69E-02
SM(34:2)	3.89E-02	-0.2260	-0.3936	-0.0585	4.80E-02	0.0185	-0.0696	0.1066	8.32E-01
SM(35:1)	2.39E-02	-0.2188	-0.3890	-0.0487	5.79E-02	0.0495	-0.0399	0.1390	4.74E-01
SM(35:2)	5.43E-02	-0.0724	-0.2409	0.0961	5.94E-01	0.1040	0.0154	0.1926	7.65E-02
SM(36:0)	9.33E-01	-0.0210	-0.1800	0.1380	9.23E-01	0.0089	-0.0747	0.0925	9.28E-01
SM(36:1)	6.36E-03	-0.2352	-0.4010	-0.0694	3.73E-02	0.0807	-0.0065	0.1679	1.72E-01
SM(36:2)	7.94E-02	-0.0283	-0.1894	0.1327	8.81E-01	0.1015	0.0168	0.1862	7.02E-02
SM(36:3)	1.63E-01	0.0063	-0.1609	0.1735	9.91E-01	0.0943	0.0063	0.1822	1.04E-01
SM(37:1)	2.30E-01	-0.1559	-0.3172	0.0054	1.59E-01	-0.0082	-0.0930	0.0766	9.28E-01
SM(37:2)	5.88E-01	-0.0935	-0.2570	0.0700	4.68E-01	-0.0281	-0.1141	0.0579	7.00E-01
SM(38:0)	6.03E-01	-0.0891	-0.2520	0.0737	4.74E-01	-0.0275	-0.1131	0.0581	7.00E-01
SM(38:1)	6.36E-03	-0.2716	-0.4401	-0.1030	1.34E-02	0.0563	-0.0323	0.1449	4.16E-01
SM(38:2)	1.72E-02	-0.1164	-0.2822	0.0494	3.46E-01	0.1118	0.0246	0.1990	5.79E-02
SM(38:3)	5.29E-01	-0.0174	-0.1871	0.1524	9.28E-01	0.0557	-0.0336	0.1449	4.22E-01
SM(39:1)	1.72E-02	-0.2789	-0.4494	-0.1084	1.34E-02	-0.0217	-0.1113	0.0680	8.02E-01
SM(39:2)	9.33E-01	-0.0363	-0.2072	0.1347	8.32E-01	-0.0025	-0.0924	0.0874	9.91E-01
SM(40:0)	9.25E-01	-0.0423	-0.2064	0.1218	7.86E-01	-0.0055	-0.0917	0.0806	9.71E-01
SM(40:1)	8.42E-04	-0.3250	-0.4910	-0.1590	5.21E-03	0.0680	-0.0192	0.1553	2.73E-01
SM(40:2)a	9.36E-02	-0.1828	-0.3529	-0.0127	1.04E-01	0.0313	-0.0581	0.1207	6.84E-01
SM(40:2)b	6.36E-03	-0.1944	-0.3625	-0.0263	7.69E-02	0.1063	0.0179	0.1947	0.07016
SM(41:0)	6.41E-01	-0.0899	-0.2566	0.07685	4.77E-01	-0.0029	-0.0912	0.08537	0.99059
SM(41:1)	1.17E-02	-0.2889	-0.4592	-0.1186	1.22E-02	0.01146	-0.0781	0.10099	0.92346
SM(41:2)a	4.49E-02	-0.2342	-0.4048	-0.0636	4.51E-02	-0.0002	-0.0899	0.08953	0.99718
SM(41:2)b	2.07E-02	-0.2167	-0.3841	-0.0492	5.79E-02	0.05282	-0.0352	0.14086	0.44637
SM(42:1)	1.55E-03	-0.2821	-0.4476	-0.1165	1.22E-02	0.08072	-0.0063	0.16777	0.17218
SM(42:2)	5.55E-03	-0.2895	-0.4582	-0.1208	1.22E-02	0.04985	-0.0389	0.13857	0.47207
SM(42:3)	2.07E-02	-0.1415	-0.3111	0.02805	2.32E-01	0.10129	0.01212	0.19047	0.08203
SM(44:1)	1.72E-02	-0.2081	-0.3783	-0.0379	7.02E-02	0.07144	-0.0181	0.16095	0.2607
SM(44:2)	1.72E-02	-0.2016	-0.3696	-0.0337	7.02E-02	0.07631	-0.012	0.16461	0.21142
SM(44:3)	1.72E-02	-0.2439	-0.414	-0.0738	3.71E-02	0.04697	-0.0425	0.13642	0.47819

