Online-Only Supplement

Manuscript Title: Association of Maternal Pre-Pregnancy Dyslipidemia with Adult Offspring Dyslipidemia In Excess of Anthropometric, Lifestyle and Genetic Factors in The Framingham Heart Study

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Supplemental Methods

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Dietary and Physical Activity Assessments

Dietary intake was ascertained in the adult offspring (Third Generation cohort) via a 126-item semi-quantitative self-reported food frequency questionnaire (FFQ). The validity of the FFQ has been reported previously.[1-3] A common unit or portion size for each food was specified, and participants were asked how often, on average, they had consumed that amount of the food or beverage during the previous year. The average daily intake of fat and other nutrients was calculated by multiplying the frequency of consumption of each item by its nutrient content per serving and totaling the nutrient intake for all food items. Data from the FFQ were considered valid if <13 food items were missing and the total energy intakes reported were 600-4200 kcal/d for men and <4000 kcal/d for women. For the present analyses, dietary trans and saturated fat intake, known major dietary contributors to serum LDL-C,[4,5] were included in the regression model as residuals after adjustment for total energy intake (nutrient residual method).[6] Total caloric intake was also included in the model to allow for isocaloric interpretation.

Physical activity was determined as the number of hours spent performing specific activities (e.g., sleep, sedentary, slight activity, moderate activity and heavy activity) during a typical day. A physical activity index (PAI), expressed in metabolic equivalents (METs), was calculated by assigning each activity category a MET value based on the oxygen consumption

required to perform activities in the category and deriving a weighted average of the MET values based on the proportion of time spent on activities in each category.[7]

Clinical and Laboratory Assessments

Height (to the nearest 0.25 inches) and weight (to the nearest 0.5 lbs) were measured at the physical examination with the participant standing, shoes off, and wearing only a hospital gown. BMI was calculated as weight (kg) divided by height (meters) squared. Participants were considered to be current smokers if they smoked on average at least 1 cigarette per day for the last year. Systolic and diastolic blood pressure readings were taken two times from the left arm with a mercury sphygmomanometer with the participant seated by a trained physician examiner.

Hypertension was defined as an average systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg or current treatment with an anti-hypertensive agent. Diabetes was defined as a fasting plasma glucose ≥126 mg/dL (7.0 mmol/L) at a FHS examination or treatment with either insulin or a hypoglycemic agent.

LDL-C Genetic Risk Score

FHS participants in the Third Generation cohort were genotyped using 550K single nucleotide polymorphism (SNP) arrays on the Affymetrix platform (Affymetrix, Santa Clara, CA) or Illumina Golden Gate assay (Illumina Inc., San Diego, CA) and imputed to 2.5 million HapMap SNPs as previously described.[8] Adult offspring SNP genotypes were available for 525 (98%) parent-offspring pairs. The LDL-C genetic risk score (GRS), which has been previously defined,[9,10] included genome-wide significant SNP and LDL-C associations reported in 2010 by the Global Lipids Genetic Consortium.[11] The LDL-C GRS for each participant included 37 SNPs

weighted by summation of genotypes (coded additively for the risk allele) multiplied by the reported effect-size estimates (**eTable 1**).

Statistical Analysis

The additional association of the maternal pre-pregnancy LDL-C as compared to paternal pre-pregnancy LDL-C was contrasted in three ways. First, a subset of the sample with both parents was analyzed with both parents' pre-pregnancy LDL-C values in the same model to determine the association of maternal pre-pregnancy and adult offspring LDL-C after adjustment for paternal pre-pregnancy LDL-C. Second, the discriminatory ability of logistic regression models was evaluated descriptively using the c-statistic with and without the parental pre-pregnancy dichotomized LDL-C in the fully-adjusted models (M4) and contrasted between parents.[12] Third, the net reclassification index (NRI) and integrated discrimination improvement (IDI) were used to assess the change in discriminatory ability of the model when the dichotomized parental pre-pregnancy LDL-C was added to the covariates-only models (M4) and contrasted between parents.[13] The NRI and IDI were developed to evaluate the additional utility of a variable for risk prediction beyond other predictors and covariates; in this context we used them to demonstrate the additive contribution of parental LDL-C beyond that of classic risk factors for elevated LDL-C. The NRI measures the correctness of reclassification of subjects and the IDI measures the new model's improvement in average sensitivity.

References for Supplemental Methods

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Supplemental Results

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Comparison of Maternal vs. Paternal Pre-Pregnancy Contribution

A third approach to contrast the maternal and paternal associations was to examine the improvement in model performance when either the maternal or paternal pre-pregnancy LDL-C is added to a covariate-only model. The c-statistic for the covariates-only fully adjusted logistic regression model (M4) predicting adult offspring's elevated LDL-C was 0.80 and increased to 0.83 when mother's pre-pregnancy elevated LDL-C was added to the model (NRI 19% [p=0.0008]; relative IDI 22% [p=0.048]). In contrast, the c-statistic only increased from 0.775 to 0.778 (NRI -3% [p=0.6]; relative IDI 17% [p=0.06]) with the addition of paternal pre-pregnancy elevated LDL-C to the covariates-only model 4.

eTable 1: LDL-C Genetic Risk Score (GRS) Components, Effect Sizes and Genotyping Details

