Genetic Variation in Immune Response and Vitamin D Metabolism and Developmental Origins of Cardiometabolic Risk

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Abstract

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The developmental origins of cardiovascular and metabolic risk (CMR) may involve interactions of genetic variants, the intrauterine environment, and life course exposures. While accumulating evidence supports the roles of the immune system and vitamin D metabolism in CMR, the role of immune- and vitamin D-related candidate gene variants in the developmental origins of CMR is not well-described. We conducted a candidate single nucleotide polymorphism (SNP) study among mother-offspring dyads representing a subset of the Jerusalem Perinatal Study birth cohort. We selected 122 SNPs in 51 genes characterizing immune or vitamin D-related genes and imprinted regions. We investigated nine CMR-related phenotypes measured in adult offspring: cytomegalovirus antibody titer (CMV IgG), 25-hydroxyvitamin D (25[OH]D) concentration, body mass index (BMI), fasting glucose, HDL cholesterol, LDL cholesterol, triglycerides, systolic blood pressure (BP), and diastolic BP. We used weighted linear regression with robust variance estimates to examine associations of 1) offspring genotype, 2) maternal

genotype, and 3) maternal-offspring genotype interactions with adult offspring CMR. We also evaluated genotype-CMV IgG and genotype-25[OH]D interactions on CMR. Multiple testing adjustments were performed using the Benjamini-Hochberg False Discovery Rate. The offspring minor allele of rs10894157 in CNTN5 was associated with BMI (β = -1.35, SE = 0.30, p = 0.00002). Two offspring SNPs in SLC2A1, rs841858 (β = -7.33, SE = 2.10, p = 0.0005) and rs3820548 (β = -5.84, SE = 1.75, p = 0.0009) were associated with lower LDL cholesterol, and rs841858 was also associated with lower natural-log transformed triglycerides (β = -0.12, SE 0.03, p = 0.0004). We found interactions between offspring rs3771170 in IL-18R1 and 25[OH]D on LDL cholesterol (p = 0.0001), and offspring rs1950902 in MTHFD1 and 25[OH]D on systolic blood pressure (p = 0.0004). We also found evidence for interactions at maternal rs4987853 in BCL2 with offspring CMV IgG on offspring HDL (p = 0.0003), maternal rs4851522 in IL-1R2 with offspring 25[OH]D on offspring LDL (p < 0.0001), and maternal rs13143866 in IL-21 with offspring 25[OH]D on offspring HDL (p = 0.0002). We found evidence of maternal-offspring genotype interactions at a SNP in MTRR on diastolic and systolic BP, and MTHFD1 on triglycerides, though these latter interactions were not statistically significant after taking into account multiple comparisons. If replicated in future studies, our findings provide evidence that maternal and offspring immune- and vitamin D metabolism- related genetic variants, and their interactions, may play a role in the developmental origins of offspring CMR. Further research in family-based genetic studies with long-term follow-up will enhance our understanding of early life origins of CMR.

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DEDICATION

Thanks, Mom and Dad.

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Chapter 1: Overview: developmental origins, immune system and vitamin D metabolism, and cardiometabolic risk

Developmental origins

The theory of the developmental origins of health and disease is rooted in the idea that the developing fetus prepares itself for the outside environment based on cues received in the intrauterine environment (1). This hypothesis has been most commonly applied to cardiometabolic risk factors (CMR) in conjunction with the idea of a "thrifty" phenotype, wherein there exists a mismatch between the nutritional quality of the intrauterine environment and that encountered later in life (2, 3). Natural selection for genetic variation that favors survival during adverse environmental exposures, such as infection or famine, may contribute to this mismatch when the post-natal environment lacks stressors (4). In addition, maternal genetic (5-9) variation and maternal-offspring genotype interactions (10-14) may modify the intrauterine environment and influence the early life origins of offspring life course disease. However, substantial gaps in the literature remain.

Developmental origins of cardiometabolic risk (CMR) and immune function

Interrelationships between the immune system and CMR development are well-described in the literature. Mature adipocytes secrete pro-inflammatory cytokines, such as IL-6, which act to regulate lipid and glucose homeostasis (15). In obese individuals, expression of innate immune genes TLR2 and TLR4 is increased and correlated with increased TNF-α and IL-6 (16). Animal studies demonstrated that pro-inflammatory cytokines influence the development of hypertension (17, 18), and that the presence of T cells is required for the development of angiotensin II-induced hypertension (19, 20). Prospective human studies have implicated inflammatory biomarkers in hypertension as well (21, 22). Studies of pro-inflammatory cytokines and various measures of dysregulated glucose metabolism have had mostly positive associations, though findings were inconsistent and inter-study comparison is difficult because of differences in study population baseline characteristics, study designs, exposure choice, and outcome assessment (23-26). Moreover, a growing body of evidence supports the hypothesis that the developing immune system responds to the intrauterine environment and influences CMR throughout the life course. Immune system development begins in utero and is therefore vulnerable to adverse conditions during

pregnancy (27). Insults to the developing immune system during pregnancy have been associated with offspring atopy, asthma, and respiratory disease (28-32).

Consequences of maternal infection and activation of the immune system can also be seen in offspring outcomes. Investigators have demonstrated in a mouse model that intrauterine growth restriction, infiltration of uteroplacental tissue by cellular innate immune effectors, and elevated maternal serum proinflammatory cytokines following induced bacterial cystitis (33). Maternal infections may contribute to nearly 20-25% of preterm births (34). Prenatal exposure to the 1918 influenza pandemic has been associated with excess incident cardiovascular disease between the ages of 60-82 years relative to those born shortly before or after this exposure window (35). Variation in genes related to immune response, such as IL-10 (36, 37), TNFA (38), and IL-1RN (38), have also been implicated in response to viral infection. Therefore, offspring and maternal genetic variants in immune-related pathways may contribute to the developmental origins of CMR in the offspring.

Cytomegalovirus (CMV) antibody titer as a measure of immune response and its relationship with CMR

Several investigations have been conducted to investigate CMV antibody titer as a biomarker of immune response. Studies have found an inverse association with CMV antibody titer and CD4+ T-cells (39, 40), and positive association with CMV antibody titer and increased numbers of CD8+ T-cells (39). A study of adolescents in the UK found lower circulating naïve T cells and increased CD28- memory T cells in CMV seropositive individuals compared to individuals who were seronegative (41). Among a subset of Multi-Ethnic Study of Atherosclerosis (MESA) participants, a cross-sectional relationship between CMV IgG and lower concentrations of naïve T cells, but higher concentrations of memory T cells, has been described (42), along with a bias towards Th1 CD4+ T cells found in otherwise healthy, seropositive participants with increasing CMV IgG (43).

Higher CMV antibody titers have also been associated with pro-inflammatory cytokines TNFA (44, 45), IL-6 (44, 46), INFG (40, 45), CRP (46), fibrinogen (46), and lower IL-4 (40). The overall picture is one in which increased CMV antibody titer is associated with pro-inflammatory cytokines, fewer naïve T-cells available to respond to new infections, and more memory T-cells devoted to suppression of latent CMV.

A negative CMV IgG test is taken to mean that an individual has never been infected with CMV, or that that individual is unable to produce an adequate antibody response due to immunosuppression. In a study of randomly selected Framingham Heart Study Offspring participants with serial measurements of CMV IgG at approximately 5-year intervals, there was a nonsignificant rise in CMV IgG over the 20 years from first to last blood draw (47). A study of new infection among initially seronegative adolescents found CMV IgG increased until nine weeks after the initial infection before stabilizing and remaining at a relatively constant level for the remaining 48 weeks of the study (48). In sum, CMV IgG is a relatively stable marker of immune senescence.

CMV seropositivity has been linked to disease where inflammation plays a role, including CMR development (49-59). For example, among participants of the Cardiovascular Risk in Young Finns Study, high versus low CMV antibody titers were associated with blood pressure in men, but not women (55). Experimental and mouse studies have shown that CMV infection is sufficient to increase blood pressure via induction of renin and angiotensin II, and that it acts synergistically with a high cholesterol diet, but not in isolation, to promote atherosclerosis (60, 61). Findings from studies in humans are varied. There were inconsistent associations of various measures of CMV infection with hypertension (55, 62-64) and a possibility of promoting glucose dysregulation in the elderly (65, 66). However, other studies have failed to find similar associations (67, 68). The recognition of CMV as a possible part of the etiology of CMR is growing, and this dissertation is an opportunity to examine relationships between immune response-related genetic variants and CMR among young adults in the context of the developmental origins of CMR.

Vitamin D, immune function, and the developmental origins of CMR

Vitamin D has widespread effects throughout the human body, as evidenced by the presence of vitamin D receptors in almost all tissue types (69) and vitamin D response elements in hundreds of genes (70-72). Vitamin D maintains balance between cell-mediated Th1 immune response and antibody-mediated Th2 immune response, with vitamin D deficiency associated with both autoimmune and atopic diseases (73, 74). Low circulating vitamin D concentrations at baseline have been associated with

incident adverse cardiovascular events in several prospective studies (75, 76), though randomized trials of supplementation have yielded inconsistent results with respect to CMR (77-85).

The role that vitamin D plays in fetal development and the developmental origins of offspring health and disease is not yet fully understood. Research into perinatal outcomes has yielded associations between inadequate maternal 25-hydroxyvitamin D (25[OH]D) concentrations and preeclampsia (86), gestational diabetes (87), and offspring birthweight (88). In studies of young children, low maternal 25[OH]D concentrations during pregnancy have been associated with reduced bone density (89) and atopy (90, 91); low cord blood 25[OH]D has been associated with various respiratory and atopic outcomes (92), and maternal self-report of low vitamin D intake during pregnancy has been associated with asthma (93). Maternal genotype at vitamin D metabolism genes has also been studied in conjunction with offspring outcomes. For example, a SNP in the maternal Vitamin D Receptor (VDR) gene has been associated with infant birthweight among non-Hispanic black mothers, but not white mothers (94), whereas another study found an association between maternal VDR and small for gestational age infants in both black and white women (88). Furthermore, Morley, et al., found that the association between maternal 25[OH]D concentrations during pregnancy and offspring birth size was modified by maternal VDR genotype (95). Collectively, the evidence suggests that maternal 25[OH]D concentrations, with some influence of vitamin D metabolism genotype, can affect the intrauterine environment in ways that have lasting consequences for the offspring. However, little is known regarding the role of vitamin D-related maternal and offspring genetic variants in adult offspring CMR.

Project Description

In this project, we identified candidate SNPs (Supplementary Table S1) belonging to immune- and vitamin D- related genes and examined associations between offspring and maternal variants in these SNPs and CMR among otherwise healthy adult offspring. We also explored the potential for effect modification of CMV-CMR and 25[OH]D-CMR associations by genotypes of candidate SNPs. Analyses were conducted among a subset of participants in the Jerusalem Perinatal Study (JPS). Offspring and their mothers, originally recruited between 1974 and1976, were re-contacted when the offspring were, on

average, 32 years old. Maternal and offspring participants gave blood samples, interviews, and underwent physical examinations.

Chapter 2 describes a study of offspring candidate SNPs and CMR using 109 candidate SNPs in or around 42 genes involved in immune response or vitamin D metabolism. We analyzed genotype-phenotype associations for nine outcomes using linear regression with sampling weights and robust variance estimates. Additional analyses were conducted to identify potential effect modification of CMV IgG-CMR or 25[OH]D-CMR associations by genotype. Exploratory analysis was conducted to identify the potential for mediation of statistically significant SNP-CMR associations by either CMV IgG or 25[OH]D concentrations.

Chapter 3 describes a study that investigated associations of maternal genotypes of selected candidate SNPs with offspring adult CMR. We investigated 111 candidate maternal SNPs characterizing variations in genes involved in innate immune function and vitamin D metabolism. We analyzed maternal genotype-offspring phenotype associations for nine outcomes using linear regression with sampling weights and robust variance estimates. Exploratory analysis was conducted to identify the potential for effect modification of offspring CMV IgG-offspring CMR or offspring 25[OH]D-offspring CMR associations by maternal genotype.

In Chapter 4, we examined the potential for mother-offspring genotype interactions at our candidate SNPs in relation to offspring CMR. We selected 97 SNPs in 42 genes in immune function, vitamin D metabolism, and imprinted regions for this analysis. We analyzed maternal genotype-offspring genotype interactions on nine offspring CMR outcomes using weighted linear regression models with robust variance estimates that contained terms for maternal genotype and offspring genotype, assuming an additive genetic model, and a simple multiplicative interaction term.

Finally, in Chapter 5, we present an overall summary of study findings and a discussion of strengths and limitations of the study, along with implications for future research.

Chapter 2: Candidate immune and vitamin D-related genetic variants and adult cardiometabolic risk: the Jerusalem Perinatal Study

Abstract

Molecular pathways related to activation of the immune system and vitamin D concentrations have been associated with cardiometabolic risk factors (CMR). However, the contribution of genetic variation in these pathways to CMR development is not well-characterized. Among participants of a population-based birth cohort study, the Jerusalem Perinatal Study, who were examined and interviewed at 32 years of age, we investigated associations of immune- and vitamin D–associated candidate single nucleotide polymorphisms (SNPs, n=109) with cytomegalovirus antibody titers (CMV IgG), 25-hydroxyvitamin D concentrations (25[OH]D, body mass index (BMI), fasting glucose, lipids, and blood pressure. We also examined effect modification of CMV IgG-CMR and 25[OH]D-CMR associations by genotype. The minor allele of rs10894157 in CNTN5 was associated with lower BMI (β = -1.31, SE = 0.31, p = 0.00002). This association was not mediated by CMV IgG nor 25[OH]D concentrations. Two SNPs in SLC2A1, rs841858 (β = -7.33, SE = 2.10, p = 0.0005) and rs3820548 (β = -5.84, SE = 1.75, p = 0.0009) were associated with lower LDL cholesterol, and rs841858 was also associated with lower natural-log transformed triglycerides (β = -0.12, SE 0.03, p = 0.0004). In the interaction analyses, the association between genotype at rs3771170 in IL-18R1 and LDL cholesterol was positive (β = 0.3.49, SE = 2.49, p = 0.161) among participants with 25[OH]D concentrations of 15.3ng/mL and negative (β = -7.00, SE = 2.13, p = 0.001) among participants with 25[OH]D concentrations of 27.4ng/mL (p for interaction = 0.0001). The association between rs1950902 in MTHFD1 and systolic blood pressure was modified by 25[OH]D concentration as well (p for interaction = 0.0004): among participants with 25[OH]D concentrations of 15.3ng/mL, the association was positive (β = 1.49, SE = 1.10, p = 0.177), while among participants with 25[OH]D concentrations of 27.4ng/mL, the association was negative (β = -1.95, SE = 1.01, p = 0.054). In this candidate SNP study, we found evidence for a novel association between a CNTN5 SNP and BMI. We also identified possible effect modification of 25[OH]D and LDL cholesterol associations by a SNP in IL-18R1. Our findings, if replicated, will inform molecular and genetic investigations of the pathogenesis of dyslipidemia and obesity.

Introduction

Systemic inflammation and activation of the immune system and low vitamin D concentrations have been implicated in the pathogenesis of cardiometabolic risk (CMR). Animal models provide strong evidence for the role of the immune system in essential hypertension (19, 20, 96). Work in the Atherosclerosis Risk In Communities cohort demonstrated that inflammation predicts Type 2 Diabetes independently of obesity and other risk factors (97) and baseline levels of C-Reactive Protein (CRP) were independently associated with the risk of incident Type 2 diabetes in the Women's Health Initiative (98). Circulating baseline CRP, but not variation within the CRP gene, has been associated with incident hypertension (99). Cytomegalovirus infection and cytomegalovirus antibody titers have been associated with CMR, including hypertension and glucose dysregulation (60-66). While aspects of lipid metabolism have been associated with genetic variants in several immune genes (100, 101), associations of immune genetic variations with other CMR have been less clear (65, 99, 102, 103).

In addition, associations between vitamin D and CMR have been reported, though findings were inconsistent. Baseline 25[OH]D has been associated with incident hypertension (82, 83, 104), though the associations do not always reach statistical significance (105) and other studies report no association (106). In humans, individuals with essential hypertension have an altered cytokine profile compared to healthy individuals (107). Meta-analysis has found lower risk of incident hypertension (108), and incident Type 2 Diabetes (108) with increasing baseline 25[OH]D, but trials of supplementation have generally found no change in blood pressure (80, 85, 109, 110), or glucose metabolism (77, 111) Cross-sectional inverse associations of vitamin D concentrations with triglycerides (112-114) and LDL (113, 114) have been reported, but there is a lack of strong association with incident hypercholesterolemia (113) and null findings in meta-analysis of supplementation trials (115).

Besides observed inconsistencies, genetic variations characterized by single nucleotide polymorphisms (SNPs), particularly those related to the innate immune system and vitamin D metabolism, remain underexplored in research on CMR development (36-38, 116). If the immunomodulating effects of CMV and/or vitamin D are indeed part of the causal pathway of cardiometabolic risk, genetic determinants of immune response may exert pleiotropic effects on CMR; alternatively, the genetic associations may be mediated by effects on CMV or vitamin D throughout the life course.

In this study of young adults, we investigated the associations of 109 candidate SNPs in or around 42 genes with CMV IgG and 25[OH]D concentration, and with multiple CMR quantitative traits (fasting glucose, body mass index (BMI), LDL, HDL, triglycerides, systolic blood pressure (BP), diastolic BP). We examined interactions of SNPs with CMV IgG and 25[OH]D concentrations on CMR. Finally, we also examined the potential for mediation of SNP-CMR associations by CMV IgG or 25[OH]D concentration.

Methods

Study setting and study population

This study is based on data collected as part of the Jerusalem Perinatal Study, including prenatal, perinatal, and post-natal data on all births (n=17,003) to residents of west Jerusalem during 1974-1976. The Jerusalem Perinatal Family Follow-up Study (JPS-1) recruited 1,500 mother-offspring dyads from the original JPS cohort, and collected additional data and biological samples from these dyads. Participants were recruited using a stratified sampling of term, singleton, live births with oversampling of low (≤2500g) and high (≥4000g) birthweight offspring and mothers with a pre-pregnancy BMI ≥ 27 kg/m^2. Mother-offspring dyads with offspring who had congenital disorders at birth or were preterm (<36 weeks gestation) were excluded.