Supplementary Discussion S10

Discussion (extended)

In the present work we investigated the associations of apoE polymorphism with plasma ceramide species with an emphasis on previously reported CAD-risk-related ceramide species. The results of this study show for the first time an association between apoE genotypes and a high-risk CAD ceramide Cer(d18:1/16:0). The effect size of ε2 would seem bigger than that of ε4, which corresponds the reference findings of ε2 cholesterol-lowering effect being 2-3 times that of the ε4 cholesterol-raising effect[(1)]. Cer(d18:1/16:0) has consistently been connected to elevated risk of cardiovascular death[(2-4)], major adverse cardiovascular events[(5,6)] and vulnerable plaque characteristics[(6)] among patients with established CAD, as well as to incident major adverse cardiovascular events among healthy population[(7,8)]. Moreover, elevated plasma levels of Cer(d18:1/16:0) have earlier been reported among patients with stable CAD compared with healthy controls[(9)]. Reported data on subclinical atherosclerotic associations of Cer(d18:1/16:0), or ceramides in general, is very limited. Nevertheless, Cer(d18:1/16:0) has been linked to an increased risk of carotid artery plaques[(10)], and sphingomyelin SM(34:1), a precursor molecule of Cer(d18:1/16:0), to an increased carotid artery intima-media thickness[(11)]. Our Supplementary analyses (S7) also indicate an association of Cer(d18:1/16:0) with elevated high-sensitivity CRP levels. In addition to Cer(d18:1/16:0), associations over apoE genotypes were indicated in six other ceramide species as well. The present results suggest a novel linkage between apoE polymorphism, lipid metabolism and CAD.

Previous GWAS has mapped apoE locus to (Ingenuity) sphingolipid metabolism pathway[(12)], which is the major hub also for ceramide metabolism[(13)]. Ceramides are mainly generated through sphingomyelinase (SMase, a sphingomyelin-specific form of phospholipase C) pathway, which breaks downs sphingomyelin in the cell membrane and releases ceramides[(14)]. Alternatively, ceramides can be generated via de novo pathway in all cell types, liver being an active site of circulating ceramide production[(15,16)]. A variety of cell types present in atherosclerotic lesions, including macrophages and endothelial cells, secretes SMase[(17)]. Inflammatory conditions such as atherosclerosis may increase via key cytokines (e.g., tumour necrosis factor-α and interleukin-2), as well as several cardiovascular risk factors/markers (e.g., oxidized LDL (ox-LDL) and homocysteine) the production of ceramides, which as signalling molecules can regulate e.g., cell differentiation, proliferation, apoptosis, ROS production and cytokine gene expression – at least some of these roles are directly involved in the molecular mechanisms of cardiovascular disease[14]. In plasma, ceramides are bound to lipoproteins and are particularly abundant in LDL[18]. Moreover, LDL isolated from human plasma has also been reported to possess SMase activity[19], being able to catalyse the formation of ceramides in the contacting, adjacent lipoproteins, as well[20]. In the de novo pathway, ceramides are synthesized from palmitate and serine, and a covalently linked fatty acid is added to the molecule by a ceramide synthase (CerS)[16]. CerS has six isoforms each with different preferences for the fatty acyl chain lengths, leading to different molecular ceramide species[16].