SNP	CHR	POS	Nearby Gene	Effect Size Per Allele on LDL-C (SE) mg/dL	MAF	Genotyping Platform	Call Rate	Imputation R^2	Additional Lipid Trait Associations
rs1030431	8	59,474,251	*CYP7A1*	0.95 (0.18)	0.34	Imputed		0.97	TC
rs10401969	19	19,268,718	*CILP2*	-3.11 (0.38)	0.07	Illumina	100		TC, TG
rs11065987	12	110,556,807	*BRAP*	-0.97 (0.18)	0.45	Illumina	100		TC
rs11136341	8	145,115,531	*PLEC1*	1.40 (0.21)	0.41	Imputed		0.8	TC
rs11153594	6	116,461,284	*FRK*	-0.89 (0.18)	0.44	Affymetrix	99		
rs11220462	11	125,749,162	*ST3GAL4*	1.95 (0.26)	0.14	Affymetrix	100		
rs1129555	10	113,900,711	*GPAM*	1.08 (0.20)	0.31	Affymetrix	100		
rs1169288	12	119,901,033	*HNF1A*	1.42 (0.19)	0.34	Imputed		0.98	TC
rs12027135	1	25,648,320	*LDLRAP1*	-1.10 (0.18)	0.48	Imputed		0.99	TC
rs12670798	7	21,573,877	*DNAH11*	1.26 (0.20)	0.23	Imputed		0.98	
rs12916	5	74,692,295	*HMGCR*	2.45 (0.18)	0.38	Imputed		0.97	TC
rs1367117	2	21,117,405	*APOB*	4.05 (0.19)	0.31	Imputed		0.98	TC
rs1564348	6	160,498,850	*LPA*	1.95 (0.24)	0.16	Affymetrix	100		TC
rs174583	11	61,366,326	*FASD1-2-3*	-1.71 (0.19)	0.33	Imputed		0.94	
rs1800562	6	26,201,120	*HFE*	-2.22 (0.39)	0.06	Affymetrix	100		TC
rs2000999	16	70,665,594	*HPR*	2.00 (0.22)	0.21	Imputed		0.91	TC
rs2126259	8	9,222,556	*PP1R3B*	-2.22 (0.29)	0.09	Imputed		1	TC
rs217386	7	44,567,220	*NPC1L1*	-1.17 (0.19)	0.46	Imputed		0.92	
rs2332328	14	23,952,898	*NYNRIN*	1.17 (0.19)	0.50	Imputed		0.5	
rs247616	16	55,547,091	*CETP*	-1.45 (0.20)	0.32	Imputed		0.94	
rs2479409	1	55,277,238	*PCSK9*	2.01 (0.22)	0.37	Affymetrix	100		TC
rs2807834	1	219,037,216	*MOSC1*	-1.09 (0.20)	0.31	Affymetrix	99		TC
rs2902941	20	38,524,928	*MAFB*	-0.98 (0.19)	0.34	Imputed		0.96	
rs2954022	8	126,551,803	*TRIB1*	-1.84 (0.17)	0.44	Imputed		0.98	TC
rs3177928	6	32,520,413	*HLA*	1.83 (0.24)	0.14	Affymetrix	99		TC
rs3757354	6	16,235,386	*MYLIP*	-1.43 (0.21)	0.20	Imputed		0.98	TC
rs3850634	1	62,823,186	*ANGPTL3*	-1.59 (0.19)	0.33	Imputed		1	TC
rs4299376	2	43,926,080	*ABCG5/8*	2.75 (0.20)	0.32	Imputed		0.89	TC
rs4420638	19	50,114,786	*APOE-C1-C2*	7.14 (0.29)	0.16	Illumina	100		TC, HDL-C
rs514230	1	232,925,220	*IRF2BP2*	-1.13 (0.18)	0.50	Imputed		0.98	TC
rs629301	1	109,619,829	*SORT1*	-5.65 (0.21)	0.21	Imputed		0.95	TC
rs649129	9	135,144,125	*ABO*	2.05 (0.21)	0.22	Imputed		0.93	
rs6511720	19	11,063,306	*LDLR*	-6.99 (0.30)	0.13	Illumina	100		TC
rs6882076	5	156,322,875	*TIMD4*	-1.67 (0.19)	0.37	Imputed		1	TC
rs7225700	17	42,746,803	*OSBPL7*	-0.87 (0.18)	0.35	Imputed		1	
rs909802	20	39,370,229	*TOP1*	1.41 (0.17)	0.45	Imputed		1	
rs964184	11	116,154,127	*APOA1–C3–A4–A5*	2.85 (0.27)	0.14	Illumina	100		TC,HDL-C,TG

SNP = Single Nucleotide Polymorphism, CHR = Chromosome, POS = Position, LDL-C = Low-Density Lipoprotein Cholesterol, MAF = Minor Allele Frequency, TC = Total Cholesterol, HDL-C = High-Density Lipoprotein Cholesterol, TG = Triglycerides.

eTable 2: Characteristics of Maternal and Paternal Study Sample at the Concurrent Examination with Adult Offspring LDL-C Measurement

	Mothers	**Fathers**
n	224	283
Age (years)	52 (4)	55 (5)
BMI (kg/m^2)	27.1 (5.7)	28.9 (3.9)
Current smokers	20 (9%)	35 (12%)
LDL-C (mg/dL)	118 (29)	124 (34)
LDL-C > 130 mg/dL	84 (37%)	117 (41%)
Lipid-lowering therapy	15 (7%)	50 (18%)
Loss between pre-pregnancy and concurrent exam due to known cardiovascular disease death	0	0
Mean length of time between offspring birth and concurrent examination (years)	22.1 (3.1)	22.4 (3.2)
Diabetes	6 (3%)	30 (10%)
Hypertension	54 (23%)	115 (40%)

*Values are mean (standard deviation) and proportions presented as n (%), unless otherwise specified. BMI = body mass index, LDL-C = low-density lipoprotein cholesterol. To convert LDL-C from mg/dL to mmol/L, multiply by 0.02586.

eTable 3: Descriptives of all participants of the Framingham Heart Study Offspring Cohort (from which the parents in the current study were drawn from) and Third Generation Cohort (from which the adult offspring in the current study were drawn from)