Information on maternal and birth characteristics, such as maternal lifestyle factors during pregnancy or birthweight of offspring, were collected from a combination of maternity ward logbooks, birth certificates, and maternal interviews conducted on the first or second day postpartum. (117). At the time of data collection for JPS-1, the mean age of offspring was 32 years (range 31-33 years). Offspring who self-reported taking medication for high blood pressure (n = 22), high cholesterol (n = 20), or diabetes (n = 22) were excluded, as well as individuals with a fasting time that was missing (n = 234) or fewer than eight hours (n = 97). A maximum of 1,194 mother-offspring dyads were available for analysis after these exclusions. Study procedures were approved by the Institutional Review Boards of the University of Washington, in Seattle (USA), and the Hadassah-Hebrew University Medical Center in Jerusalem (Israel). All participants provided informed consent.

Data Collection

Data collection procedures have been published previously (117). To summarize, data on anthropomorphic measurements, lifestyle, and sociodemographic characteristics were collected during telephone interviews conducted by trained interviewers and physical exams conducted between 2007 and 2009. BMI was calculated as weight (kg) divided by squared height (m^2). Blood pressure was measured as the average of three consecutive measurements performed after five minutes of sitting (Omron M7 automated sphygmomanometer). Fasting blood samples were taken using standard procedures. Plasma

glucose, LDL, HDL, and triglycerides were measured on the VITROS 5,1FS Chemistry System (Ortho Clinical Diagnostics, Raritan, NJ). Plasma vitamin D (25[OH]D) was measured using liquid chromatography tandem mass spectroscopy at the Nutrition and Obesity Research Center, University of Washington (Seattle, WA). The sum of 25[OH]D_2 and 25[OH]D_3 was used in analyses to represent total plasma vitamin D concentration. CMV antibody titers were determined by enzyme immunoassay technology using the Immunosimplicity® Is-CMV IgG Test Kit (Diamedix Corp. Miami, Florida). Antibody titers are reported as EU/mL, with a value ≥ 10.0EU/mL considered positive for anti-CMV IgG. CMV assays were performed at the University of Vermont Laboratory for Clinical Biochemistry Research (Burlington, VT). All covariates, except for maternal smoking during pregnancy and offspring birthweight, were measured in JPS-1 offspring at the average age of 32 years.

Gene/SNP selection and genotyping

Genes and SNPs were selected based on previously published and unpublished work investigating their associations with phenotypes of interest: immune functions, vitamin D metabolism, BMI, fasting glucose, LDL cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, and diastolic blood pressure. A detailed list of SNPs is shown in Supplementary Table S1.

Extraction and amplification of DNA from offspring blood samples was performed using standard methods. Genotyping was performed using the Illumina, Inc., BeadArray™ or the TaqmanOpenArray assay systems at the University of California, San Francisco. SNPs were required to be in Hardy-Weinberg Equilibirum ($p > 0.000439$) for inclusion. 109 SNPs were included for analysis.

Statistical Analyses

We examined offspring characteristics in the entire study cohort. Means and standard deviations were calculated for continuous variables; numbers and percentages for categorical variables. We used weighted multiple linear regression models to examine associations of genotypes, modeled additively, with CMR (BMI, fasting glucose, triglycerides, LDL, HDL, systolic BP, and diastolic BP), CMV IgG, and 25[OH]D. Sampling weights were based on maternal pre-pregnancy BMI and offspring birthweight recruitment strata and non-response rates. Ninety-five percent confidence intervals of beta estimates and

p-values were calculated using robust standard error estimates. Triglyceride concentration was natural log-transformed to improve model fit, due to the extreme right-skewness of the distribution of triglycerides. CMV IgG was square root-transformed to improve model fit due to the extreme right-skewness of CMV IgG and the large proportion of participants (21%) with a CMV IgG titer of zero EU/mL, making a log transformation impractical.

Our models were adjusted for season of blood draw (December-February, March-May, June-August, September-November) and additional covariates for smoking status (current, former, and never), alcohol abstinence (yes/no), intense physical activity participation (yes/no), employment status (yes/no), religiosity (Ultra-Orthodox, Religious, and Traditionalist/Secular/Other), socio-economic status (low, medium, and high), birthweight, maternal smoking during pregnancy (yes/no), sex, and grandparent country of origin (Yemen, Kurdistan, Morocco, Iran, Iraq, Other African, Other Asian, and Ashkenazi, using Israel as baseline), and BMI unless BMI was the outcome of interest.

We used the Benjamini-Hochberg step-up procedure to adjust for multiple comparisons (118) in our analyses of genotype-CMR associations. Briefly, this procedure controls the False Discovery Rate (FDR) at our chosen level of 0.05. P-values are ranked from smallest to largest and compared to $0.05*k/m$, where m is the total number of tests performed and k is the rank of the test. All tests with k smaller than the largest k such that $p_{(k)} \leq 0.05*k/m$ are rejected. Each of the nine outcomes was evaluated separately.

We examined the potential for effect modification of the relationship of CMV IgG and 25[OH]D with CMR by genotypes of all 109 SNPs. Genotype was coded as having 0, 1, or 2 minor alleles. CMV IgG and 25[OH]D were continuous variables. Besides the outcome variables, the models included main effects terms for both genotype and either CMV IgG or 25[OH]D, the multiplicative interaction term, and the same adjustment covariates used in the main effects models. Statistical significance of the interaction term was determined using the significance level of 0.05.

In an exploratory analyses, we performed the Sobel-Goodman mediation test to examine whether CMV IgG or 25[OH]D concentrations, separately, mediate associations between genotype(s) and CMR (119). Only those SNP-CMR associations that met our criteria for statistical significance in the main

effects analyses, after adjustment for multiple testing, were carried forward for these mediation analyses. A significance level of 0.05 was used to evaluate mediation.

In sensitivity analyses, in the case where multiple SNPs within the same gene were found among our top hits in association with one or more CMR, we examined associations of genotype combinations with the relevant CMR. Finally, to assess whether any of our candidate SNPs were associated with CMV infection rather than immune response, we conducted an analysis of our candidate SNPs and CMV seropositivity (≥10EU/mL) as a binary outcome variable.

Statistical analyses were performed using Stata v10.0 (StataCorp, College Station, TX).

Results

Selected study participant characteristics are presented in Table 2.1. Mean 25[OH]D concentrations and CMV IgG were 21.8ng/mL (SD = 9.0) and 179.9EU/mL (SD = 242.7), respectively. The average BMI of participants was 26.2kg/m^2 (SD = 5.1). Funnel plots (Supplementary Figures 2.1 – 2.9) summarize the results of genotype-CMR associations. Top hits (unadjusted p-value < 0.01) from analyses evaluating SNP-CMR associations are presented in Table 2.2. Four SNPs met our criteria for statistical significance using the Benjamini-Hochberg correction for multiple testing. A copy of the minor allele of the top hit, rs10894157 (Minor Allele Frequency (MAF) =24%), a SNP in the CNTN5 gene, was associated with an average 1.31kg/m^2 decrease in BMI (SE = 0.31, p = 0.00002). A SNP (rs841858) in the SCL2A1 gene was associated with decreases in both the natural logarithm of triglycerides (β = -0.12, SE = 0.03, p = 0.0004) and LDL (β = -7.33, SE = 2.10, p = 0.0005), while another SCL2A1 SNP, rs3820548, was associated with a decrease in LDL (β = -5.84, SE = 1.75, p = 0.0009).

Table 2.1. Study Participant Characteristics.

			Total	
			N = 1194*	
CMV IgG, EU/mL (mean, sd)			179.9	242.7
Total D, ng/mL (mean, sd)			21.8	9.0
BMI, kg/m^2 (mean, sd)			26.2	5.1
Glucose, mg/dL (mean, sd)			80.0	15.3
Triglycerides, mg/dL (mean, sd)			105.1	72.9
LDL, mg/dL (mean, sd)			112.3	28.8
HDL, mg/dL (mean, sd)			50.1	14.6
Systolic BP, mmHg (mean, sd)			105.7	11.9
Diastolic BP, mmHg (mean, sd)			71.2	8.1
Birthweight, grams (mean, sd)			3409.1	618.7
Smoking Status (n, %)	non		691	59.4
	former		154	13.2
	current		318	27.3
Alcohol Abstain (n, %)	yes		544	46.6
Intense Physical Activity (n, %)	yes		343	29.4
Employed (n, %)	yes		963	83.7
Orthodoxy (n, %)	Ultra-orthodox		226	18.9
	Religious		242	20.3
Traditionalist, Secular, or Other			726	60.8
Season (n, %)	winter		332	27.8
	spring		191	16.0
	summer		340	28.5
	autumn		331	27.7
SES (n, %)	low		236	22.7
	medium		369	35.6
	high		592	41.8
maternal smoking (n, %)	yes		181	17.4

s.d. = Standard Deviation

*May not sum to total due to missing data

†Defined as exercise that brings about labored breathing, increased pulse rate, and sweating, lasting for at least twenty minutes.

Table 2.2. Top hits (unadjusted p < 0.01) for candidate SNP analysis of CMR outcomes.

Outcome	Gene	SNP	Coefficient	Robust SE	p-value
Square root (CMV IgG)	MTRR	rs1801394	1.56	0.55	0.0048
25[OH]D (ng/mL)	CYP2R1	rs12794714	-1.46	0.47	0.0018
25[OH]D (ng/mL)	IL-21	rs2055979	-1.20	0.49	0.0155
25[OH]D (ng/mL)	GC	rs2282679	-1.60	0.52	0.0023
BMI (kg/m^2)	CNTN5	rs10894157	-1.31	0.31	0.00002*
BMI (kg/m^2)	RXRA	rs1045570	-0.95	0.33	0.0046
Glucose (mg/dL)	TNFRSF1A	rs4149578	2.42	0.96	0.0121
Glucose (mg/dL)	IL-6R	rs4075015	1.77	0.66	0.0079
HDL (mg/dL)	MTRR	rs10380	4.13	1.31	0.0017
HDL (mg/dL)	RXRA	rs1045570	2.71	0.98	0.0058
LDL (mg/dL)	SLC2A1	rs841858	-7.33	2.10	0.0005*
LDL (mg/dL)	SLC2A1	rs3820548	-5.84	1.75	0.0009*
ln(Triglycerides)	SLC2A1	rs841858	-0.12	0.03	0.0004*
ln(Triglycerides)	SLC2A1	rs3820546	0.09	0.03	0.0036
Systolic BP (mmHg)	IL-6	rs1800797	-1.84	0.69	0.0079
Diastolic BP (mmHg)	TLR4	rs11536889	-2.03	0.66	0.0023

*Met Benjamini-Hochberg multiple testing correction for statistical significance.

Table 2.3 presents the results for the SNP-CMV IgG interaction analyses. Forty-five SNPs were associated with changes in the relationship between CMV IgG and CMR at the exploratory significance level of 0.05. In one specific example, the difference in LDL cholesterol associated with each additional minor allele of TNFRSF1A SNP rs4149578 among participants who had CMV IgG titers of 26.1EU/mL was 0.23 (SE = 2.72, p = 0.934), 4.70 (SE = 2.88, p = 0.104) among participants who had CMV IgG titers of 103.8EU/mL, and 9.26 (SE = 3.68, p = 0.012) among participants who had CMV IgG titers of 237.8EU/mL.

Table 2.4 presents the results for the SNP-25[OH]D interaction analyses. Sixty-one SNPs were associated with changes in the relationship between 25[OH]D and CMR at the exploratory significance level of 0.05. In one specific example, among individuals deficient for vitamin D (25[OH]D concentration of 15.3ng/mL), each minor allele of rs3771170 was associated with a 3.49mg/dL increase in LDL cholesterol (SE = 2.49, p = 0.161). At a 25[OH]D concentration of 21.3, each minor allele was associated with a 1.71mg/dL decrease in LDL cholesterol (SE = 1.90, p = 0.367), and at a 25[OH]D concentration of 27.4,

with a 7.00mg/dL decrease in LDL cholesterol (SE = 2.13, p = 0.001, p for interaction = 0.0001). (Figure 2.1). While we did not specify *a priori* a multiple testing correction for this analysis, this interaction and the interaction between rs1950902 in MTHFD1L and 25[OH]D on systolic BP (Figure 2.2) met the Benjamini-Hochberg FDR correction used in the earlier analysis of genotype main effects.

In sensitivity analysis, we examined the associations of candidate SNPs and CMV seropositivity and found that the minor allele of CD14 rs5744455 was associated with slightly increased odds of CMV seropositivity (OR = 1.04, SE = 0.26, p = 0.0001, Supplementary Table S2.4).

Since two SNPs in SLC2A1 were among our top hits in both LDL cholesterol and triglycerides (Table 2.2), we performed analyses of genotype combinations at the two relevant loci and LDL and triglycerides, respectively. The magnitude of the association between minor alleles in SLC2A1 and LDL cholesterol grew larger with an increasing number of minor alleles, ranging from -2.89 (SE = 3.28, p = 0.3790) for one minor allele in rs3820548 compared to no minor alleles in either SNP, to -15.61 (SE = 6.54, p = 0.0170) when both SNPs were homozygous for the minor alleles (Supplementary Table 2.5). In the analysis of rs841858 and rs3820548 with triglycerides, no particular minor allele combination was significantly associated with triglycerides (Supplementary Table S2.6).

Table 2.3. Top hit (p < 0.05) offspring SNP X offspring CMV IgG interactions in association with offspring CMR outcomes.

			SNP association			CMV IgG association			SNP X CMV IgG Interaction		
Outcome	Gene	SNP	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
25[OH]D (ng/mL)	CUBN	rs1801222	2.83	1.17	0.0154	0.15	0.05	0.0061	-0.27	0.08	0.0014
25[OH]D (ng/mL)	BCL2	rs4987853	1.83	0.82	0.0251	0.19	0.06	0.0007	-0.18	0.06	0.0015
25[OH]D (ng/mL)	BCL2	rs1564483	-2.35	0.96	0.0143	0.00	0.05	0.9338	0.22	0.09	0.0127
25[OH]D (ng/mL)	MTHFD1L	rs9478878	1.36	0.78	0.0809	0.16	0.06	0.0048	-0.14	0.06	0.0274
25[OH]D (ng/mL)	MTHFD1L	rs572522	-1.34	0.67	0.0455	-0.02	0.06	0.7427	0.11	0.05	0.0306
25[OH]D (ng/mL)	ABCA1	rs1883025	-1.40	0.93	0.1331	-0.02	0.06	0.7655	0.14	0.07	0.0361
25[OH]D (ng/mL)	LTA	rs1041981	1.36	1.00	0.1745	0.14	0.06	0.0218	-0.15	0.08	0.0425
BMI (kg/m^2)	SLC2A1	rs3820546	-0.82	0.41	0.0448	-0.09	0.04	0.0235	0.11	0.03	0.0012
BMI (kg/m^2)	LRP2	rs4667591	-1.28	0.54	0.0183	-0.06	0.03	0.0517	0.14	0.04	0.0019
BMI (kg/m^2)	CD14	rs2563298	0.32	0.46	0.4931	0.09	0.04	0.0219	-0.11	0.04	0.0030
BMI (kg/m^2)	SLC2A1	rs3820548	1.04	0.47	0.0263	0.06	0.03	0.0630	-0.09	0.04	0.0149
BMI (kg/m^2)	IL-18R1	rs7558013	1.36	0.63	0.0320	0.04	0.03	0.1405	-0.13	0.05	0.0154
BMI (kg/m^2)	FCGR2A	rs1801274	-0.55	0.48	0.2498	-0.09	0.04	0.0221	0.08	0.03	0.0187
BMI (kg/m^2)	IL-1A	rs3783546	0.80	0.51	0.1145	0.06	0.03	0.0671	-0.10	0.04	0.0190
BMI (kg/m^2)	SLC2A1	rs751210	0.72	0.41	0.0843	0.07	0.04	0.0541	-0.08	0.03	0.0214
BMI (kg/m^2)	BCL2	rs4940574	-0.85	0.65	0.1946	-0.03	0.03	0.2882	0.10	0.04	0.0232
BMI (kg/m^2)	IL-18R1	rs11465596	1.44	0.90	0.1097	0.03	0.03	0.2891	-0.16	0.07	0.0288
BMI (kg/m^2)	SLC2A1	rs841858	1.02	0.53	0.0554	0.04	0.03	0.1822	-0.09	0.04	0.0427
BMI (kg/m^2)	IL-1A	rs2856836	-0.62	0.40	0.1175	-0.04	0.03	0.2553	0.07	0.03	0.0442
BMI (kg/m^2)	CD35	rs1953299	0.90	0.41	0.0291	0.07	0.04	0.0646	-0.07	0.03	0.0448
Glucose (mg/dL)	MTHFD1L	rs4869713	3.29	1.20	0.0061	0.25	0.08	0.0033	-0.27	0.09	0.0024
Glucose (mg/dL)	IL-18R1	rs7579737	-2.53	1.07	0.0183	-0.06	0.08	0.4856	0.17	0.08	0.0210
Glucose (mg/dL)	MTHFD1L	rs175866	-0.83	1.20	0.4909	-0.09	0.10	0.3598	0.18	0.09	0.0441
Glucose (mg/dL)	CRP	rs1130864	1.79	1.12	0.1096	0.17	0.08	0.0453	-0.16	0.08	0.0455
HDL (mg/dL)	BCL2	rs4987853	-3.10	1.38	0.0248	-0.13	0.10	0.1810	0.31	0.09	0.0010
HDL (mg/dL)	CYP27B1	rs4646536	3.19	1.60	0.0469	0.22	0.10	0.0220	-0.34	0.14	0.0155
HDL (mg/dL)	IL-1A	rs3783546	1.80	1.50	0.2305	0.21	0.09	0.0264	-0.26	0.11	0.0215
HDL (mg/dL)	SLC2A1	rs710221	-3.05	1.25	0.0147	-0.13	0.11	0.2616	0.22	0.10	0.0218
HDL (mg/dL)	BCL2	rs1564483	3.34	1.80	0.0633	0.17	0.09	0.0503	-0.31	0.14	0.0312
HDL (mg/dL)	CRP	rs1130864	-1.48	1.25	0.2374	-0.05	0.11	0.6158	0.21	0.10	0.0380
HDL (mg/dL)	TLR4	rs7873784	-1.05	1.57	0.5038	0.01	0.10	0.9403	0.25	0.13	0.0459