Ceramides have been found to enrich in LDL of atherosclerotic lesions compared with plasma LDL[21], and a recent study showed that LDL particles with elevated sphingolipid content (including certain risk ceramides) are prone to aggregate and accumulate within the arterial intima promoting future CAD death – via various plausible mechanisms triggered by SMase-induced modifications in lipoprotein particles[22]. Macrophage-derived SMase has also been shown to stimulate the aggregation of LDL, as well as very-low-density lipoproteins (VLDL)[14,23]. Uptake of lipoproteins by macrophages is increased by aggregation and accumulation of LDL, thus potently inducing cholesterol-rich foam cell formation and atherosclerotic plaque development[14]. Also, ox-LDL loading of macrophages has been demonstrated to increase intracellular ceramide contents – Cer(d18:1/16:0) and Cer(d18:1/18:0) among reported species[24]. On the other hand, deficiency and inhibition of LDL-receptor (LDLR) degrading proprotein convertase subtilisin/kexin type 9 (PCSK9) enzyme has been found to decrease certain plasma ceramide levels, including Cer(d18:1/16:0) and Cer(d18:1/18:0)[25,26]. These changes in plasma ceramides with specific fatty acyl chain lengths depend on different ceramide distributions in the lipoprotein fractions[25]. Also, by PCSK9 inhibition certain ceramide contents have been reported to decrease in LDL and VLDL particles, including all the seven ceramide species we found to associate over apoE genotypes[25]. Our supplementary analyses (Supplementary tables S4 and S5) are further indicating a strong connection between these ceramides and apoB-containing LDL, as well as VLDL (+ IDL) particles, whereas the associations with HDL are weak to none (Supplementary Table S6). A consistent association of apoE polymorphism with all the apoB-containing lipoproteins along with their subfractions have been shown recently in the same YFS cohort[27]. Altogether, ceramides would seem as a plausible and novel common nominator in the abundantly investigated chain of apoE polymorphism, plasma LDL metabolism and the risk of CAD.

In addition to SMase pathway, it may be possible that the altered concentrations of certain ceramide species could also result from changes in de novo pathway due to altered CerS activities. For example, de novo pathway has been reported to be influenced by high-fat-diet administration (especially saturated fatty acids) and/or palmitate treatment, which in several reference studies have been shown to increase molecular ceramide contents, most consistently C16:0 and C18:0 species, independent of tissue or cell type – due to increased amount of substrate, as well as upregulation of certain CerS isomers[28].

To our knowledge, previous studies of apoE interactions with sphingolipid metabolism in atherogenesis have not investigated the effect of apoE polymorphism. However, it has been noticed that apoE increases the macrophage uptake of ceramides, mediated by cell surface heparan sulphate proteoglycans and low-density lipoprotein receptor-related protein pathways[29]. Moreover, apoE has been reported to prevent SMase-induced hydrophobic interaction and aggregation of lipoproteins by a proposed mechanism of binding on ceramide-enriched domains exposed on particle surfaces, without affecting the SMase activity[30]. ApoE has also been shown to prefer binding to ceramides compared to sphingomyelins[30], and SMase stimulated ceramide formation can, therefore, induce the membrane remodelling activity of apoE and free cholesterol. In addition, it has been demonstrated that majority of apoE in atherosclerotic lesions is synthesized locally by lesion macrophages, and the local apoE expression by macrophages is atheroprotective[31] – via a suggested role of preventing the SMase-induced aggregation of lipoproteins[30]. On the other hand, increased cellular ceramides have been reported to reduce macrophage-derived apoE secretion (demonstrated with E3 phenotype) without increased cell retention of nascent apoE[32]. Furthermore, apoE has been demonstrated to prefer binding on ox-LDL-loading-induced, ceramide-enriched microdomain surfaces of human macrophages, which could contribute to atherogenesis by impaired lipid efflux and enhancement of inflammatory processes in macrophages[24]. If hypothesized, all these reported findings would be related to the apoE parent isoform E3, and E2 or E4 might, therefore, present altered interactions worth investigating – possibly affecting atherogenic processes via e.g., lipoprotein particle aggregation, or adjustment of sphingolipid transfer balance in macrophages. In any case, apoE E4 (but not E3) in macrophages has already been demonstrated to enhance atherosclerotic plaque development in an LDLR-dependent manner, as higher LDLR binding affinity of E4 increases cholesterol delivery to macrophages, accompanied by reduced sterol efflux[33].