	FHS Offspring Cohort (exam 1) 1971-1975	**FHS Third Generation Cohort (exam 1) 2002-2005**
n	5124	4095
Age (years)	37 (4)	40 (9)
Sex (female)	2641 (52%)	2183 (53%)
BMI (kg/m^2)	25.1 (4.4)	26.9 (5.6)
Current smokers	2269 (44%)	635 (16%)
LDL-C (mg/dL)	128 (37)	112 (32)
LDL-C > 130 mg/dL	2162 (44%)	1052 (26%)
Lipid-lowering therapy	28 (0.5%)	355 (9%)
HDL-C (mg/dL)	52 (16)	54 (16)
Triglycerides (mg/dL) - median (IQR)	81 (63)	110 (72)
Diabetes	95 (2%)	124 (3%)
Hypertension	991 (19%)	762 (19%)

*Values are mean (standard deviation) and proportions presented as n (%), unless otherwise specified. BMI = body mass index, LDL-C = low-density lipoprotein cholesterol. To convert LDL-C from mg/dL to mmol/L, multiply by 0.02586. Mother-offspring and father-offspring pairs are not mutually exclusive and include 116 adult offspring for which the offspring are represented in both maternal and paternal pairs.

eTable 4: Full model output from successive linear regression models with adult offspring LDL-C (mg/dL) specified as the dependent variable and maternal pre-pregnancy LDL-C (mg/dL) as the independent variable of interest. In order to present model r^2, standard linear regression models and not GEE models are presented. There are slight differences in coefficients for maternal pre-pregnancy LDL-C compared to coefficients reported in table 2 due to differences in the GEE models adjusted for family structure.

Variable	**Model 1**		**Model 2**		**Model 3**		**Model 4**	
n	241		241		216		209	
	beta (SE)	p-value	beta (SE)	p-value	beta (SE)	p-value	beta (SE)	p-value
Intercept	12 (18)	0.5	-14 (21)	0.5	5 (25)	0.8	-48 (31)	0.1
Maternal pre-pregnancy LDL-C (mg/dL)	0.4 (0.06)	<0.0001	0.4 (0.06)	<0.0001	0.4 (0.06)	<0.0001	0.3 (0.06)	<0.0001
Maternal age (yrs)	0.3 (0.4)	0.4	0.5 (0.4)	0.2	0.6 (0.4)	0.2	0.4 (0.4)	0.3
Offspring age (yrs)	2.4 (0.5)	<0.0001	2.0 (0.5)	0.0003	1.7 (0.6)	0.002	2.0 (0.6)	0.0005
Offspring sex (F)	-15 (3)	<0.0001	-12 (4)	0.004	-12 (4)	0.002	-13 (4)	0.0006
Maternal BMI (kg/m^2)	-		0.05 (0.6)	0.9	-1.0 (0.6)	0.1	-0.7 (0.6)	0.3
Maternal smoking (Y)	-		-1.3 (3)	0.7	0.5 (4)	0.9	1.7 (3.5)	0.6
Offspring BMI (kg/m^2)	-		1.2 (0.3)	0.0006	1.4 (0.4)	0.0001	1.2 (0.4)	0.002
Offspring trans fat intake (g/day)	-		-		1.5 (2.5)	0.6	1.2 (2.5)	0.6
Offspring saturated fat intake (g/day)	-		-		-0.4 (0.3)	0.2	-0.4 (0.3)	0.2
Offspring total calorie intake (kcal/d)	-		-		0.006 (0.003)	0.1	0.005 (0.004)	0.2
Offspring physical activity index (METS)	-		-		-0.2 (0.2)	0.4	-0.1 (0.2)	0.6
Offspring LDL-C GRS (sum weighted allele score)	-		-		-		0.7 (0.2)	0.003
Model adjusted r^2	0.26		0.29		0.31		0.33	

*LDL-C = low-density lipoprotein cholesterol, BMI = body mass index, GRS = Genetic risk score, GEE = generalized estimating equation. Dashes (-) indicate that variable was not included in that specific successive regression model.

eTable 5: Full model output from successive logistic regression models with adult offspring LDL-C (>130 mg/dL) specified as the dependent variable and maternal pre-pregnancy LDL-C (>130 mg/dL) as the independent variable of interest. In order to present c-statistics, standard logistic regression models and not GEE models are presented. There are slight differences in coefficients for maternal pre-pregnancy LDL-C compared to coefficients reported in table 2 due to differences in the GEE models adjusted for family structure.

Variable	**Model 1**		**Model 2**		**Model 3**		**Model 4**	
n	241		241		216		209	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Maternal pre-pregnancy LDL-C (>130 mg/dL)	5.0 (2.2, 11)	<0.0001	4.7 (2.0, 11)	0.0004	4.7 (1.8, 12)	0.002	3.8 (1.4, 10)	0.008
Maternal age (yrs)	1.0 (0.9, 1.1)	0.6	1.0 (0.9, 1.1)	0.7	1.0 (0.9, 1.1)	0.6	1.0 (0.9, 1.1)	0.7
Offspring age (yrs)	1.2 (1.1, 1.4)	0.005	1.2 (1.0, 1.3)	0.02	1.2 (1.0, 1.4)	0.03	1.2 (1.0, 1.4)	0.03
Offspring sex (F)	0.3 (0.1, 0.7)	0.003	0.3 (0.1, 0.7)	0.007	0.4 (0.2, 1.0)	0.045	0.3 (0.1, 0.9)	0.03
Maternal BMI (kg/m^2)	-		1.0 (0.9, 1.1)	0.8	0.9 (0.8, 1.1)	0.4	0.9 (0.8, 1.1)	0.5
Maternal smoking (Y)	-		0.6 (0.3, 1.3)	0.2	0.6 (0.3, 1.6)	0.3	0.7 (0.3, 1.7)	0.4
Offspring BMI (kg/m^2)	-		1.1 (1.0, 1.2)	0.04	1.1 (1.0, 1.2)	0.02	1.1 (1.0, 1.2)	0.07
Offspring trans fat intake (g/day)	-		-		1.5 (0.8, 2.6)	0.2	1.4 (0.8, 2.6)	0.3
Offspring saturated fat intake (g/day)	-		-		1.0 (0.9, 1.0)	0.2	1.0 (0.9, 1.0)	0.4
Offspring total calorie intake (kcal/d)	-		-		1.0 (1.0, 1.0)	0.99	1.0 (1.0, 1.0)	0.8
Offspring physical activity index (METS)	-		-		1.0 (1.0, 1.1)	0.4	1.0 (1.0, 1.1)	0.2
Offspring LDL-C GRS (sum weighted allele score)	-		-		-		1.1 (1.0, 1.2)	0.02
Model c-statistic	0.787		0.808		0.815		0.825	