Table 2.3. Top hit (p < 0.05) offspring SNP X offspring CMV IgG interactions in association with offspring CMR outcomes. (continued)

			SNP association			CMV IgG association			SNP X CMV IgG Interaction		
Outcome	Gene	SNP	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
LDL (mg/dL)	TNFRSF1A	rs4149578	-4.25	3.26	0.1929	-0.09	0.17	0.6201	0.88	0.28	0.0020
LDL (mg/dL)	MTHFD1L	rs572522	-4.67	2.84	0.1000	-0.40	0.23	0.0813	0.53	0.19	0.0066
LDL (mg/dL)	IL-6	rs1554606	-4.35	2.96	0.1418	-0.27	0.21	0.2164	0.62	0.27	0.0202
LDL (mg/dL)	TLR3	rs3775291	6.13	3.83	0.1102	0.31	0.21	0.1398	-0.66	0.30	0.0302
ln(Triglycerides)	IL-6R	rs2229238	-0.13	0.06	0.0297	0.00	0.00	0.1410	0.01	0.00	0.0076
ln(Triglycerides)	IL-6	rs1554606	-0.08	0.05	0.1206	-0.01	0.00	0.0545	0.01	0.00	0.0101
ln(Triglycerides)	BCL2	rs1542578	0.07	0.05	0.1798	0.01	0.00	0.0987	-0.01	0.00	0.0139
ln(Triglycerides)	IL-18R1	rs2080289	-0.12	0.06	0.0280	0.00	0.00	0.1179	0.01	0.00	0.0191
ln(Triglycerides)	BCL2	rs7242542	-0.19	0.07	0.0081	0.00	0.00	0.1931	0.01	0.01	0.0275
ln(Triglycerides)	MTHFR	rs1801131	-0.12	0.05	0.0190	-0.01	0.00	0.0736	0.01	0.00	0.0295
ln(Triglycerides)	IL-1R2	rs2110562	0.06	0.05	0.1992	0.01	0.00	0.1439	-0.01	0.00	0.0331
ln(Triglycerides)	RXRA	rs1045570	-0.12	0.07	0.0762	-0.01	0.00	0.0312	0.01	0.00	0.0459
Systolic BP (mmHg)	IL-18	rs549908	-2.08	0.95	0.0284	-0.07	0.06	0.2592	0.17	0.08	0.0250
Systolic BP (mmHg)	BCL2	rs4987853	0.97	1.03	0.3457	0.14	0.08	0.0653	-0.18	0.08	0.0260

Table 2.4. Top hit ($p < 0.05$) SNP X Vitamin D interactions in association with CMR outcomes.

			SNP association			Vitamin D association			SNP X Vitamin D Interaction		
Outcome	Gene	SNP	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
Square root (CMV IgG)	ABCA1	rs1883025	-4.66	1.55	0.0028	-0.04	0.06	0.5030	0.19	0.06	0.0027
Square root (CMV IgG)	CYP2R1	rs12794714	-3.16	1.45	0.0296	-0.04	0.07	0.5189	0.15	0.06	0.0120
Square root (CMV IgG)	BCL2	rs4987853	3.12	1.46	0.0327	0.15	0.05	0.0023	-0.14	0.06	0.0134
Square root (CMV IgG)	MTHFD1L	rs4341013	-3.14	1.61	0.0507	0.02	0.05	0.7686	0.16	0.07	0.0170
Square root (CMV IgG)	CUBN	rs1801222	2.09	1.63	0.2012	0.11	0.06	0.0531	-0.13	0.06	0.0389
Square root (CMV IgG)	IL-1R2	rs4851522	-2.86	1.49	0.0563	0.05	0.05	0.3271	0.12	0.06	0.0426
Square root (CMV IgG)	MTHFD1L	rs803471	-2.91	1.38	0.0351	0.02	0.05	0.6982	0.11	0.05	0.0487
Square root (CMV IgG)	IL-21	rs13143866	2.47	1.40	0.0779	0.10	0.06	0.0858	-0.10	0.05	0.0498
BMI (kg/m^2)	IL-1R2	rs3218984	2.34	0.85	0.0063	0.00	0.03	0.8981	-0.09	0.03	0.0063
BMI (kg/m^2)	BCL2	rs4940574	2.14	0.94	0.0230	-0.01	0.03	0.6589	-0.08	0.04	0.0300
BMI (kg/m^2)	MTHFD1	rs1076991	-1.67	0.75	0.0273	-0.13	0.04	0.0011	0.07	0.03	0.0306
BMI (kg/m^2)	LRP2	rs2075252	-1.11	0.89	0.2099	-0.11	0.03	0.0004	0.07	0.03	0.0379
BMI (kg/m^2)	MTHFD1L	rs4869713	0.99	0.79	0.2126	-0.01	0.03	0.7663	-0.06	0.03	0.0401
BMI (kg/m^2)	CD14	rs2569190	1.53	0.74	0.0394	0.01	0.04	0.7726	-0.06	0.03	0.0467
Glucose (mg/dL)	RXRA	rs1045570	-7.30	2.35	0.0020	-0.21	0.09	0.0301	0.30	0.10	0.0026
Glucose (mg/dL)	MTHFR	rs1801133	-3.16	1.67	0.0584	-0.23	0.09	0.0087	0.16	0.07	0.0232
Glucose (mg/dL)	CYP24A1	rs6013897	4.97	2.00	0.0133	0.01	0.09	0.9171	-0.19	0.08	0.0253
Glucose (mg/dL)	IL-12(B)	rs3212227	4.28	2.29	0.0625	0.05	0.09	0.5711	-0.22	0.10	0.0300
Glucose (mg/dL)	BCL2	rs4940574	-4.71	2.17	0.0303	-0.19	0.08	0.0207	0.21	0.10	0.0346
Glucose (mg/dL)	CNTN5	rs10894157	-4.19	2.09	0.0454	-0.19	0.10	0.0563	0.20	0.09	0.0348
Glucose (mg/dL)	MTR	rs1805087	4.92	2.55	0.0542	-0.01	0.08	0.9479	-0.23	0.11	0.0394
Glucose (mg/dL)	BCL2	rs4940576	-4.04	2.03	0.0469	-0.21	0.09	0.0283	0.17	0.08	0.0433
Glucose (mg/dL)	IL-21	rs2055979	-4.44	2.14	0.0380	-0.22	0.11	0.0472	0.17	0.09	0.0475
HDL (mg/dL)	TNFRSF1A	rs4149578	-9.99	2.86	0.0005	0.00	0.08	0.9579	0.45	0.14	0.0008
HDL (mg/dL)	SLC2A1	rs841858	6.23	2.72	0.0222	0.16	0.09	0.0763	-0.26	0.11	0.0153
HDL (mg/dL)	BCL2	rs4987736	-6.69	2.58	0.0098	-0.03	0.12	0.7988	0.23	0.10	0.0242
HDL (mg/dL)	IL-18R1	rs2287033	-6.28	2.39	0.0088	-0.12	0.12	0.3254	0.21	0.10	0.0368
HDL (mg/dL)	CYP2R1	rs1993116	-5.07	2.49	0.0417	-0.10	0.11	0.3553	0.19	0.09	0.0372
HDL (mg/dL)	ABCA1	rs1883025	-6.22	2.76	0.0244	-0.07	0.10	0.4805	0.21	0.11	0.0441

Table 2.4. Top hit (p < 0.05) SNP X Vitamin D interactions in association with CMR outcomes. (continued)

			SNP association			Vitamin D association			SNP X Vitamin D Interaction		
Outcome	Gene	SNP	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
LDL (mg/dL)	IL-18R1	rs3771170	16.75	5.34	0.0018	0.67	0.23	0.0033	-0.87	0.22	0.0001*
LDL (mg/dL)	BCL2	rs1564483	-21.20	5.90	0.0003	-0.17	0.20	0.4020	0.77	0.25	0.0022
LDL (mg/dL)	TLR3	rs3775291	-14.73	5.58	0.0085	-0.14	0.20	0.5028	0.62	0.23	0.0088
LDL (mg/dL)	MTHFD1L	rs4869959	-14.78	6.21	0.0175	-0.05	0.22	0.8174	0.69	0.27	0.0095
LDL (mg/dL)	LRP2	rs2075252	-17.40	6.40	0.0067	-0.10	0.21	0.6361	0.70	0.27	0.0100
LDL (mg/dL)	IL-1R2	rs3218984	12.53	6.00	0.0372	0.43	0.22	0.0445	-0.53	0.24	0.0276
LDL (mg/dL)	SLC2A1	rs710221	-14.07	5.18	0.0067	-0.26	0.24	0.2695	0.50	0.23	0.0291
LDL (mg/dL)	IL-1R2	rs4851522	10.70	6.01	0.0752	0.34	0.20	0.0937	-0.52	0.25	0.0369
LDL (mg/dL)	IL-18R1	rs2287033	8.52	4.59	0.0636	0.55	0.24	0.0214	-0.40	0.19	0.0387
LDL (mg/dL)	MTHFD1L	rs6940322	9.11	5.50	0.0981	0.51	0.22	0.0214	-0.43	0.22	0.0496
ln(Triglycerides)	CD14	rs5744455	0.32	0.11	0.0031	0.00	0.00	0.7180	-0.01	0.00	0.0092
ln(Triglycerides)	MTHFD1L	rs4341013	-0.22	0.10	0.0354	-0.01	0.00	0.0402	0.01	0.00	0.0106
ln(Triglycerides)	BCL2	rs1801018	0.20	0.07	0.0065	0.00	0.00	0.4108	-0.01	0.00	0.0113
ln(Triglycerides)	IL-6	rs1554606	-0.15	0.07	0.0490	-0.01	0.00	0.0385	0.01	0.00	0.0223
ln(Triglycerides)	CNTN5	rs10894157	-0.16	0.10	0.1151	-0.01	0.00	0.0623	0.01	0.00	0.0228
ln(Triglycerides)	TNFRSF1A	rs4149578	0.26	0.13	0.0520	0.00	0.00	0.8421	-0.01	0.01	0.0293
ln(Triglycerides)	IL-18R1	rs2287033	0.16	0.08	0.0512	0.00	0.00	0.3360	-0.01	0.00	0.0373
ln(Triglycerides)	IL-6	rs1800797	-0.15	0.09	0.0939	-0.01	0.00	0.1437	0.01	0.00	0.0377
Systolic BP (mmHg)	MTHFD1	rs1950902	5.85	2.03	0.0041	0.08	0.06	0.1721	-0.28	0.08	0.0004*
Systolic BP (mmHg)	MTHFD1L	rs1555179	3.93	1.50	0.0088	0.11	0.06	0.0695	-0.15	0.06	0.0116
Systolic BP (mmHg)	IL-6	rs2069840	4.97	1.52	0.0011	0.10	0.06	0.1103	-0.17	0.07	0.0119
Systolic BP (mmHg)	MTHFD1	rs1076991	2.42	1.55	0.1183	0.10	0.07	0.1521	-0.13	0.06	0.0249
Systolic BP (mmHg)	SLC2A10	rs7251505	-3.66	2.73	0.1808	-0.04	0.06	0.4641	0.24	0.11	0.0254
Systolic BP (mmHg)	RXRA	rs1045570	-3.96	1.78	0.0261	-0.10	0.06	0.1287	0.16	0.07	0.0258
Systolic BP (mmHg)	MTRR	rs1532268	-2.72	1.48	0.0668	-0.11	0.07	0.0923	0.14	0.06	0.0309
Systolic BP (mmHg)	LTA	rs1041981	3.39	1.59	0.0339	0.06	0.06	0.3166	-0.13	0.06	0.0376
Systolic BP (mmHg)	IL-6	rs1554606	-4.71	1.65	0.0044	-0.08	0.07	0.2402	0.15	0.07	0.0495

Table 2.4. Top hit (p < 0.05) SNP X Vitamin D interactions in association with CMR outcomes. (continued)

			SNP association			Vitamin D association			SNP X Vitamin D Interaction		
Outcome	Gene	SNP	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
Diastolic BP (mmHg)	MTHFD1	rs1950902	3.69	1.62	0.0232	0.00	0.05	0.9889	-0.22	0.06	0.0005
Diastolic BP (mmHg)	IL-6	rs2069840	4.54	1.26	0.0003	0.03	0.05	0.5189	-0.17	0.05	0.0014
Diastolic BP (mmHg)	LTA	rs1041981	3.57	1.29	0.0057	0.01	0.05	0.8222	-0.15	0.05	0.0032
Diastolic BP (mmHg)	IL-6	rs1554606	-3.09	1.33	0.0208	-0.12	0.05	0.0237	0.12	0.05	0.0357
Diastolic BP (mmHg)	CD53	rs2885805	-2.25	1.15	0.0510	-0.15	0.06	0.0176	0.10	0.05	0.0435

*Met Benjamini-Hochberg-adjusted criteria for statistical significance.

Figure 2.1. 25[OH]D modifies the association between IL-18R1 SNP rs3771170 and LDL cholesterol among study participants.

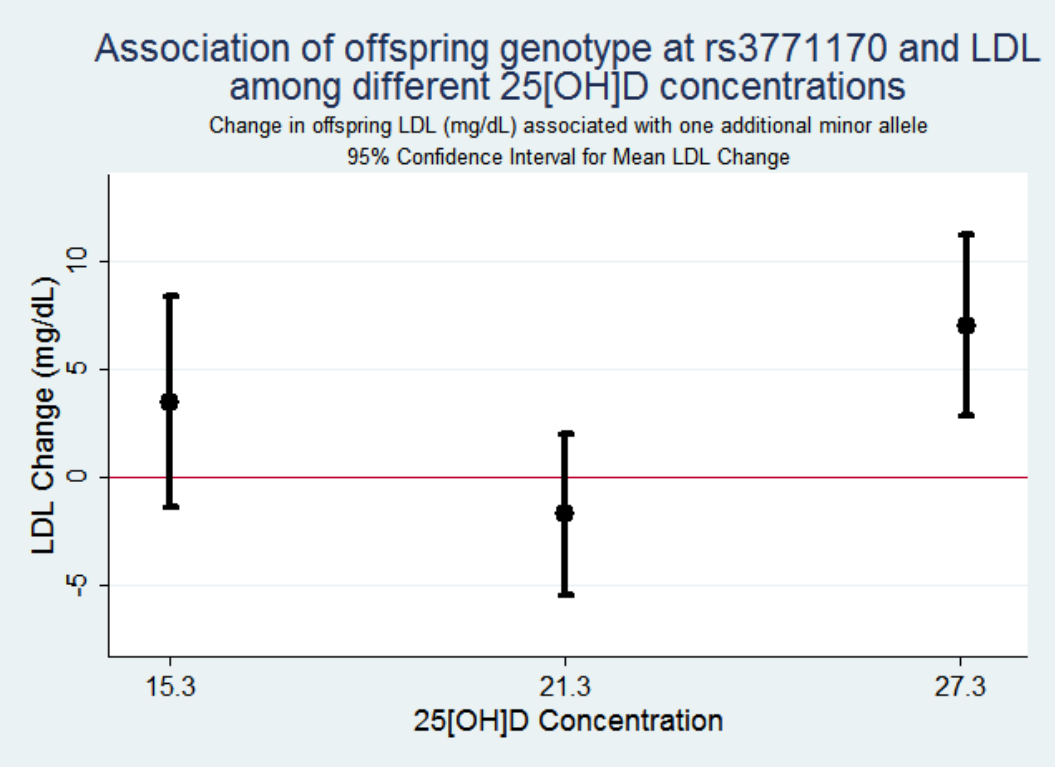
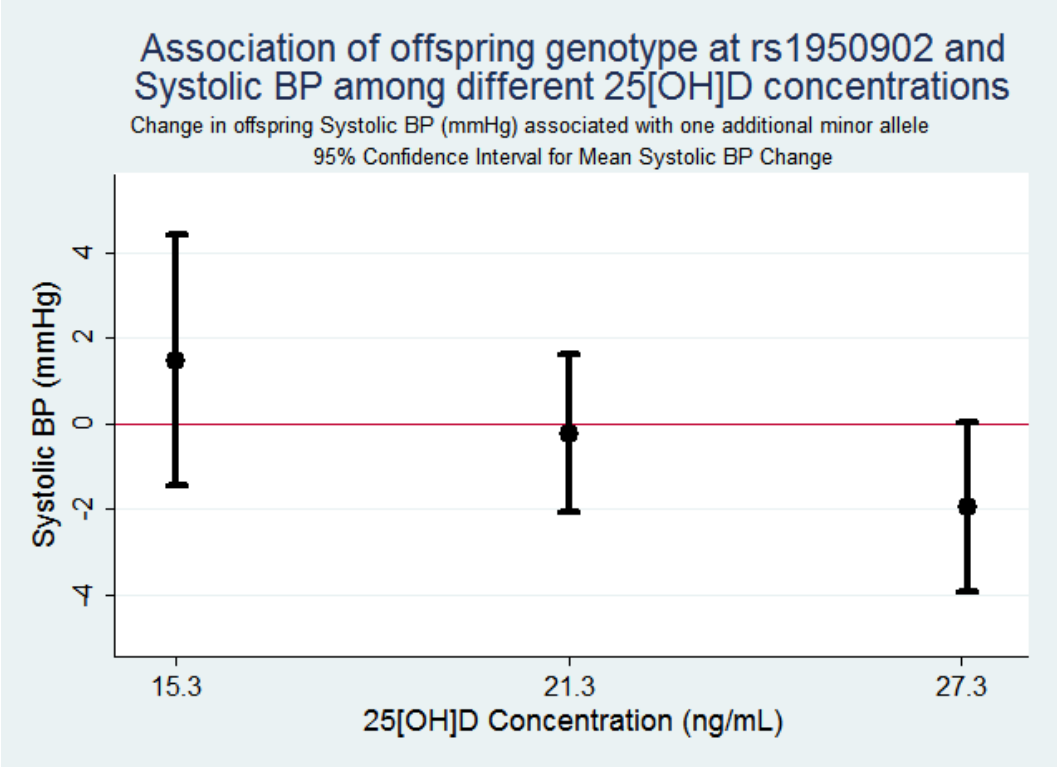


Figure 2.2. 25[OH]D modifies the association between MTHFD1 SNP rs1950902 and Systolic BP among study participants.