Our study has several important strengths. Our study cohort has a long and systematic follow-up, offering a representative cross-sectional sample of relatively young Finnish adults. Also, the comprehensive background information gathered from our cohort allowed a thorough analysis and consideration of the most expected confounding factors. Furthermore, our analyses reflect genotype-related differences in a basically healthy general population – avoiding confounding/confusing expressions of illnesses expected when using morbid study subjects. In addition, the modern and sophisticated mass spectrometry technology enabled the measurement of circulating ceramides with good accuracy. We also acknowledge certain limitations in our cross-sectional study. Our present scope did not allow direct analyses of subclinical atherosclerosis measures. As previously reported in Finnish general population[34,35], subgroups of apoE ε2/2, ε4/2, as well as ε4/4 were small also in our population-based setting and did not allow complete analyses of all the six genotypes separately. Also, a completely comprehensive comparison between (sex-stratified) men and women might require even a bigger sample size for increased statistical power – albeit, the absence of apoE x sex interactions was confirmed in the analyses of all subjects. Nevertheless, based on reference studies (e.g.,[1,34]) these recognized limitations are not expected to alter the effect directions related to different apoE alleles, and are thus less likely to have any major impact on our main discoveries presented and discussed here.

In conclusion, based on quantified molecular ceramide species from plasma, apoE polymorphism is associated with risk of developing CAD, especially by indicating altered levels of previously reported clinical high-risk marker Cer(d18:1/16:0) in all subjects, accompanied by another known CAD-marker Cer(d18:1/18:0) in women – both also shown to increase in macrophages by ox-LDL loading[24] and to decrease in plasma by PCSK9 inhibition[25,26]. Furthermore, the concentration of every apoE-associating ceramide species in our study has been reported to be reduced in LDL- and VLDL-particles by PCSK9 inhibition[26], whereas excessive sphingolipid loading has been reported to induce LDL aggregation and accumulation within the arterial intima, hence promoting future CAD death[22]. These findings suggest a plausible linkage between ceramide metabolism, apoE polymorphism, plasma LDL metabolism and atherogenesis. Discovered changes are already evident in a basically healthy general population and extend our understanding about the role of apoE in health and disease. For further work, similar analyses could be performed with patients at incident CAD and established CAD to discover, whether the observed associations of apoE polymorphism would change. Associations with subclinical atherosclerosis measures (e.g., carotid artery intima-media thickness or plaques) should also be investigated for further indications. In addition, longitudinal follow-up studies could be performed, with older test subjects included, as well. Furthermore, effects of apoE polymorphism on sphingolipid/ceramide metabolism pathway might be worth investigating – whether e.g., the macrophage functions or lipoprotein aggregation tendency would become different depending on the apoE isoform. Additional inflammatory marker correlations might also be worth investigating, and the ceramide levels could also be correlated with sphingomyelinase activities.

References

(1) Davignon, J., R. E. Gregg, and C. F. Sing. 1988. Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis* **8:** 1-21.

(2) Laaksonen, R., K. Ekroos, M. Sysi-Aho, M. Hilvo, T. Vihervaara, D. Kauhanen, M. Suoniemi, R. Hurme, W. März, H. Scharnagl, T. Stojakovic, E. Vlachopoulou, M. Lokki, M. S. Nieminen, R. Klingenberg, C. M. Matter, T. Hornemann, P. Jüni, N. Rodondi, L. Räber, S. Windecker, B. Gencer, E. R. Pedersen, G. S. Tell, O. Nygård, F. Mach, J. Sinisalo, and T. F. Lüscher. 2016. Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes beyond LDL-cholesterol. *Eur. Heart J.* **37:** 1967-1976.

(3) Tarasov, K., K. Ekroos, M. Suoniemi, D. Kauhanen, T. Sylvänne, R. Hurme, I. Gouni-Berthold, H. K. Berthold, M. E. Kleber, R. Laaksonen, and W. März. 2014. Molecular lipids identify cardiovascular risk and are efficiently lowered by simvastatin and PCSK9 deficiency. *J. Clin. Endocrinol. Metab.* **99:** 45.

(4) Sigruener, A., M. E. Kleber, S. Heimerl, G. Liebisch, G. Schmitz, and W. Maerz. 2014. Glycerophospholipid and sphingolipid species and mortality: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *PLoS ONE* **9:** e85724.