*LDL-C = low-density lipoprotein cholesterol, BMI = body mass index, GRS = Genetic risk score, GEE = generalized estimating equation. Dashes (-) indicate that variable was not included in that specific successive regression model.

eTable 6: Full model output from successive linear regression models with adult offspring LDL-C (mg/dL) specified as the dependent variable and paternal pre-pregnancy LDL-C (mg/dL) as the independent variable of interest. In order to present model r^2, standard linear regression models and not GEE models are presented. There are slight differences in coefficients for paternal pre-pregnancy LDL-C compared to coefficients reported in table 2 due to differences in the GEE models adjusted for family structure.

Variable	**Model 1**		**Model 2**		**Model 3**		**Model 4**	
n	297		297		271		263	
	beta (SE)	p-value	beta (SE)	p-value	beta (SE)	p-value	beta (SE)	p-value
Intercept	61 (20)	0.002	40 (23)	0.08	73 (28)	0.01	3 (33)	0.9
Paternal pre-pregnancy LDL-C (mg/dL)	0.25 (0.05)	<0.0001	0.26 (0.05)	<0.0001	0.25 (0.05)	<0.0001	0.15 (0.05)	0.003
Paternal age (yrs)	-0.1 (0.4)	0.7	-0.1 (0.4)	0.7	-0.2 (0.4)	0.6	-0.02 (0.4)	0.97
Offspring age (yrs)	1.1 (0.5)	0.04	1.1 (0.5)	0.04	0.9 (0.5)	0.1	1.2 (0.5)	0.02
Offspring sex (F)	-12 (3)	0.0006	-8 (3)	0.02	-12 (4)	0.003	-9 (4)	0.02
Paternal BMI (kg/m^2)	-		-1.1 (0.5)	0.04	-1.2 (0.6)	0.04	-1.3 (0.5)	0.01
Paternal smoking (Y)	-		-2 (3)	0.5	-2 (4)	0.003	-3 (3)	0.4
Offspring BMI (kg/m^2)	-		1.7 (0.3)	<0.0001	1.6 (0.4)	<0.0001	1.8 (0.4)	<0.0001
Offspring trans fat intake (g/day)	-		-		-4 (3)	0.1	-3 (3)	0.2
Offspring saturated fat intake (g/day)	-		-		0.5 (0.3)	0.09	0.3 (0.3)	0.3
Offspring total calorie intake (kcal/d)	-		-		-0.003 (0.004)	0.4	-0.001 (0.004)	0.7
Offspring physical activity index (METS)	-		-		-0.3 (0.2)	0.2	-0.3 (0.2)	0.2
Offspring LDL-C GRS (sum weighted allele score)	-		-		-		0.8 (0.2)	0.0004
Model adjusted r^2	0.12		0.18		0.18		0.20	

*LDL-C = low-density lipoprotein cholesterol, BMI = body mass index, GRS = Genetic risk score, GEE = generalized estimating equation. Dashes (-) indicate that variable was not included in that specific successive regression model.

eTable 7: Full model output from successive logistic regression models with adult offspring LDL-C (>130 mg/dL) specified as the dependent variable and paternal pre-pregnancy LDL-C (>130 mg/dL) as the independent variable of interest. In order to present c-statistics, standard logistic regression models and not GEE models are presented. There are slight differences in coefficients for paternal pre-pregnancy LDL-C compared to coefficients reported in table 2 due to differences in the GEE models adjusted for family structure.

Variable	**Model 1**		**Model 2**		**Model 3**		**Model 4**	
n	297		297		271		263	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Paternal pre-pregnancy LDL-C (>130 mg/dL)	1.8 (0.9, 3.6)	0.08	1.9 (0.9, 3.9)	0.07	1.7 (0.8, 3.7)	0.2	1.8 (0.8, 4.0)	0.2
Paternal age (yrs)	1.0 (0.9, 1.1)	0.7	1.0 (0.9, 1.1)	0.6	1.0 (1.0, 1.1)	0.5	1.0 (1.0, 1.1)	0.3
Offspring age (yrs)	1.1 (0.9, 1.2)	0.3	1.1 (0.9, 1.2)	0.4	1.0 (0.9, 1.2)	0.5	1.1 (0.9, 1.2)	0.4
Offspring sex (F)	0.4 (0.2, 0.7)	0.004	0.4 (0.2, 0.9)	0.02	0.4 (0.2, 0.9)	0.02	0.4 (0.2, 1.0)	0.049
Paternal BMI (kg/m^2)	-		0.9 (0.8, 1.1)	0.2	0.9 (0.8, 1.0)	0.1	0.9 (0.7, 1.0)	0.04
Paternal smoking (Y)	-		1.2 (0.6, 2.5)	0.6	1.0 (0.5, 2.3)	0.9	0.9 (0.4, 2.3)	0.9
Offspring BMI (kg/m^2)	-		1.1 (1.1, 1.2)	0.0004	1.1 (1.0, 1.2)	0.004	1.1 (1.0, 1.2)	0.002
Offspring trans fat intake (g/day)	-		-		1.3 (0.7, 2.5)	0.4	1.3 (0.7, 2.6)	0.4
Offspring saturated fat intake (g/day)	-		-		1.0 (0.9, 1.1)	0.9	1.0 (0.9, 1.1)	0.9
Offspring total calorie intake (kcal/d)	-		-		1.0 (1.0, 1.0)	0.2	1.0 (1.0, 1.0)	0.3
Offspring physical activity index (METS)	-		-		1.0 (0.9, 1.0)	0.9	1.0 (0.9, 1.1)	0.9
Offspring LDL-C GRS (sum weighted allele score)	-		-		-		1.1 (1.0, 1.1)	0.01
Model c-statistic	0.667		0.749		0.733		0.778	