In an exploratory analysis, we examined the potential for mediation of SNP-CMR relationships by CMV IgG and 25[OH]D concentration among SNPs that met our pre-specified significance criterion based on the Benjamini-Hochberg FDR multiple testing adjustment. Using the Sobel-Goodman test, we found no evidence of mediation by CMV IgG titer nor 25[OH]D in the association of rs10894157 and BMI (results not shown).

The associations between rs10894157 and BMI, the interaction between rs3771170 and 25[OH]D on LDL, and the interaction between rs1950902 and 25[OH]D on systolic BP were robust with respect to covariate inclusion/exclusion (Supplementary Tables S2.1, S2.2, and S2.3, respectively).

Discussion

In the current study, we investigated immune- and vitamin D-related candidate SNP-CMR associations, along with SNP-CMV IgG and SNP-25[OH]D interactions on CMR. We report evidence for novel associations of SNPs with BMI and LDL cholesterol. We also found possible evidence of effect modification of CMV IgG-CMR and 25[OH]D-CMR associations by several SNPs, including an interaction between a novel IL-18R1SNP (rs3771170) and 25[OH]D on LDL cholesterol

Our report of an association between a SNP in CNTN5 and lower BMI is novel. While the intronic SNP rs10894157 in CNTN5 was originally chosen for inclusion in our candidate list because of a previous report of the association of CNTN5 gene expression changes with viral infection by CMV and other herpesviruses (120), we found no evidence that the mechanism for its association with BMI is mediated through CMV IgG. Expression of CNTN5 in adults is predominantly in the brain and thyroid (121) and believed to be critical for normal brain development (122). Haplotype analysis of a block of CNTN5 SNPs, not including our candidate SNP, was associated with anorexia among Japanese females (123). Our finding of variation in CNTN5 associated with lower BMI ($B = -1.35$, $p < 0.0001$) is consistent in direction with association of the rarer CNTN5 haplotype with anorexia that was reported before.

We also found multiple SNPs in SLC2A1 associated with LDL cholesterol and triglycerides in our population; the relevant SNPs had linkage disequilibrium of 0.25 and 0.39, respectively. These associations, to our knowledge, have not been previously reported in the literature, nor were they reported in the recent Global Lipids Consortium results (124). The SLC2A1 gene, also known as GLUT1, codes for the glucose transporter protein type 1 (125). GLUT1 is a highly-conserved protein that facilitates the transport of glucose across the plasma membrane in humans, as well as other mammals, and, notably, is primarily responsible for the transport of glucose across the blood-brain barrier (126). Mutations in SLC2A1 are responsible for GLUT1 deficiency, characterized by low cerebrospinal fluid glucose concentration (127) and a specific type of epilepsy (128). Furthermore, Vazquez-Chantada, et al., found that two SNPs in SLC2A1 were associated with Non-Alcoholic Fatty Liver Disease (NAFLD) and that decreases in SLC2A1 mRNA expression are associated with triglyceride accumulation in hepatocytes, though the direction of this association is unclear (129). One of the SNPs studied and validated by Vazquez-Chantada, et al., rs841856, is in high (0.809) LD with rs841858, a SNP among our

top hits for both triglycerides and LDL (Table 2.2). In their case-control study including individuals with biopsy-proven NAFLD, the minor alleles in rs841856 and rs4658 were associated with increased odds of NAFLD. However, in our study, the minor allele of rs841856 was associated with decreases in both triglycerides and LDL (Table 2.2). Our results may be due to chance. Or, this may be due to population differences with respect to the development of overt disease such as NAFLD. Our population is younger and non-diseased compared to that of Vazquez-Chantada, et al., despite the inclusion of some individuals with high fasting triglycerides and/or LDL. To speculate along this line of reasoning, in our younger population, individuals have not yet developed overt NAFLD and instead, the lower fasting lipids associated with the risk alleles could represent a dysregulation of fatty acid uptake and release by hepatocytes (130). We speculate that individuals with the risk alleles inappropriately sequester fatty acids in the liver during times of fasting rather than releasing them into the bloodstream as triglycerides at this point in risk or disease development, leading to lower fasting lipids compared to individuals without the risk allele.

In exploratory analyses of SNP-CMV IgG and SNP-25[OH]D interactions on CMR outcomes, we found preliminary evidence of statistical interaction with several SNPs in each case (Tables 2.3, 2.4). Although we did not specify an *a priori* multiple testing correction for our exploratory interaction analyses, the interaction between 25[OH]D and rs3771170 met the Benjamini-Hochberg criteria for statistical significance that was used in our main effects analysis. We are the first to report a statistical interaction between IL-18R1 and 25[OH]D concentration on LDL. The SNP rs3771170 is within IL-18R1, a IL-18 receptor, which binds to IL-18 and is required for signal transduction (131). SNP rs3771170 is intronic and has not previously been associated with any phenotype, to our knowledge, nor is it predicted to cause a change in the protein product. Functional polymorphisms in IL-18R1 have been reported previously in conjunction with vaccine response (132), but our rs3771170 is not in linkage disequilibrium with any of these polymorphisms. Its partner, IL-18, is a proinflammatory cytokine in the IL-1 family that induces the production of Th1 cytokines, enhances cell-mediated cytotoxicity, and is involved in the defense against intracellular pathogens (133). Associations between CMR and IL-18 have been reported in the literature. Weight loss via dietary changes in obese, premenopausal women resulted in lowering of both LDL cholesterol and IL-18 (134). Among individuals with Type 2 Diabetes, IL-18 is associated with preclinical

atherosclerosis (135). It has been shown to be proatherogenic in mice only in the presence of a high-fat diet (136). IL-18 levels have also been shown to decrease with statin therapy given for hypercholesterolemia (137). However, Kong, et al., demonstrated that the active metabolite of 25[OH]D suppressed IL-18 synthesis in keratinocytes in a VDR-dependent manner(137). Enhanced expression of IL-18, among other cytokines, is found in CYP27B1 knockout mouse placentas (138), indicating a role for the active form of vitamin D in balancing the immune system. However, 25[OH]D concentrations are not correlated with IL-18 concentration in morbidly obese subjects (139). Thus, the literature, while indicating that insufficient 1,25$[OH]_2$D, the active metabolite of vitamin D, may promote inflammation via increasing IL-18 expression, it does not indicate how a non-deficient 25[OH]D concentration could alter the relationship between IL-18 or its receptor and LDL cholesterol concentration. Indeed, while IL-18 has been associated with atherogenesis, associations with circulating LDL cholesterol, an involved precursor to atherosclerotic lesions, are lacking in previous studies. Overall, there is indirect evidence for the plausibility of interactions between IL-18R1 and 25[OH]D on LDL, but further studies are needed to confirm the nature and direction of the interaction reported in the current study.

We report here an interaction between MTHFD1 SNP rs1950902 and 25[OH]D concentration on systolic BP. MTHFD1 encodes a protein involved in folate metabolism (140); homozygous knock-out mice do not survive past the embryonic stage (141). This SNP has been studied in conjunction with migraines (142), tetralogy of Fallot (143), and ovarian cancer (144) with null findings, and a borderline association with non-small cell lung cancer (145). While the SNP results in an amino acid change, it is not predicted to result in a change to the protein product function (SIFT: http://sift.jcvi.org/). MTHFD1 has not been studied previously with respect to blood pressure, but other genes in this pathway, such as MTHFR, have been associated with hypertension (146, 147). It has been hypothesized that exposure to ultraviolet light, such as that necessary for endogenous vitamin D production, may reduce serum folate levels, thus possibly accounting for the statistical interaction observed in the present study. One study has reported a decrease in folate levels among patients treated with UVB phototherapy for psoriasis (148), but a pilot study on the topic that included healthy volunteers found no such decrease (149). Thus, while the literature does not preclude an interaction between MTHFD1 and 25[OH]D concentration on systolic BP, the evidence for a biological interaction is somewhat weak to date.

In a sensitivity analysis of CMV seropositivity, defined as an IgG titer greater than or equal to 10Eu/mL, we found that offspring rs5744455 in CD14 was associated with increased odds of CMV seropositivity (OR = 2.83, SE = 0.74, p = 0.0001). CD14 is a pattern recognition receptor of the innate immune system, which is believed to sensitize cells to the presence of Gram-negative lipopolysaccharide via delivery to the TLR4 signaling complex (150). Infected peripheral blood monocytes are primarily CD14+ cells (151), and and CMV virons trigger inflammatory cytokines in a CD14-dependent manner (152), consistent with the fact that cellular detection for CMV is primarily based on the combination of CD14 and TLR2 (152); however, CMV is capable of entering and establishing infection in cell lines that are both CD14 and TLR2 negative (152). Krichen, et al., found no association of CD14 promotor polymorphism and CMV infection after kidney transplantation (153), but this is the only other study known to the authors to examine CD14 polymorphisms and CMV infection. In light of our negative results of CMV IgG and rs574455 (B = 0.62, SE = 0.61, p = 0.3077), and previous literature showing that CD14 is not necessary for CMV entry into cells (152), our findings suggest that CD14 variation may influence the ability of CMV to establish infection, but additional work is necessary.

Our study has a number of strengths. JPS-1 participants came from a well-characterized cohort that was oversampled for individuals at high risk for CMR from a developmental origins perspective, with higher maternal pre-pregnancy BMI and low birth weight. CMR were measured before most of the participants had developed overt disease, allowing analysis to be performed without complex exclusions or adjustment for multiple comorbidities. The study has a number of limitations as well. As a candidate SNP study, we analyzed a limited number of SNPs and in many cases have tag SNPs rather than functional variants. Specific lifestyle and demographic characteristics of our population may limit the generalizability of our findings. Our novel findings require independent replication. Our study is of modest size, which limits statistical power for minor allele frequencies less than 20%, particularly in the setting of a large number of statistical tests. The power limitation is illustrated by the non-significant association of rs2282679 in the GC gene, a gene widely associated with 25[OH]D in a variety of populations (154-159). The estimate of association found in this analysis, B = -1.45, is about 0.16 standard deviations and is reasonably consistent with prior studies (155, 160). With our population size, we had inadequate power to detect statistical significance at this locus.

In conclusion, we report a novel association between rs10894157 in CNTN5 and BMI. We also identified possible effect modification of the association between 25[OH]D and LDL cholesterol by rs3771170 genotype in IL-18R1. Our findings, if replicated, will inform molecular and genetic explorations of the origins and pathogenesis of dyslipidemia and obesity.

Chapter 3. Maternal candidate immune- and vitamin D-related genetic variants and offspring adult cardiometabolic risk

Abstract

Maternal genotype effects are closely linked to the intrauterine environment which may in turn influence offspring adult health and disease. The role of maternal genetic variants in candidate immune- and vitamin D- related genes on adult offspring cardiometabolic risk factors (CMR) has not been studied previously. Among a subset of participants from a population-based birth cohort study, the Jerusalem Perinatal Study, we investigated associations of immune- and vitamin D–related candidate maternal SNPs with adult CMR (fasting glucose, lipids, body mass index, blood pressure), cytomegalovirus antibody titers (CMV IgG), and 25[OH]D concentrations. We found little evidence for associations between maternal genotype and offspring adult CMR. However, we found suggestive evidence of maternal genotype-offspring CMV IgG and vitamin D interactions on CMR. The maternal BCL2 SNP rs4987853 interacted with offspring CMV IgG on offspring HDL cholesterol ($p = 0.0003$), with a positive association between maternal genotype and offspring HDL ($B = 0.76$, $SE = 0.90$, $p = 0.402$) among offspring with CMV IgG of 26.1EU/mL, and a negative association ($B -1.22$, $SE = 0.75$, $p = 0.102$) among offspring with CMV IgG of 237.8EU/mL.The maternal IL-1R2 SNP rs4851522 interacted with offspring 25[OH]D concentrations on offspring LDL cholesterol ($p < 0.0001$). Among offspring with 25[OH]D concentrations of 15.3ng/mL, maternal minor allele at rs4851522 with a 9.06mg/dL increase in offspring LDL ($SE = 2.79$, $p = 0.001$). Among offspring with 25[OH]D concentrations of 21.3ng/mL, each maternal minor allele was associated with a 2.33mg/dL increase in offspring LDL ($SE = 2.18$, $p = 0.286$), and among offspring with 25[OH]D of 27.4ng/mL, with a 4.51mg/dL decrease in LDL ($SE = 2.44$, $p = 0.065$). Finally, we report an interaction between maternal rs13143866 in IL-21 with offspring 25[OH]D on offspring HDL (p for interaction $= 0.0002$). Findings, if replicated, suggest potential interactions of maternal genotype with offspring adult characteristics on CMR development. Larger, multigenerational studies have the potential to enhance our understanding of the developmental origins of CMR through detailed characterization of maternal and offspring factors that influence the intrauterine environment.

Introduction

Maternal factors that influence the intrauterine environment, such as maternal genetic variation, may play a role in early life offspring programming that will affect later life disease risk. A maternal genetic effect on offspring health may be observed due to simple transmission of the causal allele to the offspring (161), its effect on the intrauterine environment (162), or through parent-of-origin effects conveyed via imprinting (163). To date, the associations between maternal genetic effects on the intrauterine environment and offspring health in humans has been most frequently studied in relation to early life outcomes. For instance, in mothers with low folate intake during pregnancy, variants in several maternal methionine synthase pathway genes are associated with the risk of neural tube defects in the offspring (162). Maternal genotype has been associated with offspring outcome independent of offspring genotype for TCF7L2 and birthweight (6), Il-6 (7) and IL1A (8) with preterm birth, TGFA and oral clefts (164), and TPH1 and attention-deficit hyperactivity disorder (9). While multiple mouse cross-fostering studies have demonstrated that maternal genetic variation has lasting effects on adult offspring body size (165-167), there is a paucity of similar data among humans.

Early life factors involved in the development of adult CMR and overt cardiovascular and metabolic disease have most often been studied in the context of maternal over- and under-nutrition and the resulting aberrations in the intrauterine environment (2, 168). Few previous studies have examined associations between maternal genetic variation and subsequent offspring adulthood CMR. Recently, our group published findings that indicate a role for maternal variation in candidate genes from fetal development and CMR pathways in the relationship between maternal gestational weight gain and offspring adult Body Mass Index (BMI) (169). Maternal genetic variation in other candidate pathways could influence offspring CMR. For instance, there is evidence for relationships between the developmental origins of the immune system and CMR (170, 171). Prenatal exposure to the 1918 influenza pandemic has been associated with excess incident cardiovascular disease among those aged 60-82 years relative to those born shortly before or after the pandemic exposure window (35). Recently, multiple genes have been implicated in host response to influenza in both mice (172) and humans (173), indicating that maternal genetic factors at the time of infection may influence the intrauterine environment and contribute to observed adult offspring cardiovascular effects. Another, related candidate pathway

involves vitamin D, which maintains balance between cell-mediated Th1 immune response and antibody-mediated Th2 immune response (74), and has widespread functions that may influence CMR (69). Low vitamin D during pregnancy have been linked to offspring health outcomes (174-179) and maternal Vitamin D Receptor (VDR) genotype has been linked to low birthweight among infants of back mothers (94). However, to our knowledge, no prior study has examined maternal genetic variations in immune- and vitamin D-related pathways and adult offspring CMR.

The current study leverages an existing cohort of maternal-offspring dyads with maternal genotypic information and well-characterized young adult offspring to investigate the associations between candidate maternal SNPs and adult offspring CMR (Body Mass Index (BMI), fasting glucose, fasting triglycerides, Low-Density Lipoproteins (LDL), High-Density Lipoproteins (HDL), systolic blood pressure (BP), diastolic BP), CMV antibody titers (CMV IgG), and 25[OH]D concentrations. We also examined the potential for effect modification of associations of offspring CMV IgG or 25[OH]D concentrations with offspring CMR by maternal genotype.

Methods

Study setting and study population

This study is based upon data of the Jerusalem Perinatal Study, which collected prenatal, perinatal, and post-natal data on all births (n=17,003) to residents of west Jerusalem during 1974-1976. In the Jerusalem Perinatal Family Follow-up Study (JPS-1), 1,500 mother-offspring dyads were recruited from the original JPS cohort and provided additional interview, physical examination, and biological samples. Participants were recruited using a stratified sampling of term (≥ 36 weeks gestation), singleton, live offspring births with oversampling of low (≤2500g) and high (≥4000g) birthweight offspring and mothers with a pre-pregnancy BMI ≥ 27 kg/m^2. Mother-offspring dyads with offspring who had congenital disorders at birth were excluded.

Information on maternal lifestyle factors during pregnancy and birthweight of offspring were collected from a combination of maternity ward logbooks, birth certificates, and maternal interviews conducted on the first or second day postpartum. (117). Maternal blood samples used for genotyping were collected during JPS-1. Offspring who self-reported taking medication for high blood pressure (n = 22), high cholesterol (n = 20), or diabetes (n = 22) were excluded, as well as individuals with a fasting time that was missing (n = 234) or fewer than eight hours (n = 97). A maximum of 1,194 mother-offspring dyads were available for analysis after these exclusions. Study procedures were approved by the Institutional Review Boards of the University of Washington, Seattle (USA), and the Hadassah-Hebrew University Medical Center in Jerusalem (Israel). All participants provided informed consent.