(5) Anroedh, S. S., M. Hilvo, K. M. Akkerhuis, D. Kauhanen, K. Koistinen, R. Oemrawsingh, P. Serruys, R. van Geuns, E. Boersma, R. Laaksonen, and I. Kardys. 2018. Plasma concentrations of molecular lipid species predict long-term clinical outcome in coronary artery disease patients. *J. Lipid Res.*

(6) Cheng, J. M., M. Suoniemi, I. Kardys, T. Vihervaara, de Boer, Sanneke P M, K. M. Akkerhuis, M. Sysi-Aho, K. Ekroos, H. M. Garcia-Garcia, R. M. Oemrawsingh, E. Regar, W. Koenig, P. W. Serruys, R. van Geuns, E. Boersma, and R. Laaksonen. 2015. Plasma concentrations of molecular lipid species in relation to coronary plaque characteristics and cardiovascular outcome: Results of the ATHEROREMO-IVUS study. *Atherosclerosis* **243:** 560-566.

(7) Havulinna, A. S., M. Sysi-Aho, M. Hilvo, D. Kauhanen, R. Hurme, K. Ekroos, V. Salomaa, and R. Laaksonen. 2016. Circulating Ceramides Predict Cardiovascular Outcomes in the Population-Based FINRISK 2002 Cohort. *Arterioscler. Thromb. Vasc. Biol.* **36:** 2424-2430.

(8) Wang, D. D., E. Toledo, A. Hruby, B. A. Rosner, W. C. Willett, Q. Sun, C. Razquin, Y. Zheng, M. Ruiz-Canela, M. Guasch-Ferré, D. Corella, E. Gómez-Gracia, M. Fiol, R. Estruch, E. Ros, J. Lapetra, M. Fito, F. Aros, L. Serra-Majem, C. Lee, C. B. Clish, L. Liang, J. Salas-Salvadó, M. A. Martínez-González, and F. B. Hu. 2017. Plasma Ceramides, Mediterranean Diet, and Incident Cardiovascular Disease in the PREDIMED Trial (Prevención con Dieta Mediterránea). *Circulation* **135:** 2028-2040.

(9) Meikle, P. J., G. Wong, D. Tsorotes, C. K. Barlow, J. M. Weir, M. J. Christopher, G. L. MacIntosh, B. Goudey, L. Stern, A. Kowalczyk, I. Haviv, A. J. White, A. M. Dart, S. J. Duffy, G. L. Jennings, and B. A. Kingwell. 2011. Plasma lipidomic analysis of stable and unstable coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* **31:** 2723-2732.

(10) Zhao, W., X. Wang, A. A. Deik, D. B. Hanna, T. Wang, S. A. Haberlen, S. J. Shah, J. M. Lazar, H. N. Hodis, A. L. Landay, B. Yu, D. Gustafson, K. Anastos, W. S. Post, C. B. Clish, R. C. Kaplan, and Q. Qi. 2019. Elevated Plasma Ceramides Are Associated With Antiretroviral Therapy Use and Progression of Carotid Artery Atherosclerosis in HIV Infection. *Circulation* **139:** 2003-2011.

(11) Saulnier-Blache, J. S., R. Wilson, K. Klavins, D. Graham, I. Alesutan, G. Kastenmüller, R. Wang-Sattler, J. Adamski, M. Roden, W. Rathmann, J. Seissler, C. Meisinger, W. Koenig, J. Thiery, K. Suhre, A. Peters, M. Kuro-O, F. Lang, G. Dallmann, C. Delles, J. Voelkl, M. Waldenberger, J. Bascands, J. Klein, and J. P. Schanstra. 2018. Ldlr-/- and ApoE-/- mice better mimic the human metabolite signature of increased carotid intima media thickness compared to other animal models of cardiovascular disease. *Atherosclerosis* **276:** 140-147.