*LDL-C = low-density lipoprotein cholesterol, BMI = body mass index, GRS = Genetic risk score, GEE = generalized estimating equation. Dashes (-) indicate that variable was not included in that specific successive regression model.

eTable 8: Distribution of HDL Cholesterol and Triglycerides among Study Sample

		HDL-C		TRIG	
		mg/dL (mean [SD])	**< 40 / 50 mg/dL (men / women) (n [%])**	**mg/dL (median [IQR])**	**> 150 mg/dL (n [%])**
Adult Offspring Assessment		54 (14)	55 (23%)	91 (58)	34 (14%)
Pre-Pregnancy Assessment	**Maternal**	57 (15)	76 (32%)	58 (39)	9 (4%)
	Paternal	44 (12)	113 (38%)	62 (63)	38 (13%)
Concurrent Assessment	**Maternal**	61 (15)	52 (23%)	93 (60)	35 (16%)
	Paternal	45 (12)	102 (36%)	104 (79)	82 (29%)

* To convert HDL-C from mg/dL to mmol/L, multiply by 0.02586. To convert TRIG from mg/dL to mmol/L, multiply by 0.01129.

eTable 9: Relationship Between Parental HDL Cholesterol and Adult Offspring HDL Cholesterol at Pre-Birth (A) and Concurrent Examinations (B)

OUTCOME: Adult Offspring HDL-C	**INDEPENDENT VARIABLE:**							
	Maternal HDL-C				**Paternal HDL-C**			
A) PRE-BIRTH EXAMINATION	**N**	**β**	**(SE)**	**p-value**	**N**	**β**	**(SE)**	**p-value**
M1: adjusted for parental age, offspring age and sex	241	0.17	0.05	0.002	297	0.27	0.07	<0.001
M2: M1 + adjusted for parental BMI and smoking, offspring BMI	241	0.16	0.05	0.002	297	0.27	0.07	<0.001
B) CONCURRENT EXAMINATION	**N**	**β**	**(SE)**	**p-value**	**N**	**β**	**(SE)**	**p-value**
M1: adjusted for parental age, offspring age and sex	224	0.20	0.05	<0.001	283	0.29	0.07	<0.001
M2: M1 + adjusted for parental BMI and smoking, offspring BMI	224	0.21	0.06	<0.001	283	0.27	0.08	<0.001

*HDL-C = high-density lipoprotein cholesterol, BMI = body mass index, β = linear model regression coefficient, SE = standard error, M# = model #. To convert HDL-C from mg/dL to mmol/L, multiply by 0.02586.

eTable 10: Relation Between Adult Offspring Triglycerides and Parental Triglycerides at Pre-Birth (A) and Concurrent Examinations (B)

OUTCOME: Adult Offspring logTG	**INDEPENDENT VARIABLE:**							
	Maternal logTG				**Paternal logTG**			
A) PRE-BIRTH EXAMINATION	**N**	**β**	**(SE)**	**p-value**	**N**	**β**	**(SE)**	**p-value**
M1: adjusted for parental age, offspring age and sex	241	0.20	0.06	0.001	297	0.16	0.07	0.02
M2: M1 + adjusted for parental BMI and smoking, offspring BMI	241	0.16	0.06	0.009	297	0.18	0.07	0.01
B) CONCURRENT EXAMINATION	**N**	**β**	**(SE)**	**p-value**	**N**	**β**	**(SE)**	**p-value**
M1: adjusted for parental age, offspring age and sex	224	0.20	0.07	0.004	283	0.14	0.05	0.004
M2: M1 + adjusted for parental BMI and smoking, offspring BMI	224	0.19	0.07	0.004	283	0.13	0.05	0.005

*logTG = log-transformed triglycerides, BMI = body mass index, β = linear model regression coefficient, SE = standard error, M# = model #

eTable 11: Sensitivity analysis using a correction factor (1.35) to estimate untreated LDL-C among individuals on lipid lowering medication during the parental pre-pregnancy assessment (n=1), parental concurrent assessment (n=15), and adult offspring assessment (n=4) in the fully adjusted GEE models (model 4) with the continuous and dichotomous LDL-C measures.