Data Collection

Data collection procedures have been published previously (117). To summarize, data on anthropomorphic measurements, lifestyle, and socio-demographic characteristics of the offspring were collected during telephone interviews conducted by trained interviewers and physical exams conducted between 2007 and 2009. At the time of data collection for JPS-1, the mean age of offspring was 32 (range 31-33 years). BMI was calculated as weight (kg) divided by squared height (m^2). Blood pressure (BP) was measured as the average of three consecutive measurements performed after five minutes of sitting (Omron M7 automated sphygmomanometer). Fasting blood samples were taken using standard

procedures. Plasma glucose, HDL, LDL, and triglycerides were measured on the VITROS 5,1FS Chemistry System (Ortho Clinical Diagnostics, Raritan, NJ). CMV antibody titers were determined by enzyme immunoassay technology using the Immunosimplicity® Is-CMV IgG Test Kit (Diamedix Corp. Miami, Florida). Antibody titers are reported as EU/mL, with a value ≥ 10.0EU/mL considered positive for anti-CMV IgG. Assays were performed at the University of Vermont Laboratory for Clinical Biochemistry Research (Burlington, VT). Plasma vitamin D (25[OH]D) was measured using liquid chromatography tandem mass spectroscopy at the Nutrition and Obesity Research Center, University of Washington (Seattle, WA). The sum of 25[OH]D2 and 25[OH]D3 was used in analyses to represent total plasma vitamin D concentration.

Gene/SNP selection, DNA extraction and genotyping

We investigated 111 candidate maternal SNPs characterizing variations in genes involved in innate immune function and vitamin D metabolism. (Supplementary Table S1). SNPs were selected from previously published studies based on hypothesized functional and biological significance, and known associations with phenotypes related to the immune system and vitamin D metabolism. Extraction and amplification of DNA from maternal blood samples was performed using standard methods. Genotyping was performed using an Illumina, Inc., BeadArray™ and the TaqmanOpenArray assay at the University of California, San Francisco. Quality control measures were taken to ensure quality of the genotyping assays. SNPs (n = 3) in violation of Hardy-Weinberg Equilibirum, as determined using a significance level that adjusts for the multiple tests performed ($p < 0.00044$), were excluded from analysis.

Statistical Analyses

We examined offspring characteristics in the entire study cohort. Means and standard deviations were calculated for continuous variables; numbers and percentages for categorical variables. We used weighted multiple linear regression models to examine associations of maternal genotype, modeled additively, with CMV IgG, 25[OH]D concentrations, BMI, fasting glucose, triglycerides, LDL, HDL, systolic BP, and diastolic BP). Ninety-five percent confidence intervals and p-values were calculated using robust standard error estimates. Inverse probability sampling weights were based on maternal pre-pregnancy

BMI and offspring birthweight sampling strata and non-response rates. The outcome triglycerides was natural log-transformed to improve model fit, due to the extreme right-skewness of the distribution of triglycerides. CMV IgG was square root-transformed to improve model fit due to the extreme right-skewness of CMV IgG concentrations and the large number of participants with a CMV IgG titer of zero EU/mL, making a log transformation impractical.

Our models were adjusted for season of blood draw (December-February, March-May, June-August, September-November) and additional covariates for smoking status (current, former, and never), alcohol abstinence (yes/no), intense physical activity participation (yes/no), employment status (yes/no), religiosity (Ultra-Orthodox, Religious, and Traditionalist/Secular/Other), socio-economic status (low, medium, and high), birthweight, maternal smoking during pregnancy (yes/no), sex, grandparent country of origin (Yemen, Kurdistan, Morocco, Iran, Iraq, Other African, Other Asian, and Ashkenazi, using Israel as baseline), and BMI except when BMI was the outcome of interest. All covariates were measured on offspring.

We used the Benjamini-Hochberg step-up procedure to adjust for multiple comparisons (118). Briefly, this procedure controls the False Discovery Rate (FDR) at our chosen level of 0.05. P-values are ranked from smallest to largest and compared to $0.05*k/m$, where m is the total number of tests performed and k is the rank of the test. All tests with k smaller than the largest k such that $p_{(k)} \leq 0.05*k/m$ are rejected. Each of the nine outcomes were evaluated separately.

We examined the potential for effect modification by maternal genotype on the relationship of offspring CMV IgG and 25[OH]D with offspring CMR for all 111 SNPs that were in Hardy-Weinberg Equilibrium (Supplementary Table S1). Maternal genotype was coded as having 0, 1, or 2 minor alleles. CMV IgG and 25[OH]D were continuous variables. Multiplicative interaction terms were used and evaluated at an exploratory p-value cutoff of 0.05. The models included the outcome, the interaction term, main effects terms for both maternal genotype and either CMV IgG or 25[OH]D, and the same adjustment covariates used in the main effects models.

We performed the Sobel-Goodman mediation test to examine CMV IgG and 25[OH]D concentrations, separately, as potential mediators in the relationship between genotype and CMR (119). An exploratory significance level of 0.05 was used to determine statistical significance. Only those SNP-

CMR associations that met our criteria for statistical significance after multiple testing adjustment in the main effects analysis were carried forward for mediation analysis.

In sensitivity analyses, we included perinatal maternal characteristics pre-pregnancy BMI and gestational weight gain to assess their roles in any associations and examined the effect of excluding individuals with a missing fasting time. In the case where multiple maternal SNPs within the same gene were found among our top hits in association with one or more CMR, we examined associations of genotype combinations with the relevant CMR. Finally, to assess whether any of our candidate maternal SNPs were associated with CMV infection rather than immune response, we conducted an analysis of our candidate SNPs and CMV seropositivity (≥10EU/mL) as a binary outcome variable.

Analyses were performed using Stata v10.0 (StataCorp, College Station, TX).

Results

Mean age of offspring participants was 32 years. Selected study offspring characteristics are presented in Table 3.1. Mean CMV IgG concentrations were 173.9EU/mL (SD = 237.0); 25[OH]D concentration was 22.2ng/dL (SD = 9.1); BMI was 26.2kg/m2 (SD = 5.1). Mean fasting glucose was 80.1 mg/dL (15.1). There were no associations of maternal genotypes with offspring CMR that met our criteria for statistical significance after Benjamini-Hochberg multiple testing correction (Supplementary Figures 3.1-3.9). The top hits from the maternal genotype-offspring CMR analyses ($p < 0.01$) are presented in Table 3.2. Of note, two SNPs (rs751210 and rs12407920) in SLC2A1 related to lower triglycerides were among these top hits (Table 3.2); the linkage disequilibrium between these two SNPs was 0.23. Since we found no maternal SNP-offspring CMR associations that met our strict criteria for statistical significance (Table 3.2), none were carried forward for mediation analysis.

Table 3.1. Offspring Study Participant Characteristics.

		Total
		N = 1038*
CMV IgG, EU/mL (mean, sd)		173.9 237.0
Total D, ng/mL (mean, sd)		22.2 9.1
BMI, kg/m^2 (mean, sd)		26.2 5.1
Glucose, mg/dL (mean, sd)		80.1 15.1
Triglycerides, mg/dL (mean, sd)		105.2 71.3
LDL, mg/dL (mean, sd)		112.2 28.4
HDL, mg/dL (mean, sd)		50.1 14.6
Systolic BP, mmHg (mean, sd)		105.6 12.0
Diastolic BP, mmHg (mean, sd)		71.2 8.0
Birthweight, grams (mean, sd)		3429.2 609.6
Smoking Status (n, %)	non	597 59.1
	former	138 13.7
	current	276 27.3
Alcohol Abstain (n, %)	yes	453 44.7
Intense Physical Activity (n, %)	yes	307 30.3
Employed (n, %)	yes	854 85.7
Orthodoxy (n, %)	Ultra-orthodox	172 16.6
	Religious	217 20.9
	Traditionalist, Secular, or Other	349 62.5
Season (n, %)	winter	282 27.2
	spring	153 14.7
	summer	296 28.5
	autumn	307 29.6
SES (n, %)	low	236 22.7
	medium	369 36.6
	high	433 40.7
maternal smoking (n, %)	yes	181 17.4

s.d. = Standard Deviation

*May not sum to total due to missing data

†Defined as exercise that brings about labored breathing, increased pulse rate, and sweating, lasting for at least twenty minutes.

Table 3.2. Top hits ($p < 0.01$) for maternal candidate SNP analysis of offspring CMR outcomes.

Outcome	Gene	SNP	Coefficient	Robust SE	p-value
Square root (CMV IgG)	BCL2	rs1564483	-1.55	0.57	0.0067
Square root (CMV IgG)	IL-10	rs3024496	-1.22	0.52	0.0185
25[OH]D (ng/dL)	CYP2R1	rs10741657	1.60	0.53	0.0027
BMI (kg/m^2)	BCL2	rs1531697	-0.91	0.32	0.0047
BMI (kg/m^2)	GC	rs2282679	0.90	0.33	0.0061
BMI (kg/m^2)	MTHFD1L	rs572522	-0.77	0.28	0.0050
Glucose (ng/dL)	IL-18	rs1834481	-3.65	1.06	0.0006
HDL (mg/dL)	MTHFD1	rs1950902	-3.53	1.23	0.0043
LDL (mg/dL)	CD53	rs2966952	6.95	2.31	0.0027
ln(Triglycerides)	BCL2	rs1564483	0.10	0.03	0.0045
ln(Triglycerides)	SLC2A1	rs751210	-0.08	0.03	0.0075
ln(Triglycerides)	SLC2A1	rs12407920	-0.10	0.04	0.0124
ln(Triglycerides)	IL-6r	rs4075015	-0.06	0.03	0.0350
Diastolic BP (mmHg)	RXRA	rs1045570	-1.52	0.58	0.0089
Diastolic BP (mmHg)	CYP27B1	rs4646536	-1.33	0.51	0.0094

No p-values presented in this table met Benjamini-Hochberg multiple testing correction for statistical significance.

Results of the interaction analyses between maternal candidate SNPs and offspring CMV IgG on offspring CMR are presented in Table 3.3. A number of interactions (n = 55) met our exploratory interaction p-value cutoff of 0.05. Of note, multiple maternal SNPs (rs1800630, rs1799964, and rs2229094) in the maternal LTA gene interacted with offspring CMV IgG on offspring 25[OH]D (interaction p-values ranging from 0.0013 to 0.006). Both rs1800630 and 1799964 are downstream intergenic variants. The maternal SNP rs4987853 in BCL2 had a statistically significant (p = 0.0003) interaction with offspring CMV IgG on offspring HDL, which met the same Benjamini-Hochberg criteria used in the primary analysis of maternal genotype associations. At an offspring CMV IgG titer of 26.1EU/mL, each maternal minor allele at rs4987853 was associated with a nonsignificant 0.76mg/dL increase in HDL (SE = 0.90, p = 0.402), with a trend towards a negative association at higher offspring CMV IgG titers: at 237.8EU/mL, each maternal minor allele was associated with a 1.22mg/dL decrease in offspring HDL (SE = 0.75, p = 0.102, Figure 3.1). Two SNPs (rs7558013 and rs7579737) in the maternal IL-18R1 gene interacted with offspring CMV IgG on offspring systolic BP (Table 3.3).

Results of the interaction analyses between maternal candidate SNPs and offspring 25[OH]D concentrations on offspring CMR are presented in Table 3.4. A number of interactions (n = 65) met our exploratory interaction significance level of 0.05 (Table 3.4). The interaction of maternal rs4851522 genotype and 25[OH]D concentration (p for interaction = 0.000004) with offspring LDL cholesterol met criteria for statistical significance after taking into account multiple comparisons. In offspring with an average 25[OH]D concentration of 15.3ng/mL, the association between maternal rs4851522 minor allele and offspring LDL was 9.06mg/dL (SE = 2.79, p = 0.001), while among offspring with a 25[OH]D concentration of 27.4ng/mL, each maternal minor allele at rs4851522 was associated with a 4.51mg/dL decrease in offspring LDL (SE = 2.44, p = 0.065) (Figure 3.2).

The interaction of maternal rs13143866 genotype in IL-21 and offspring 25[OH]D concentration (p for interaction = 0.0002) with offspring HDL cholesterol met criteria for statistical significance after taking into account multiple comparisons. In offspring with an average 25[OH]D concentration of 15.3ng/mL, the association between maternal rs13143866 minor allele and offspring HDL was 3.60mg/dL (SE = 1.21, p = 0.003), while among offspring with a 25[OH]D concentration of 27.4ng/mL, each maternal minor allele at

rs13143866 was associated with a 0.59mg/dL decrease in offspring HDL (SE = 0.89, p = 0.510) (Figure 3.3).

Sensitivity analysis of maternal genotype and offspring CMV seropositivity did not demonstrate any statistically significant associations (Supplementary Table S3.4), and exploration of the effects of covariate adjustment on statistically significant interactions between maternal genotype and offspring CMV IgG or 25[OH]D failed to show major impact of covariate adjustment, including adjusting for the maternal perinatal characteristics pre-pregnancy BMI and gestational weight gain (Supplementary Tables S3.1, S3.2, S3.3).

Table 3.3. Top hit ($p < 0.05$) maternal SNP X offspring CMV IgG interactions in association with offspring CMR outcomes.

			SNP association			CMV IgG association			SNP X CMV IgG Interaction		
Outcome	Gene	SNP	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
25[OH]D (ng/mL)	LTA	rs1800630	-2.11	0.97	0.0292	0.03	0.05	0.4995	0.18	0.06	0.0045
25[OH]D (ng/mL)	LTA	rs1799964	-1.92	0.98	0.0499	0.01	0.05	0.8679	0.17	0.07	0.0106
25[OH]D (ng/mL)	IL-18	rs1834481	2.41	1.09	0.0272	0.13	0.05	0.0086	-0.21	0.08	0.0126
25[OH]D (ng/mL)	CUBN	rs1801222	2.43	1.03	0.0185	0.14	0.05	0.0056	-0.19	0.08	0.0176
25[OH]D (ng/mL)	LTA	rs2229094	-1.85	0.95	0.0528	0.01	0.06	0.8683	0.16	0.07	0.0286
25[OH]D (ng/mL)	MTR	rs1805087	1.40	1.04	0.1812	0.15	0.05	0.0053	-0.16	0.08	0.0410
25[OH]D (ng/mL)	MTHFD1L	rs572522	-1.69	0.78	0.0305	-0.03	0.07	0.6636	0.12	0.06	0.0461
BMI (kg/m^2)	LTA	rs1041981	1.53	0.50	0.0021	0.07	0.03	0.0519	-0.10	0.03	0.0030
BMI (kg/m^2)	CYP2R1	rs1993116	1.26	0.53	0.0187	0.06	0.04	0.0975	-0.11	0.04	0.0083
BMI (kg/m^2)	IL-19	rs3024496	-0.60	0.50	0.2297	-0.05	0.03	0.1626	0.08	0.04	0.0279
BMI (kg/m^2)	LTA	rs2229094	-1.18	0.48	0.0136	-0.03	0.03	0.3058	0.09	0.04	0.0309
BMI (kg/m^2)	CYP2R1	rs10741657	0.92	0.53	0.0849	0.03	0.04	0.4189	-0.09	0.04	0.0371
BMI (kg/m^2)	CD14	rs2563298	0.40	0.48	0.4075	0.06	0.04	0.1131	-0.08	0.04	0.0475
Glucose (mg/dL)	MTHFR	rs1801133	-1.39	1.17	0.2320	-0.08	0.10	0.4162	0.22	0.09	0.0146
Glucose (mg/dL)	MTHFD1L	rs175866	-2.30	1.23	0.0620	-0.09	0.09	0.3414	0.19	0.08	0.0224
Glucose (mg/dL)	IL-18	rs1834481	-6.54	1.84	0.0004	-0.01	0.07	0.8764	0.30	0.14	0.0278
Glucose (mg/dL)	GATA-3	rs1058240	-2.24	1.47	0.1283	0.01	0.08	0.9299	0.22	0.10	0.0283
Glucose (mg/dL)	MTHFD1L	rs4869713	1.85	1.12	0.0988	0.21	0.10	0.0300	-0.18	0.09	0.0353
Glucose (mg/dL)	IL-1R2	rs3218984	-2.75	1.38	0.0470	-0.02	0.08	0.8079	0.21	0.10	0.0370
Glucose (mg/dL)	IL-18R1	rs7579737	-2.32	1.30	0.0743	-0.06	0.09	0.5049	0.18	0.09	0.0392
HDL (mg/dL)	BCL2	rs4987853	-3.34	1.48	0.0241	-0.16	0.08	0.0430	0.40	0.11	0.0003*
HDL (mg/dL)	CD14	rs2569190	1.59	1.21	0.1877	0.25	0.13	0.0487	-0.20	0.09	0.0275
HDL (mg/dL)	IL-6	rs1800797	2.33	1.65	0.1585	0.19	0.10	0.0553	-0.27	0.13	0.0302
HDL (mg/dL)	CD53	rs2885805	-2.62	1.37	0.0569	-0.10	0.12	0.3970	0.21	0.10	0.0493
LDL (mg/dL)	CD36	rs997906	-2.58	2.69	0.3375	-0.36	0.24	0.1359	0.50	0.20	0.0121
LDL (mg/dL)	MTHFD1L	rs3818056	5.08	2.80	0.0693	0.41	0.21	0.0488	-0.45	0.18	0.0133
LDL (mg/dL)	CD14	rs5744455	5.12	3.33	0.1242	0.27	0.19	0.1455	-0.57	0.24	0.0151
LDL (mg/dL)	MTHFD1L	rs572522	-3.91	2.97	0.1883	-0.36	0.24	0.1393	0.51	0.21	0.0160
LDL (mg/dL)	IL-6	rs2069840	-5.14	2.81	0.0674	-0.19	0.20	0.3381	0.48	0.22	0.0273
LDL (mg/dL)	FOXP3	rs3761549	7.45	4.60	0.1055	0.12	0.20	0.5653	-0.65	0.32	0.0452

Table 3.3. Top hit (p < 0.05) maternal SNP X offspring CMV IgG interactions in association with offspring CMR outcomes. (continued)