(12) Demirkan, A., C. M. van Duijn, P. Ugocsai, A. Isaacs, P. P. Pramstaller, G. Liebisch, J. F. Wilson, Å Johansson, I. Rudan, Y. S. Aulchenko, A. V. Kirichenko, Janssens, A Cecile J W, R. C. Jansen, C. Gnewuch, F. S. Domingues, C. Pattaro, S. H. Wild, I. Jonasson, O. Polasek, I. V. Zorkoltseva, A. Hofman, L. C. Karssen, M. Struchalin, J. Floyd, W. Igl, Z. Biloglav, L. Broer, A. Pfeufer, I. Pichler, S. Campbell, G. Zaboli, I. Kolcic, F. Rivadeneira, J. Huffman, N. D. Hastie, A. Uitterlinden, L. Franke, C. S. Franklin, V. Vitart, C. P. Nelson, M. Preuss, J. C. Bis, C. J. O'Donnell, N. Franceschini, J. C. M. Witteman, T. Axenovich, B. A. Oostra, T. Meitinger, A. A. Hicks, C. Hayward, A. F. Wright, U. Gyllensten, H. Campbell, and G. Schmitz. 2012. Genome-wide association study identifies novel loci associated with circulating phospho- and sphingolipid concentrations. *PLoS Genet.* **8:** e1002490.

(13) Hannun, Y. A., L. M. Obeid. 2002. The Ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind. *J. Biol. Chem.* **277:** 25847-25850.

(14) Bismuth, J., P. Lin, Q. Yao, and C. Chen. 2008. Ceramide: a common pathway for atherosclerosis? *Atherosclerosis* **196:** 497-504.

(15) Larsen, P. J., N. Tennagels. 2014. On ceramides, other sphingolipids and impaired glucose homeostasis. *Mol Metab* **3:** 252-260.

(16) Wattenberg, B. W. 2018. The long and the short of ceramides. *J. Biol. Chem.* **293:** 9922-9923.

(17) Marathe, S., S. L. Schissel, M. J. Yellin, N. Beatini, R. Mintzer, K. J. Williams, and I. Tabas. 1998. Human vascular endothelial cells are a rich and regulatable source of secretory sphingomyelinase. Implications for early atherogenesis and ceramide-mediated cell signaling. *J. Biol. Chem.* **273:** 4081-4088.

(18) Chatterjee, S. 1998. Sphingolipids in atherosclerosis and vascular biology. *Arterioscler. Thromb. Vasc. Biol.* **18:** 1523-1533.

(19) Holopainen, J. M., O. P. Medina, A. J. Metso, and P. K. Kinnunen. 2000. Sphingomyelinase activity associated with human plasma low density lipoprotein. *J. Biol. Chem.* **275:** 16484-16489.

(20) Kinnunen, P. K. J., J. M. Holopainen. 2002. Sphingomyelinase activity of LDL: a link between atherosclerosis, ceramide, and apoptosis? *Trends Cardiovasc. Med.* **12:** 37-42.

(21) Schissel, S. L., J. Tweedie-Hardman, J. H. Rapp, G. Graham, K. J. Williams, and I. Tabas. 1996. Rabbit aorta and human atherosclerotic lesions hydrolyze the sphingomyelin of retained low-density lipoprotein. Proposed role for arterial-wall sphingomyelinase in subendothelial retention and aggregation of atherogenic lipoproteins. *J. Clin. Invest.* **98:** 1455-1464.

(22) Ruuth, M., S. D. Nguyen, T. Vihervaara, M. Hilvo, T. D. Laajala, P. K. Kondadi, A. Gisterå, H. Lähteenmäki, T. Kittilä, J. Huusko, M. Uusitupa, U. Schwab, M. J. Savolainen, J. Sinisalo, M. Lokki, M. S. Nieminen, A. Jula, M. Perola, S. Ylä-Herttula, L. Rudel, A. Öörni, M. Baumann, A. Baruch, R. Laaksonen, D. F. J. Ketelhuth, T. Aittokallio, M. Jauhiainen, R. Käkelä, J. Borén, K. J. Williams, P. T. Kovanen, and K. Öörni. 2018. Susceptibility of low-density lipoprotein particles to aggregate depends on particle lipidome, is modifiable, and associates with future cardiovascular deaths. *Eur. Heart J.* **39:** 2562-2573.