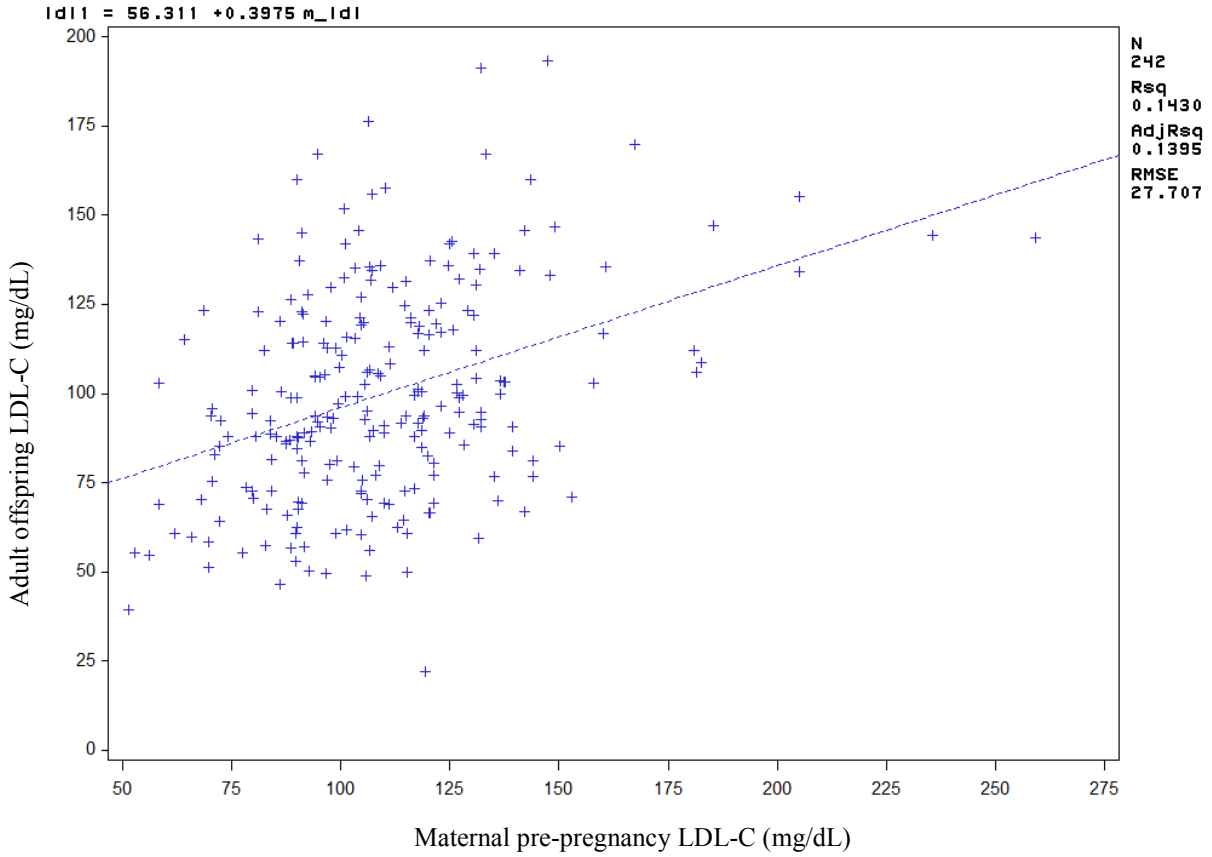
OUTCOME:	**PRE-PREGNANCY EXAM LEVEL:**							
	Maternal LDL-C (mg/dL)				**Paternal LDL-C (mg/dL)**			
Adult Offspring LDL-C (mg/dL)	**N**	**β**	**(SE)**	**p-value**	**N**	**β**	**(SE)**	**p-value**
M4: adjusted for parental age, BMI and smoking, offspring age, sex, BMI, diet, physical activity and LDL-C genetic risk score	209	0.32	(0.05)	<0.0001	267	0.12	(0.06)	0.03
OUTCOME:	**PRE-PREGNANCY EXAM LEVEL:**							
	Elevated Maternal LDL-C (>130 mg/dL)				**Elevated Paternal LDL-C (>130 mg/dL)**			
Elevated Adult Offspring LDL-C (>130 mg/dL)	**N**	**OR**	**(95% CI)**	**p-value**	**N**	**OR**	**(95% CI)**	**p-value**
M4: adjusted for parental age, BMI and smoking, offspring age, sex, BMI, diet, physical activity and LDL-C genetic risk score	209	3.8	(1.5, 9.8)	0.005	267	1.8	(0.8, 4.1)	0.2
OUTCOME:	**CONCURRENT EXAM LEVEL:**							
	Maternal LDL-C (mg/dL)				**Paternal LDL-C (mg/dL)**			
Adult Offspring LDL-C (mg/dL	**N**	**β**	**(SE)**	**p-value**	**N**	**β**	**(SE)**	**p-value**
M4: adjusted for parental age, BMI and smoking, offspring age, sex, BMI, diet, physical activity and LDL-C genetic risk score	196	0.19	(0.06)	0.002	257	0.06	(0.06)	0.4
OUTCOME:	**CONCURRENT EXAM LEVEL:**							
	Elevated Maternal LDL-C (>130 mg/dL)				**Elevated Paternal LDL-C (>130 mg/dL)**			
Elevated Adult Offspring LDL-C (>130 mg/dL)	**N**	**OR**	**(95% CI)**	**p-value**	**N**	**OR**	**(95% CI)**	**p-value**
M4: adjusted for parental age, BMI and smoking, offspring age, sex, BMI, diet, physical activity and LDL-C genetic risk score	196	1.9	(0.8, 4.4)	0.1	257	1.7	(0.7, 3.9)	0.2

*LDL-C = low-density lipoprotein cholesterol, BMI = body mass index, OR = Odds ratio, CI = confidence interval, M# = model #.