			SNP association			CMV IgG association			SNP X CMV IgG Interaction		
Outcome	Gene	SNP	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
ln(Triglycerides)	ABCA1	rs1883025	0.12	0.06	0.0462	0.00	0.00	0.2425	-0.01	0.00	0.0078
ln(Triglycerides)	MTRR	rs162036	-0.14	0.08	0.0712	-0.01	0.00	0.0313	0.01	0.01	0.0296
ln(Triglycerides)	MTRR	rs10380	-0.14	0.08	0.0840	-0.01	0.00	0.0810	0.01	0.01	0.0314
ln(Triglycerides)	RXRA	rs1045570	-0.15	0.06	0.0120	-0.01	0.00	0.1196	0.01	0.00	0.0317
ln(Triglycerides)	MTHFR	rs1801131	-0.12	0.05	0.0236	-0.01	0.00	0.0609	0.01	0.00	0.0352
ln(Triglycerides)	FOXP3	rs3761549	0.13	0.09	0.1380	0.00	0.00	0.6816	-0.02	0.01	0.0458
Systolic BP (mmHg)	IL-18R1	rs7558013	-3.88	1.25	0.0019	-0.07	0.06	0.2233	0.35	0.11	0.0013
Systolic BP (mmHg)	IL-18R1	rs7579737	-2.69	0.98	0.0063	-0.15	0.08	0.0633	0.23	0.08	0.0043
Systolic BP (mmHg)	BCL2	rs4987736	3.47	1.10	0.0017	0.21	0.09	0.0199	-0.23	0.08	0.0071
Systolic BP (mmHg)	IL-21	rs13143866	1.52	1.06	0.1528	0.19	0.07	0.0116	-0.21	0.08	0.0081
Systolic BP (mmHg)	MTHFD1L	rs803471	-1.30	0.90	0.1489	-0.07	0.07	0.2811	0.18	0.07	0.0158
Systolic BP (mmHg)	IL-18R1	rs11465596	-2.28	1.52	0.1326	-0.03	0.06	0.6415	0.32	0.14	0.0190
Systolic BP (mmHg)	IL-18R1	rs2287033	1.66	0.98	0.0906	0.18	0.09	0.0363	-0.18	0.08	0.0198
Systolic BP (mmHg)	MTHFD1L	rs4869713	1.68	0.95	0.0776	0.15	0.08	0.0456	-0.17	0.07	0.0211
Systolic BP (mmHg)	BCL2	rs4987853	1.74	1.22	0.1550	0.13	0.07	0.0492	-0.19	0.08	0.0216
Systolic BP (mmHg)	MTRR	rs162036	-2.03	1.44	0.1574	-0.04	0.07	0.5532	0.29	0.13	0.0229
Systolic BP (mmHg)	CUBN	rs1801231	2.96	1.24	0.0172	0.13	0.06	0.0354	-0.21	0.10	0.0344
Systolic BP (mmHg)	CD53	rs2966952	2.30	1.16	0.0488	0.14	0.07	0.0580	-0.20	0.10	0.0436
Diastolic BP (mmHg)	MTHFD1L	rs4869713	1.84	0.76	0.0161	0.21	0.06	0.0007	-0.16	0.06	0.0067
Diastolic BP (mmHg)	MTRR	rs162036	-1.73	1.19	0.1481	0.02	0.05	0.7041	0.22	0.10	0.0265
Diastolic BP (mmHg)	IL-6	rs1554606	-1.19	0.76	0.1179	0.02	0.05	0.6574	0.12	0.06	0.0382
Diastolic BP (mmHg)	IL-6R	rs4553185	-1.14	0.72	0.1165	-0.01	0.07	0.8393	0.12	0.06	0.0452
Diastolic BP (mmHg)	IL-6	rs1800797	-1.46	0.80	0.0674	0.04	0.06	0.5721	0.13	0.06	0.0470
Diastolic BP (mmHg)	MTR	rs1805087	1.06	0.91	0.2474	0.12	0.05	0.0236	-0.13	0.07	0.0474
Diastolic BP (mmHg)	CUBN	rs1801231	1.67	0.99	0.0917	0.15	0.05	0.0031	-0.16	0.08	0.0483
Diastolic BP (mmHg)	TLR4	rs7873784	-2.74	0.89	0.0021	0.04	0.05	0.4546	0.15	0.08	0.0499

*Met Benjamini-Hochberg-adjusted criteria for statistical significance.

Table 3.4. Top hit ($p < 0.05$) maternal SNP X offspring 25[OH]D interactions in association with offspring CMR outcomes.

			SNP association			Vitamin D association			SNP X Vitamin D Interaction		
Outcome	Gene	SNP	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
Square Root (CMV IgG)	CUBN	rs1801222	3.65	1.68	0.0297	0.17	0.06	0.0037	-0.18	0.07	0.0077
Square Root (CMV IgG)	MTHFD1L	rs175866	2.29	1.12	0.0411	0.20	0.07	0.0026	-0.11	0.05	0.0158
Square Root (CMV IgG)	LTA	rs1799964	-3.23	1.49	0.0310	0.03	0.05	0.6023	0.15	0.06	0.0198
Square Root (CMV IgG)	IL-18R1	rs2080289	3.67	1.54	0.0171	0.14	0.05	0.0026	-0.14	0.06	0.0286
Square Root (CMV IgG)	IL-18R1	rs7579737	-2.46	1.22	0.0448	0.00	0.06	0.9352	0.11	0.05	0.0310
Square Root (CMV IgG)	CYP2R1	rs10741657	2.41	1.26	0.0565	0.19	0.06	0.0027	-0.10	0.05	0.0414
Square Root (CMV IgG)	MTR	rs1805087	2.66	1.49	0.0751	0.14	0.05	0.0078	-0.11	0.05	0.0494
BMI (kg/m^2)	RXRA	rs1045570	-3.40	1.01	0.0008	-0.09	0.03	0.0058	0.11	0.04	0.0044
BMI (kg/m^2)	IL-18R1	rs7579737	1.71	0.65	0.0083	0.00	0.03	0.9298	-0.06	0.03	0.0199
BMI (kg/m^2)	IL-1R2	rs2110562	-1.19	0.73	0.1023	-0.11	0.04	0.0039	0.07	0.03	0.0224
BMI (kg/m^2)	CUBN	rs1801231	-1.98	0.99	0.0451	-0.08	0.03	0.0183	0.09	0.04	0.0290
BMI (kg/m^2)	CD36	rs1537593	-1.87	1.04	0.0711	-0.06	0.03	0.0267	0.09	0.04	0.0298
BMI (kg/m^2)	TLR4	rs11536889	2.84	1.49	0.0572	-0.02	0.03	0.5094	-0.12	0.06	0.0336
BMI (kg/m^2)	MTRR	rs1801394	1.30	0.75	0.0841	0.01	0.04	0.7341	-0.06	0.03	0.0452
BMI (kg/m^2)	IL-18R1	rs3732127	-1.34	0.73	0.0661	-0.08	0.03	0.0048	0.06	0.03	0.0469
BMI (kg/m^2)	IL-18R1	rs7558013	1.67	0.97	0.0869	-0.03	0.03	0.3101	-0.07	0.04	0.0486
Glucose (mg/dL)	MTHFR	rs1801133	-4.56	1.82	0.0125	-0.28	0.09	0.0014	0.24	0.08	0.0025
Glucose (mg/dL)	BCL2	rs4940574	-3.48	2.15	0.1055	-0.19	0.08	0.0214	0.19	0.09	0.0260
Glucose (mg/dL)	GATA-3	rs1058240	-5.39	2.43	0.0270	-0.19	0.09	0.0267	0.22	0.10	0.0349
Glucose (mg/dL)	BCL2	brs4940576	-5.45	2.20	0.0136	-0.21	0.09	0.0244	0.18	0.09	0.0372
Glucose (mg/dL)	MTR	rs1805087	4.39	2.06	0.0336	-0.07	0.09	0.4623	-0.20	0.10	0.0406
HDL (mg/dL)	IL-21	rs13143866	8.90	2.45	0.0003	0.26	0.09	0.0034	-0.35	0.09	0.0002*
HDL (mg/dL)	CD14	rs2563298	7.13	2.47	0.0040	0.25	0.09	0.0039	-0.25	0.09	0.0072

Table 3.4. Top hit (p < 0.05) maternal SNP X offspring 25[OH]D interactions in association with offspring CMR outcomes. (continued)

			SNP association			Vitamin D association			SNP X Vitamin D Interaction		
Outcome	Gene	SNP	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
HDL (mg/dL)	MTHFD1L	rs175866	6.57	2.54	0.0099	0.31	0.11	0.0060	-0.24	0.10	0.0139
HDL (mg/dL)	CYP2R1	rs1993116	-6.75	2.73	0.0135	-0.07	0.13	0.5950	0.23	0.11	0.0381
HDL (mg/dL)	BCL2	rs1801018	-3.16	2.38	0.1845	-0.05	0.10	0.6033	0.20	0.10	0.0413
HDL (mg/dL)	LTA	rs1041981	6.80	2.87	0.0180	0.20	0.09	0.0177	-0.23	0.11	0.0414
HDL (mg/dL)	IL-1R2	rs3218984	-7.27	2.96	0.0141	-0.01	0.09	0.8780	0.25	0.13	0.0482
LDL (mg/dL)	IL-1R2	rs4851522	26.22	5.89	0.00001	0.61	0.19	0.0017	-1.12	0.24	0.000004*
LDL (mg/dL)	IL-1R2	rs4851526	13.61	5.01	0.0067	0.65	0.23	0.0040	-0.62	0.20	0.0018
LDL (mg/dL)	IL-18R1	rs3771170	12.11	5.72	0.0344	0.51	0.23	0.0246	-0.68	0.23	0.0030
LDL (mg/dL)	IL-18R1	rs2287033	8.00	4.58	0.0813	0.56	0.25	0.0240	-0.41	0.18	0.0191
LDL (mg/dL)	IL-18R1	rs7579737	-8.02	5.38	0.1367	-0.16	0.22	0.4608	0.46	0.22	0.0338
LDL (mg/dL)	IL-1A	rs3783546	11.43	6.03	0.0584	0.46	0.24	0.0537	-0.49	0.25	0.0490
ln(Triglycerides)	BCL2	rs1801018	0.20	0.08	0.0095	0.00	0.00	0.3360	-0.01	0.00	0.0080
ln(Triglycerides)	CD14	rs5744455	0.24	0.10	0.0137	0.00	0.00	0.8687	-0.01	0.00	0.0081
ln(Triglycerides)	IL-1B	rs1143634	0.15	0.09	0.0708	0.00	0.00	0.6366	-0.01	0.00	0.0246
ln(Triglycerides)	IL-1A	rs2856836	0.19	0.09	0.0304	0.00	0.00	0.5310	-0.01	0.00	0.0381
ln(Triglycerides)	TNFRSF1A	rs4149578	0.25	0.12	0.0408	0.00	0.00	0.6838	-0.01	0.01	0.0387
ln(Triglycerides)	CD53	rs2966952	0.21	0.11	0.0531	0.00	0.00	0.2988	-0.01	0.00	0.0470
ln(Triglycerides)	IL-18	rs549908	0.17	0.10	0.0848	0.00	0.00	0.6516	-0.01	0.00	0.0495
Systolic BP (mmHg)	IL-6R	rs4845374	5.34	1.82	0.0034	0.10	0.06	0.0804	-0.23	0.07	0.0015
Systolic BP (mmHg)	IL-6	rs2069840g	5.50	1.59	0.0005	0.13	0.06	0.0428	-0.19	0.06	0.0032
Systolic BP (mmHg)	IL-21	rs2055979	-3.04	1.51	0.0443	-0.15	0.07	0.0417	0.18	0.06	0.0034
Systolic BP (mmHg)	IL-18	rs3882891	3.27	1.50	0.0293	0.17	0.07	0.0178	-0.16	0.06	0.0068
Systolic BP (mmHg)	CD14	rs5744455	2.85	1.66	0.0874	0.07	0.06	0.2159	-0.16	0.07	0.0129
Systolic BP (mmHg)	LRP2	rs2075252	3.36	1.77	0.0577	0.08	0.06	0.2175	-0.18	0.08	0.0162
Systolic BP (mmHg)	IL-12(B)	rs3212227	-4.46	1.83	0.0151	-0.07	0.06	0.2512	0.19	0.08	0.0222
Systolic BP (mmHg)	IL-10	rs3024498	3.35	1.95	0.0866	0.04	0.06	0.5224	-0.18	0.08	0.0257
Systolic BP (mmHg)	IL-6R	rs4845623	-3.14	1.33	0.0185	-0.07	0.07	0.2785	0.12	0.05	0.0261
Systolic BP (mmHg)	IL-18	rs5744280	-2.28	1.46	0.1188	-0.07	0.07	0.3118	0.12	0.06	0.0411

Table 3.4. Top hit (p < 0.05) maternal SNP X offspring 25[OH]D interactions in association with offspring CMR outcomes. (continued)

			SNP association			Vitamin D association			SNP X Vitamin D Interaction		
Outcome	Gene	SNP	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
Diastolic BP (mmHg)	LRP2	rs2075252	4.10	1.46	0.0050	0.04	0.06	0.4735	-0.20	0.06	0.0012
Diastolic BP (mmHg)	MTRR	rs162036	-3.75	1.70	0.0278	-0.13	0.05	0.0177	0.19	0.06	0.0032
Diastolic BP (mmHg)	IL-6	rs2069840	3.69	1.29	0.0043	0.04	0.05	0.4132	-0.15	0.05	0.0043
Diastolic BP (mmHg)	IL-12(B)	rs3212227	-4.44	1.86	0.0168	-0.13	0.05	0.0124	0.20	0.08	0.0088
Diastolic BP (mmHg)	MTRR	rs10380	-3.47	1.77	0.0509	-0.11	0.05	0.0371	0.16	0.06	0.0128
Diastolic BP (mmHg)	FOXP3	rs3761549	6.33	2.27	0.0053	0.00	0.05	0.9808	-0.21	0.08	0.0135
Diastolic BP (mmHg)	IL-6R	rs4845374	3.36	1.58	0.0339	0.01	0.05	0.8423	-0.14	0.06	0.0183
Diastolic BP (mmHg)	MTHFD1L	rs572522	-2.03	1.19	0.0882	-0.14	0.05	0.0120	0.10	0.04	0.0196
Diastolic BP (mmHg)	MTHFR	rs1801131	2.91	1.13	0.0104	0.03	0.06	0.6578	-0.10	0.04	0.0219
Diastolic BP (mmHg)	IL-6R	rs4845623	-2.43	1.17	0.0384	-0.12	0.06	0.0402	0.10	0.05	0.0319
Diastolic BP (mmHg)	TLR3	rs3775291	2.93	1.35	0.0306	0.01	0.06	0.8829	-0.11	0.05	0.0323
Diastolic BP (mmHg)	IL-18	rs3882891	2.43	1.22	0.0464	0.06	0.06	0.3310	-0.10	0.05	0.0394

*Met Benjamini-Hochberg-adjusted criteria for statistical significance.

Figure 3.1. Offspring CMV IgG modifies the association between maternal rs987853 genotype and offspring Systolic BP among study participants

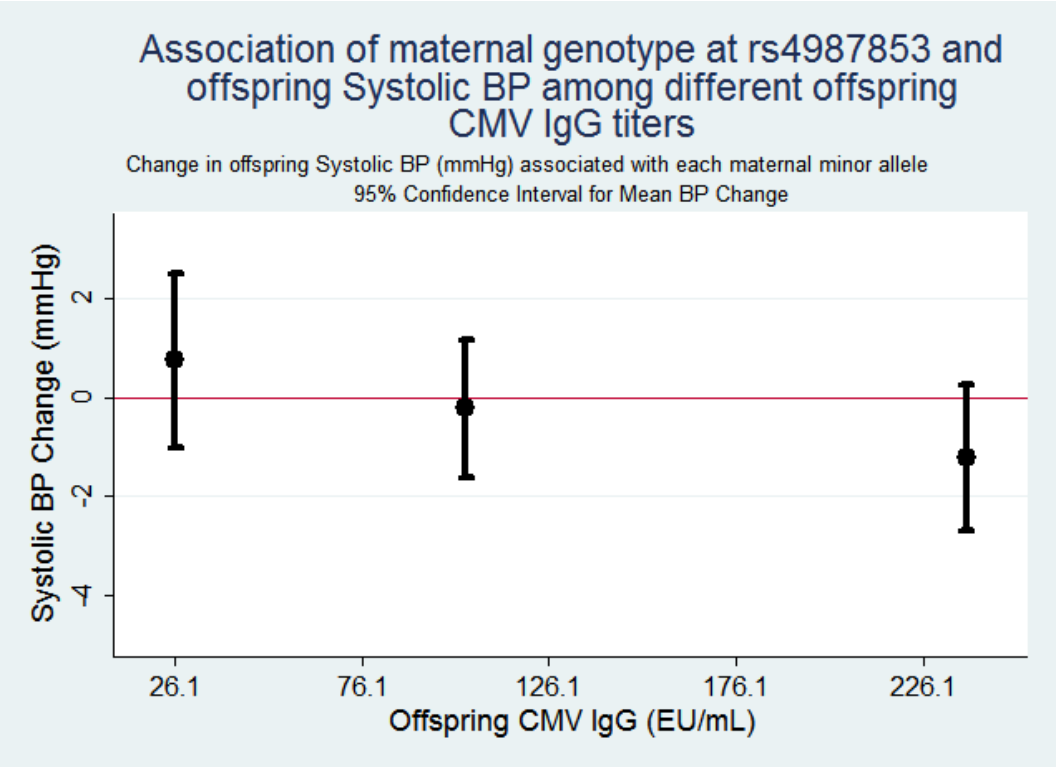


Figure 3.2. Offspring 25[OH]D modifies the association between maternal rs4851522 genotype and offspring LDL cholesterol among study participants