(23) Jeong, T., S. L. Schissel, I. Tabas, H. J. Pownall, A. R. Tall, and X. Jiang. 1998. Increased sphingomyelin content of plasma lipoproteins in apolipoprotein E knockout mice reflects combined production and catabolic defects and enhances reactivity with mammalian sphingomyelinase. *J. Clin. Invest.* **101:** 905-912.

(24) Wallner, S., M. Grandl, G. Liebisch, M. Peer, E. Orsó, A. Sigrüner, A. Sobota, and G. Schmitz. 2016. oxLDL and eLDL Induced Membrane Microdomains in Human Macrophages. *PLoS ONE* **11:** e0166798.

(25) Hilvo, M., H. Simolin, J. Metso, M. Ruuth, K. Öörni, M. Jauhiainen, R. Laaksonen, and A. Baruch. 2018. PCSK9 inhibition alters the lipidome of plasma and lipoprotein fractions. *Atherosclerosis* **269:** 159-165.

(26) Jänis, M. T., K. Tarasov, H. X. Ta, M. Suoniemi, K. Ekroos, R. Hurme, T. Lehtimäki, H. Päivä, M. E. Kleber, W. März, A. Prat, N. G. Seidah, and R. Laaksonen. 2013. Beyond LDL-C lowering: distinct molecular sphingolipids are good indicators of proprotein convertase subtilisin/kexin type 9 (PCSK9) deficiency. *Atherosclerosis* **228:** 380-385.

(27) Karjalainen, J., N. Mononen, N. Hutri-Kähönen, M. Lehtimäki, M. Juonala, M. Ala-Korpela, M. Kähönen, O. Raitakari, and T. Lehtimäki. 2019. The effect of apolipoprotein E polymorphism on serum metabolome - a population-based 10-year follow-up study. *Sci Rep* **9:** 458.

(28) Choi, S., A. J. Snider. 2015. Sphingolipids in High Fat Diet and Obesity-Related Diseases. *Mediators Inflamm.* **2015:** 520618.

(29) Morita, S., M. Kawabe, A. Sakurai, K. Okuhira, A. Vertut-Doï, M. Nakano, and T. Handa. 2004. Ceramide in lipid particles enhances heparan sulfate proteoglycan and low density lipoprotein receptor-related protein-mediated uptake by macrophages. *J. Biol. Chem.* **279:** 24355-24361.

(30) Morita, S., M. Nakano, A. Sakurai, Y. Deharu, A. Vertut-Doï, and T. Handa. 2005. Formation of ceramide-enriched domains in lipid particles enhances the binding of apolipoprotein E. *FEBS Lett.* **579:** 1759-1764.

(31) Fazio, S., V. R. Babaev, A. B. Murray, A. H. Hasty, K. J. Carter, L. A. Gleaves, J. B. Atkinson, and M. F. Linton. 1997. Increased atherosclerosis in mice reconstituted with apolipoprotein E null macrophages. *Proc. Natl. Acad. Sci. U. S. A.* **94:** 4647-4652.

(32) Lucic, D., Z. H. Huang, D. Gu, P. V. Subbaiah, and T. Mazzone. 2007. Cellular sphingolipids regulate macrophage apolipoprotein E secretion. *Biochemistry* **46:** 11196-11204.

(33) Altenburg, M., L. Johnson, J. Wilder, and N. Maeda. 2007. Apolipoprotein E4 in macrophages enhances atherogenesis in a low density lipoprotein receptor-dependent manner. *J. Biol. Chem.* **282:** 7817-7824.

(34) Lehtimäki, T., T. Moilanen, J. Viikari, H. K. Akerblom, C. Ehnholm, T. Rönnemaa, J. Marniemi, G. Dahlen, and T. Nikkari. 1990. Apolipoprotein E phenotypes in Finnish youths: a cross-sectional and 6- year follow-up study. *Journal of Lipid Research* **31:** 487.

(35) Ehnholm, C., M. Lukka, T. Kuusi, E. Nikkilä, and G. Utermann. 1986. Apolipoprotein E polymorphism in the Finnish population: gene frequencies and relation to lipoprotein concentrations. *J. Lipid Res.* **27:** 227-235.