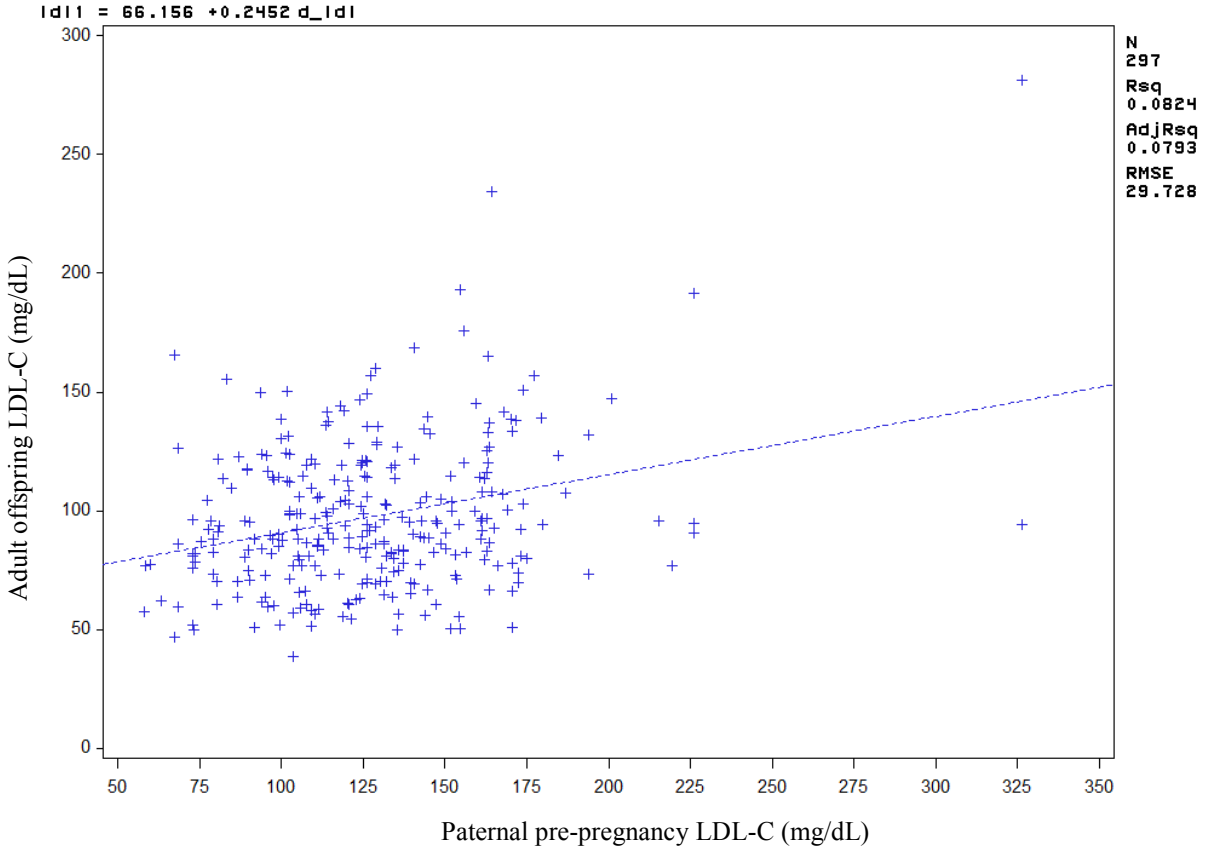
eTable 12: Range of Scenarios for the Effect Size and Distribution of Unmeasured Confounding Required to Explain the Association between Elevated Maternal Pre-Pregnancy LDL-C and Elevated Adult Offspring LDL-C

Effect estimate for unmeasured confounding factor, γ (x-fold increase in elevated adult offspring LDL-C)	None	1.5	2	3	2	2	3
Prevalence of unmeasured confounder, *U*, in elevated maternal pre-pregnancy LDL-C group, P(U=1|α_1,c)	None	70%	70%	70%	60%	80%	80%
Prevalence of unmeasured confounder, U, in non-elevated maternal pre-pregnancy LDL-C group, P(U=1|α_0,c)	None	30%	30%	30%	40%	20%	20%
Bias factor calculation $B_{mult}(c) = \frac{1+(\gamma-1)P(U=1\|a_1,c)}{1+(\gamma-1)P(U=1\|a_0,c)}$	None	[1 + (1.5-1)*(0.7)] / [1 + (1.5-1)*(0.3)]	[1 + (2-1)*(0.7)] / [1 + (2-1)*(0.3)]	[1 + (3-1)*(0.7)] / [1 + (3-1)*(0.3)]	[1 + (2-1)*(0.6)] / [1 + (2-1)*(0.4)]	[1 + (2-1)*(0.8)] / [1 + (2-1)*(0.2)]	[1 + (3-1)*(0.8)] / [1 + (3-1)*(0.2)]
Bias factor	None	1.174	1.308	1.5	1.143	1.5	1.857
Corrected effect estimate for the odds ratio for elevated adult offspring LDL-C among those exposure to elevated maternal pre-pregnancy LDL-C compared to unexposed - OR (95% CI)	3.8 (1.5-9.8)	3.2 (1.3-8.3)	2.9 (1.2-7.5)	2.5 (1.0-6.5)	3.3 (1.3-8.6)	2.5 (1.0-6.5)	2.0 (0.8-5.3)