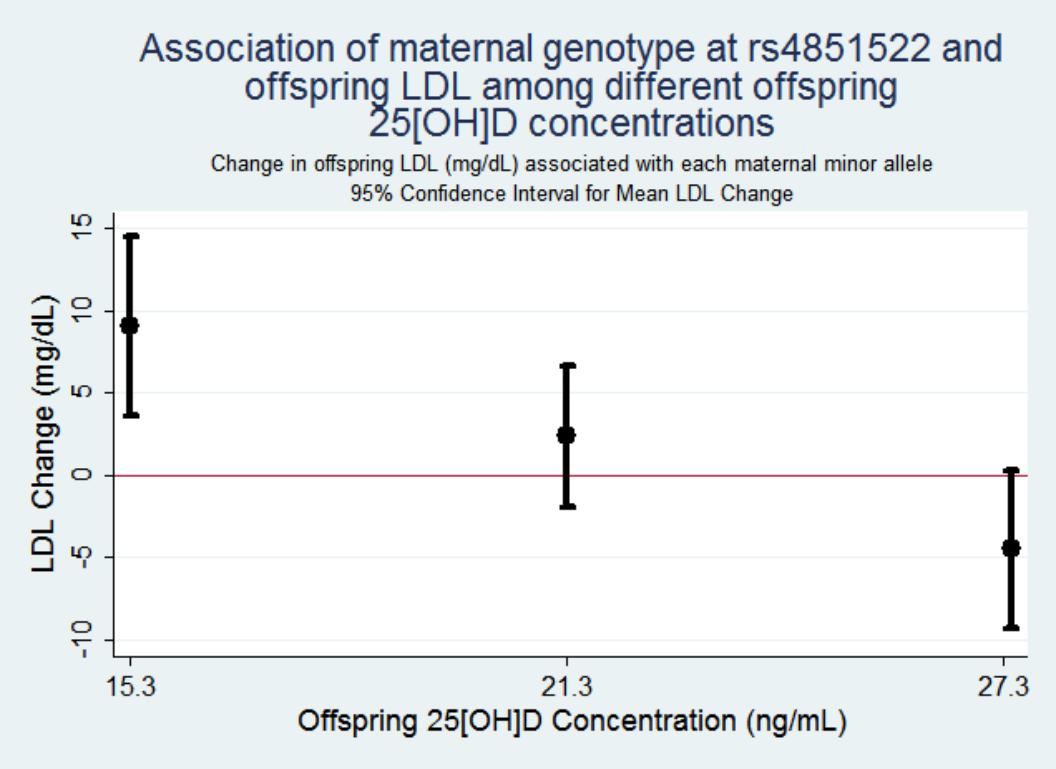
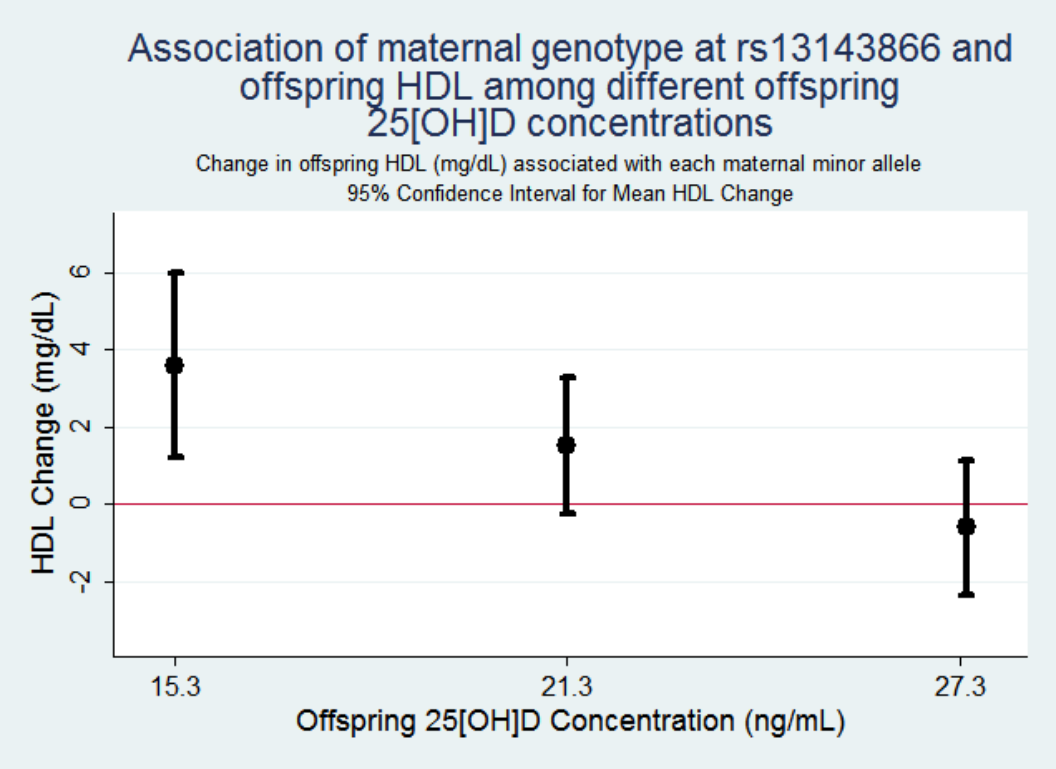


Figure 3.3. Offspring 25[OH]D modifies the association between maternal rs13143866 genotype and offspring HDL cholesterol among study participants



Discussion

In the current study, we investigated associations of candidate maternal SNPs in immune/vitamin D genes with various offspring adult CMR, along with exploratory investigation of maternal SNP-offspring CMV IgG and maternal SNP-offspring 25[OH]D interactions on offspring CMR outcomes. In our population, we failed to find evidence for associations of maternal genotype with offspring CMR. However, we found some evidence that maternal genetic variants interact with offspring adult CMV IgG or 25[OH]D concentration on adult CMR. Maternal genotype at the rs4987853 SNP in the BCL2 gene interacted with offspring CMV IgG on HDL cholesterol ($p = 0.0003$). Maternal genotype at the rs4851522 locus in the IL-1R2 gene interacted with offspring 25[OH]D concentration on LDL cholesterol ($p < 0.0001$), and maternal rs13143866 in IL-21 with offspring 25[OH]D concentration on HDL cholesterol ($p = 0.0002$). In addition, several SNP-CMV IgG and SNP-vitamin D interactions were identified with p-values < 0.05, our exploratory significance level.

To our knowledge, we present the first analyses of maternal immune- and vitamin D-related genotype and offspring CMR associations outside of the context of obesity. In a previous report in the JPS-1 population, maternal variation in candidate genes related to fetal development and CMR, not examined in this analysis, were found to explain some of the relationship between maternal gestational weight gain and offspring adult BMI (169). In that study by Wander, et al., genetic risk scores constructed from their candidate genes attenuated the magnitude of the association between maternal gestational weight gain and adult offspring BMI by 41%, but did not attenuate the relationship between maternal pre-preganancy BMI and offspring BMI. In our study, we did not find statistically significant associations between maternal genetic variation and offspring adult CMR, after multiple testing correction. Additional exploration of our negative findings suggests that for the sizes of the associations (Table 3.2), our study was underpowered. If indeed maternal genetic variation in SNPs of immune- and vitamin D –related genes are associated with adult offspring CMR, effect sizes may be smaller than we anticipated. We did find two maternal SNPs in SLC2A1 among our top ($p < 0.01$) hits in association with offspring triglycerides. Exploratory analysis of genotype combinations at these two loci (Supplementary Table S3.5) did not provide evidence for a haplotype association of maternal SLC2A1 and offspring triglycerides.

In exploratory analyses of maternal SNP-offspring CMV IgG and maternal SNP-offspring 25[OH]D interactions on adult offspring CMR, we found evidence of statistical interaction with a few SNPs in each case (Tables 3.3, 3.4). We report here an interaction between the maternal BCL2 SNP rs4987853 and offspring CMV IgG on offspring HDL. This particular SNP is in the 3'UTR region, but is not known to cause functional changes in the BCL2 protein, nor has it been reported to be associated with other disease phenotypes. This association was fairly robust to changes in covariate adjustment and addition of a variable representing offspring rs4987853 genotype to the model, but was attenuated by the addition of the interaction between offspring rs4987853 genotype and offspring CMV IgG to the model (Supplementary Table S3.2). In addition, the interaction between offspring rs4987853 genotype and offspring CMV IgG on offspring HDL was observed in our population (p for interaction = 0.001), though not statistically significant after multiple testing correction. It is possible that the statistically significant interaction with maternal genotype is truly an interaction between offspring CMV IgG and offspring genotype, as maternal and offspring genotypes are correlated. BCL2 codes for a protein that suppresses apoptosis of cells (180), and maternal BCL2 is important in embryo implantation and survival (181) and fetal-maternal alignment of regulatory T cells correlates with BCL2 upregulation during pregnancy (182), indicating that maternal BCL2 may have intergenerational effects. Regulatory T cells, or an imbalance between regulatory and Th1 T cells, have been implicated in lipid metabolism (183) and atherosclerotic changes (183, 184). A study of low birthweight, overweight children found a relationship between BCL2 function, measured by a biomarker of apoptosis, and lipid homeostasis (185). Furthermore, there is evidence that CMV may inhibit apoptosis of infected cells by modulating host BCL2 family proteins (185); specifically, that viral proteins reduce BAX levels (186); BAX itself heterodimerizes with BCL2 during the apoptosis process (180). Our findings of an association between a maternal BCL2 variant and lower offspring HDL cholesterol at higher levels of offspring CMV IgG may relate to complex interactions between regulatory T cells, immune-directed apoptosis of virally infected cells, and intergenerational immune programming.

Of note, the interaction between maternal IL1R2 SNP rs4851522 and offspring 25[OH]D concentration on offspring LDL met the criteria for statistical significance that we applied to our main effects analyses. Specifically, we found that the presence of one or more maternal minor alleles inverts

the association of offspring 25[OH]D and LDL (Figure 3.2). This association did not change with the addition of offspring genotype at this locus to the model (Supplementary Table 3.3). Additional exploration of models with dominant and recessive genotype demonstrated that the interaction was fairly robust to changes in the genotype model (Supplementary Table S3.7). The SNP rs4851522 is an intronic variant within IL-1R2, a decoy receptor that regulates the pro-inflammatory cytokine IL-1 by binding to it and preventing its binding to signaling receptors (187). It is not associated with any phenotypes in the literature to date, nor is this intronic SNP predicted to result in functional protein changes. IL-1R2 expression is induced by the Th2 cytokine, IL-4 (187). Its expression is lower in monocytes from individuals with familial combined hyperlipidemia , and *in vitro* studies show a decrease in expression of IL-4, an inducer of IL-R2 (188), when incubated with low density lipoproteins (187). The active form of 25[OH]D increases the transcription of IL-1B, a ligand of IL-1R2 (189), but also induces production of IL-4 (190), an inducer of IL-1R2, in a VDR-dependent manner (188). Interaction between 25[OH]D and IL-1R2 is therefore plausible, but replication of our finding and biological study is necessary to elucidate the exact mechanism. The link between vitamin D, lipids, and the immune system is demonstrated in several rare conditions. For instance, in individuals with familial combined hypercholesterolemia often have low 25[OH]D concentrations, which has been shown to improve with statin therapy (191). In patients receiving treatment not including statins for rheumatoid arthritis, treatment reduces dyslipidemia along with disease symptoms (192, 193). Khoo, et al., showed that both 25[OH]D and IL-1B, among other cytokines, exhibit an inverse pattern of seasonal variation (194). IL-1B, a ligand of IL-1R2, has been shown *in vitro* to be downregulated (195) and upregulated (26, 189) in activated macrophages. In reference to the somewhat contradictory results of 1,25[OH]2D3 on various aspects of the immune system, Cantorna, et al., suggest that host vitamin D status, local expression of vitamin D metabolism genes, and other factors impact the effects of vitamin D on immune function (196), which is consistent with the results presented here, if not especially illuminating.

We report here an interaction between the maternal IL-21 SNP rs13143866 and offspring 25[OH]D concentration on offspring HDL. This interaction is fairly robust to the inclusion or exclusion of covariates in the model (Supplementary Table S3.3), including the offspring SNP and its interaction with offspring 25[OH]D. IL-21 is a Th2 cytokine that inhibits the differentiation of naïve helper T cells into Th1

cells (197). Deficiency of the active form of vitamin D increases differentiation into Th17 cells, which in turn normally have upregulated IL-21 production, but does not specifically increase transcription of IL-21 in Th17 cells (198). IL-21 can down-regulate IL-2 expression and partially substitute for the role of IL-2, except for the differentiation of regulatory T cells (199). This particular SNP has been associated with Graves' Disease (200), recurrent spontaneous miscarriage (201), and Juvenile Idiopathic Arthritis (202), indicating that it could play a role in altering the intrauterine environment via the maternal immune system, though as an intronic SNP it does not yet have a known functional consequence. Previous studies have demonstrated the role of an inappropriate Th1 bias in atherosclerosis (43, 203), and HDL cholesterol inhibits the development of atherosclerotic lesions via cholesterol transport and inhibition of foam cells (204). The interaction presented here reports an association between maternal rs13143866 and increased HDL cholesterol only in the presence of vitamin D deficiency. An imbalance between Th17 and regulatory T cells has been implicated in a mouse model of atherogenesis (184), providing some support for how variation in IL-21 could contribute to altered cholesterol levels within the same individual; however, the literature does not currently provide studies either supporting or eliminating the role of maternal IL-21 variation on adult offspring cholesterol levels, nor the modulating role of offspring 25[OH]D. While our findings require replication before more focused investigation can take place, our results, in combination with previous studies (162, 205) emphasize the importance of effect modification by offspring-related environmental factors when studying the lasting effects of maternal genotype on their offspring. In general, maternal genetic effects can occur in two ways: inheritance or effects on the intrauterine environment. We do not see evidence that the effect modification of maternal IL-1R2 is mediated in some way by the offspring allele (Supplementary Table 3.3), nor is IL-1R2 known to be in an imprinted region, which precludes the suspicion of parent-of-origin effects. The other mechanism for the effect is due to influence of genotype on the intrauterine environment. In our particular results, the perturbation in the intrauterine environment resulting from maternal allelic variation of IL-1R2 would need to program an offspring LDL cholesterol pathway to respond differently to the active metabolite of 25[OH]D. Such mechanistic evidence does not currently exist and is needed to determine whether the association reported here is causal.

Strengths of the current study include the following. Detailed records of maternal and offspring characteristics obtained at several timepoints were available in the JPS. We measured CMR in offspring at a relatively young age, before the development of multiple comorbidities that would potentially complicate the analysis. Because of availability of measures of adult offspring biomarkers, CMV IgG and 25[OH]D concentration, that represent offspring exposures, we were able to examine gene-environment interactions between maternal genotype and offspring characteristics on offspring CMR.

There are also a number of limitations to this study. We analyzed a limited number of candidate SNPs. Specific lifestyle and demographic characteristics of our population, such as the narrow geographical range and nearly 17% of our participants self-identifying as of the Ultra-Orthodox Jewish religion may limit the generalizability of our findings. We lack biomarker measurements of the intrauterine environment that would facilitate the distinction between direct and indirect effects of maternal genotype. Finally, all of our measures of CMR were taken at the same time point, making the temporality of CMV IgG and 25[OH]D associations with CMR questionable.

In conclusion, our findings indicate that maternal genetic effects may play a role in adult offspring CMR development. Future work should involve additional genetic variants to better characterize immune- and vitamin D-related pathways in larger study populations to enable the investigation of small associations. Mechanisms behind observed associations also constitute other potential future areas of research. Similar work in carefully designed studies has the potential to better our understanding of the life course and developmental origins of CMR and cardiovascular diseases.

Chapter 4. Maternal-offspring candidate immune and vitamin D-related genotype interactions and offspring adult cardiometabolic risk

Abstract

While previous work demonstrates the early life origins of cardiometabolic risk factors (CMR), the role of interaction between maternal and offspring genetic variants in this process remains under-investigated. In a population-based birth cohort study, the Jerusalem Perinatal Study, we examined associations of immune- and vitamin D–associated candidate single nucleotide polymorphisms (n = 91) with offspring adult (age 32) cytomegalovirus antibody titers, 25[OH]D concentrations, body mass index, glucose, fasting lipids, and blood pressure. Maternal-offspring genotype interactions were assessed using interaction terms included in weighted linear regression models. We found evidence for maternal-offspring genotype interaction in relation to offspring diastolic blood pressure at the MTRR locus rs1532268 (p = 0.0004), with suggestive interaction on offspring systolic blood pressure (p = 0.0008). Other suggestive interactions were found at MTHFD1L SNPs rs572522 and rs4869959 in offspring triglycerides (p = 0.0.0005, 0.0020, respectively). Future larger studies have the potential to enhance understanding of contributions of maternal-offspring genotype interactions to the developmental origins of CMR.

Introduction

While previous work demonstrates the early life origins of cardiometabolic risk (CMR) and cardiovascular diseases (CVD) (2, 3), the role of maternal-offspring genotype interactions in this process remains underexplored. Previous research investigating interactions between maternal and offspring genetic variation has largely focused on a small but diverse group of offspring outcomes: hemolytic disease of the newborn (10), neural tube defects (13), and schizophrenia (14). For example, in maternal-fetal Rh incompatibility a sensitized Rh-negative mother produces antibodies against her Rh-positive fetus; this results in hemolytic disease of the newborn and was formerly a major cause of neonatal morbidity and mortality (10). On the other hand, maternal and offspring genotype interactions have been reported for maternal outcomes that directly influence the intrauterine environment, and thus fetal growth and programming. For example, in preeclampsia, maternal-offspring genetic interactions involving multiple genes in different pathways have been implicated, including IGF1 involved in offspring growth (11), LTA involved in inflammation, VWF in coagulation, and COL4A2 in tissue structure (12).

Intergenerational gene-gene interaction studies in adult CMR development have been limited by the long time period it takes for the phenotype to manifest and the difficulty of accruing appropriate study populations with genotypic data on both mothers and their offspring. The importance of identifying "missing heritability" in the post-Genome-Wide Association Study era motivates further work in this area. Interestingly, recent evidence suggests the importance of mother-offspring genotype interactions in the persistence of risk alleles in the population (206). Maternal-offspring genotype interactions may influence outcomes through their effect on the intrauterine environment, both of which can have long term consequences on offspring CMR.