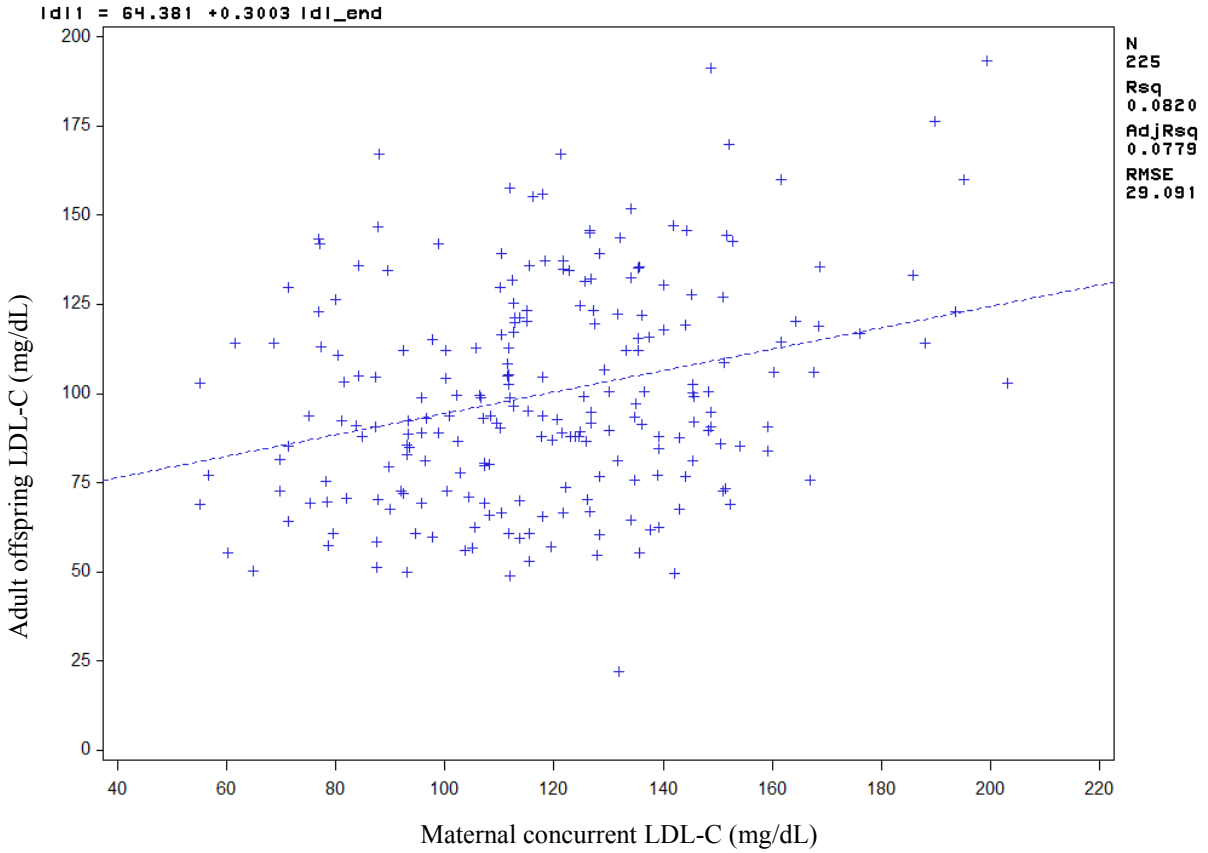
LDL-C = low-density lipoprotein cholesterol, c = measured confounders, U = unmeasured confounder, γ = effect size estimate for unmeasured confounding factor, α_1 = individuals exposed to elevated maternal pre-pregnancy LDL-C, α_1 = individuals unexposed to elevated maternal pre-pregnancy LDL-C, $B_{mult}(c)$ = Bias factor given the measured confounders.[34]



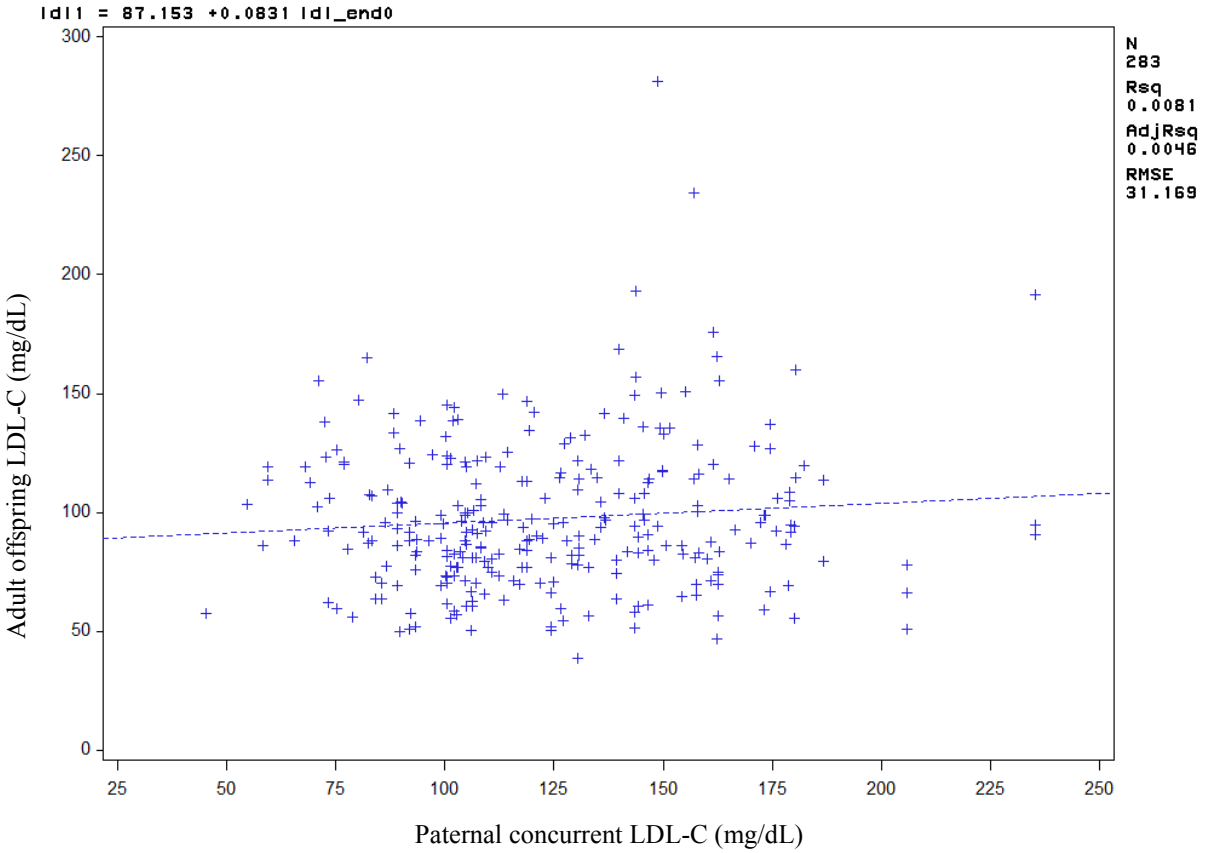
eFigure 1: Scatterplot of maternal pre-pregnancy LDL-C vs adult offspring LDL-C



eFigure 2: Scatterplot of paternal pre-pregnancy LDL-C vs adult offspring LDL-C



eFigure 3: Scatterplot of maternal concurrent LDL-C vs adult offspring LDL-C



eFigure 4: Scatterplot of paternal concurrent LDL-C vs adult offspring LDL-C