Immune response and vitamin D concentration are related to a diverse set of physiologic functions and abnormalities have been linked to adverse consequences on maternal and perinatal health with direct implications on the intrauterine environment in both animal (33, 207) and human (86-93, 208-211) studies. Menon, et al., report a three-locus interaction between maternal TNFA, IL-6, and IL-6R and spontaneous preterm birth (211). Variation in genes that influence immune response and inflammation (212-215), as well as vitamin D metabolism (158, 216, 217), have also been associated with CMR. However, maternal-offspring genotype interactions have not been examined in the context of early life

origins of offspring adult CMR. To this end, we selected 97 SNPs in 42 genes in immune function, vitamin D metabolism, and imprinted regions for maternal and offspring genotype interaction analyses with respect to offspring adult CMR in an existing cohort of mother-offspring dyads.

Methods

Study setting and study population

The current study was conducted in the setting of the Jerusalem Perinatal Study (JPS). JPS is a population-based birth cohort study that includes detailed post-partum records of all births to residents of Jerusalem between 1974 and 1976. Detailed information on data collection has been published previously (117). Data on sociodemographics, maternal medical conditions, and offspring birthweight was abstracted from medical records at the time of birth. Additional information on lifestyle and maternal medical conditions was collected during interviews on the first or second day postpartum. The JPS Family Follow-Up Study (JPS-1) recruited a stratified sub-sample of 1,500 offspring and their mothers from the original 1974-1976 JPS birth cohort who were recontacted, interviewed, and examined between 2007 and 2009. Offspring in JPS-1 were singleton, term (≥ 36 weeks) live births without congenital malformations. Offspring were oversampled for low (≤2500g) and high (≥4000g) birthweight and for having had mothers categorized as overweight (≥27kg/m^2 BMI) before pregnancy.

For the current study, offspring who self-reported taking medication for high blood pressure (n = 22), high cholesterol (n = 20), or diabetes (n = 22) were excluded, as were offspring with a fasting time that was missing (n = 234) or fewer than eight hours (n = 97). A maximum of 1,194 mother-offspring dyads were available for analysis after these exclusions. Study procedures were approved by the Institutional Review Boards of the University of Washington, Seattle (USA), and the Hadassah-Hebrew University Medical Center in Jerusalem (Israel). All participants provided informed consent.

Data collection

At the time of data collection for JPS-1, the mean age of offspring was 32 (range 31-33 years). Data on offspring sociodemographic, lifestyle, and anthropomorphic characteristics were collected during physical exams and telephone interviews conducted between 2007 and 2009. BMI was calculated as weight (kg) divided by squared height (m^2). Blood pressure (BP) was measured as the average of three consecutive measurements performed after five minutes of sitting (Omron M7 automated sphygmomanometer). Fasting blood samples were taken using standard procedures. Plasma glucose, HDL, LDL, and triglycerides were measured on the VITROS 5,1FS Chemistry System (Ortho Clinical

Diagnostics, Raritan, NJ). Plasma vitamin D (25[OH]D) was measured using liquid chromatography tandem mass spectroscopy at the Nutrition and Obesity Research Center, University of Washington (Seattle, WA). The sum of 25[OH]D2 and 25[OH]D3 was used in analyses to represent total plasma vitamin D concentration. CMV antibody titers (CMV IgG) were determined by enzyme immunoassay technology using the Immunosimplicity® Is-CMV IgG Test Kit (Diamedix Corp. Miami, Florida). Antibody titers are reported as EU/mL, with a value ≥ 10.0EU/mL considered positive for anti-CMV IgG. CMV IgG assays were performed at the University of Vermont Laboratory for Clinical Biochemistry Research (Burlington, VT).

Gene/SNP selection and genotyping

SNPs were selected based on previously published work, known functional and/or biological significance, relation to the immune system and vitamin D metabolism, and/or previously described imprinting regions. Extraction and amplification of DNA from maternal and offspring blood samples was conducted using standard methods. Genotyping was conducted using an Illumina, Inc., BeadArray™ (Illumina, City, State) and TaqmanOpenArray assays (Life Sciences, Foster City, CA) at the University of California, San Francisco. An expected minor allele frequency of at least 20% among mothers and offspring was used to select the SNPs for the final set. SNPs were required to be in Hardy-Weinberg Equilibrium in both generations. A detailed list of the 91 SNPs is shown in Supplementary Table S1.

Statistical Analyses

We examined participant characteristics in the entire study cohort. Means and standard deviations were calculated for continuous variables; numbers and percentages for categorical variables. We used weighted multiple linear regression models to examine associations between genotype, modeled additively, and CMR (BMI, fasting glucose, HDL, LDL, triglycerides, systolic blood pressure (BP), and diastolic BP), CMV IgG, and 25[OH]D.

Interaction terms were created as the product of maternal genotype and offspring genotype, assuming an additive genetic model. An example regression equation is shown in equation 1:

(1)

$$E[Y] = B_0 + B_1 O_G + B_2 M_G + B_3 (O_G * M_G) + B_4 * covariate$$

The interaction term (M_G*O_G) can take on the values 0, 1, 2, and 4. In general, the coefficient of interest, β_3, can be interpreted as the expected change in the outcome *Y* associated with each additional minor allele, whether belonging to the mother or the offspring, among dyads where both mother and offspring already have at least one copy of the minor allele, beyond the expected change in *Y* associated with the additional minor allele in dyads where either mother or offspring is homozygous for the major allele. Main effects terms for both offspring and maternal genotypes (β_1 and β_2, respectively) were also included in the model. Ninety-five percent confidence intervals and p-values were calculated using robust standard error estimates. The outcome triglycerides was natural log-transformed to improve model fit. CMV IgG was square root-transformed to improve model fit due to several orders of magnitude contained within the range of CMV IgG concentrations.

Our models were adjusted for season of blood draw (December-February, March-May, June-August, September-November) and additional covariates for smoking status (current, former, and never), alcohol abstinence (yes/no), intense physical activity participation (yes/no), employment status (yes/no), religiosity (Ultra-Orthodox, Religious, and Traditionalist/Secular/Other), socio-economic status (low, medium, and high), birthweight, maternal smoking during pregnancy (yes/no), sex, and grandparent country of origin (Yemen, Kurdistan, Morocco, Iran, Iraq, Other African, Other Asian, and Ashkenazi, using Israel as baseline), and BMI except when BMI was the outcome of interest. All covariates, except offspring birthweight and maternal smoking during pregnancy, were measured in offspring at approximately age 32.

We used the Benjamini-Hochberg step-up procedure to adjust for multiple comparisons (118). Briefly, this procedure controls the False Discovery Rate (FDR) at our chosen level of 0.05. P-values are ranked from smallest to largest and compared to $0.05*k/m$, where *m* is the total number of tests performed and *k* is the rank of the test. All tests with *k* smaller than the largest *k* such that $p_{(k)} \leq 0.05*k/m$ are rejected. Each of the nine outcomes was evaluated separately.

Analyses were performed using Stata v10.0 (StataCorp, College Station, TX).

Results

The list of evaluated SNPs (n = 91) for this analysis is shown in Supplementary Table S1 and offspring characteristics in Table 4.1. Top hits ($p < 0.01$) for the candidate SNP maternal-offspring interaction analyses are presented in Table 4.2. No interactions met our pre-specified criteria for statistical significance after multiple testing correction.

Table 4.1. Offspring Study Participant Characteristics.

		Total	
		N = 1194*	
CMV IgG, EU/mL (mean, sd)		179.9	242.7
Total D, ng/mL (mean, sd)		21.8	9.0
BMI, kg/m^2 (mean, sd)		26.2	5.1
Glucose, mg/dL (mean, sd)		80.0	15.3
Triglycerides, mg/dL (mean, sd)		105.1	72.9
LDL, mg/dL (mean, sd)		112.3	28.8
HDL, mg/dL (mean, sd)		50.1	14.6
Systolic BP, mmHg (mean, sd)		105.7	11.9
Diastolic BP, mmHg (mean, sd)		71.2	8.1
Birthweight, grams (mean, sd)		3409.1	618.7
Smoking Status (n, %)	non	691	59.4
	former	154	13.2
	current	318	27.3
Alcohol Abstain (n, %)	yes	544	46.6
Intense Physical Activity (n, %)	yes	343	29.4
Employed (n, %)	yes	963	83.7
Orthodoxy (n, %)	Ultra-orthodox	226	18.9
	Religious	242	20.3
	Traditionalist, Secular, or Other	726	60.8
Season (n, %)	winter	332	27.8
	spring	191	16.0
	summer	340	28.5
	autumn	331	27.7
SES (n, %)	low	236	22.7
	medium	369	35.6
	high	592	41.8
maternal smoking (n, %)	yes	181	17.4

s.d. = Standard Deviation

*May not sum to total due to missing data

†Defined as exercise that brings about labored breathing, increased pulse rate, and sweating, lasting for at least twenty minutes.

Table 4.2. Results of maternal-offspring genotype interaction analysis. Top hits (p-value < 0.01)

			Offspring SNP association			Maternal SNP association			Maternal-Offspring Genotype Interaction		
Outcome	Gene	SNP	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
25[OH]D (ng/mL)	TLR3	rs3775291	-0.70	0.90	0.4346	-2.03	0.73	0.0057	2.05	0.73	0.0049
25[OH]D (ng/mL)	IL-1R2	rs3218984	-2.02	0.84	0.0160	-0.92	0.91	0.3104	2.75	1.00	0.0063
BMI (kg/m^2)	BCL2	rs4940574	-0.54	0.50	0.2781	-1.40	0.52	0.0076	1.80	0.60	0.0029
BMI (kg/m^2)	SLC2A1	rs710221	-0.93	0.52	0.0741	-1.07	0.51	0.0370	1.16	0.44	0.0090
HDL (mg/dL)	IL-10	rs3024498	3.56	2.15	0.0980	1.95	1.51	0.1979	-5.64	1.97	0.0043
LDL (mg/dL)	BCL2	rs4940576	5.47	3.28	0.0953	6.53	3.18	0.0404	-8.72	3.17	0.0061
ln(Triglycerides)	MTHFD1L	rs572522	0.21	0.06	0.0002	0.12	0.05	0.0263	-0.16	0.05	0.0005
ln(Triglycerides)	MTHFD1L	rs4869959	0.17	0.06	0.0044	0.06	0.05	0.2322	-0.17	0.05	0.0020
Systolic BP (mmHg)	MTRR	rs1532268	-2.23	1.19	0.0605	-4.01	1.20	0.0009	3.49	1.03	0.0008
Systolic BP (mmHg)	BCL2	rs2046136	2.74	1.10	0.0126	2.33	1.06	0.0277	-3.44	1.12	0.0022
Diastolic BP (mmHg)	MTRR	rs1532268	-2.11	0.99	0.0328	-3.45	0.96	0.0003	2.99	0.84	0.0004
Diastolic BP (mmHg)	KCNQ1	rs231362	-2.21	0.97	0.0236	-2.37	0.94	0.0117	2.37	0.89	0.0081

Table 4.3. Linear combinations of coefficients comparing the expected change in systolic blood pressure for mother-offspring genotype combinations at MTRR SNP rs1532268, compared to both mother and offspring homozygous for the major allele.

Number of maternal alleles	Number of offspring alleles	Number of dyads	Coefficient	Standard Error	p-value
2	2	43	1.49	1.47	0.3120
1	2	76	-1.49	1.42	0.2950
0	1	91	-2.23	1.19	0.0610
1	1	162	-2.75	1.07	0.0110
2	1	69	-3.26	1.44	0.0240
1	0	119	-4.01	1.20	0.0010

Table 4.4. Linear combinations of coefficients comparing the expected change in diastolic blood pressure for mother-offspring genotype combinations at MTRR SNP rs1532268, compared to both mother and offspring homozygous for the major allele.

Number of maternal alleles	Number of offspring alleles	Number of dyads	Coefficient	Standard Error	p-value
2	2	43	0.85	1.25	0.4940
1	2	76	-1.68	1.17	0.1520
0	1	91	-2.11	0.99	0.0330
1	1	162	-2.57	0.85	0.0030
2	1	69	-3.02	1.15	0.0090
1	0	119	-3.45	0.96	< 0.0005

The maternal-offspring interaction at rs1532268 in MTRR was also among the top hits for both systolic (p = 0.0031, Table 4.5) and diastolic (p = 0.0017, Table 4.6) BP, and the interaction coefficient β_3 is in the opposite direction from the main effects (Table 4.2). The stratum where both mother and offspring were homozygous for the minor allele contained 43 dyads. Compared to dyads where both mother and offspring are homozygous for the major allele, offspring who are also homozygous for the major allele, but whose mothers are heterozygous, have an expected systolic blood pressure 4.11mmHg lower (SE = 1.23, p = 0.001) and expected diastolic blood pressure 3.50mmHg lower (SE = 0.98, p < 0.0005). Offspring belonging to dyads where the only copy of the minor allele in the dyad is of paternal origin have blood pressures that are not expected to be different from dyads in which both are homozygous for the major allele (systolic p = 0.198, diastolic p = 0.081). However, in both types of blood pressure, the presence of additional minor alleles attenuates the relationship.

The mother-offspring genotype interaction at a MTHFD1L SNP (rs572522) on triglycerides had a p-value for interaction of 0.0009. Offspring of mother-offspring dyads where both were homozygous for the major allele had similar triglyceride levels compared with offspring in mother-offspring dyads where both were homozygous for the minor allele (ratio = 1.02) (Table 4.5). In contrast, dyads with other genotype combinations had slightly elevated triglycerides compared to dyads where both mother and offspring were homozygous for the major allele or the minor allele. Furthermore, nonsignificant interaction was observed at another MTHFD1L SNP locus (rs4869959) (p-value = 0.0020) in relation to triglycerides, with similar magnitudes of association for offspring main effects and maternal-offspring interaction to those observed for the MTHFD1L SNP rs572522 (Table 4.2).

Sensitivity analysis to determine the impact of covariate adjustment did not reveal substantial influence of BMI (Supplementary Table 4.1) nor of other covariates (Supplementary Table 4.2).

Table 4.5. Expected ratio of triglycerides compared to a mother-offspring dyad where both are homozygous for the major allele at rs572522.

		Number of offspring minor alleles		
		0	1	2
Number of maternal minor alleles	0	reference	1.23	NA
	1	1.12	1.18	1.25
	2	NA	1.14	1.02

Discussion

In this study, we found limited evidence for maternal-offspring genotype interactions at candidate SNPs in candidate genes of immune- and vitamin D-related pathways. We observed a significant maternal-offspring interaction at MTRR SNP rs1532268 on both diastolic BP, and a nonsignificant interaction in systolic BP and at multiple SNPs (rs572522 and rs4869959) in the MTHFD1L gene on offspring triglyceride levels. To our knowledge, this is the first study to investigate maternal-offspring immune- and vitamin D-related genotype interactions with respect to adult offspring CMR.

We found evidence of interaction at MTRR SNP rs1532268 on offspring diastolic blood pressure, and suggestive, though nonsignificant, interaction on offspring systolic blood pressure. This SNP, also known as the C524T polymorphism, results in an amino acid change from serine to leucine and is expected to result in a less functional methionine synthase reductase protein (218). Pushpakumar, et al., demonstrated that dietary folic acid supplementation in a mouse model of induced hypertension could reduce hypertension (219), but other evidence for the methionine synthase pathway and BP is mixed. While rs153268 has not been studied in the context of blood pressure previously, another MTRR polymorphism was found not to be associated with essential hypertension (220). Another gene in the same pathway, MTHFR, has been associated with hypertension (146, 221), but definitive evidence is still lacking. If indeed the methionine synthase pathway is related to blood pressure in this manner, we might expect polymorphisms resulting in less-functional MTRR to result in increased blood pressure, but this was not the case in our analysis of offspring rs153268 and offspring BP (results not shown). In the analysis of maternal-offspring genotype interactions, no statistical difference was seen in the BP of offspring in dyads where both mother and offspring were homozygous for the minor allele compared to when both were homozygous for the major allele (Tables 4.3, 4.4), and the strongest association was seen to reduce systolic ($B = -4.01$, $SE = 1.20$, $p = 0.001$) and diastolic (-3.45, $SE = 0.96$, $p < 0.0005$) BP among offspring when only the mother had a copy of the minor allele, compared to double homozygous major dyads. In both interaction analyses, the coefficient for maternal genotype effect was negative, and for the maternal-offspring genotype effect positive (Table 4.2), which is not readily consistent with any hypothesized mechanism of maternal-offspring genotype interaction. While finding an association in

rs153268 of MTRR on BP is not inconsistent with some of the literature on the subject, chance must be ruled out before this finding is investigated further.

It is noteworthy that the top two SNPs (p-values<0.01) where we observed potential mother-offspring genetic interactions in relation to triglycerides were both in the MTHFD1L gene. After exploring linear combinations of model coefficients, we did not observe a consistent and obvious interaction pattern, such as maternal-fetal incompatibility. Formyltetrahydrofolate synthetase, encoded by the MTHFD1L gene, is involved in tetrahydrofolate synthesis, which in turn supports methylation reactions via the methionine pathway. Functional polymorphisms within this gene have been associated with neural tube defects (222). Maternal folic acid intake has been shown to alter DNA methylation in offspring in both human (223) and animal studies (224); we therefore included a number of folic acid metabolism SNPs in our candidate analysis to investigate potential maternal-offspring genotype interactions, which we hypothesized would act via changes in DNA methylation in other genes. Previous associations between MTHFD1L SNP rs6922269, a SNP tagged by SNP rs4869959 in our study, and coronary heart disease have been described in a meta-analysis (225, 226). However, the SNP was not associated with any tested biomarkers of CMR, including fasting lipids and measures of glycemic control.

Some strengths of this study deserve mention. The JPS-1 subset of the JPS birth cohort had both maternal and offspring genotypes and long term follow-up information until young adulthood, which makes it uniquely suitable for the current study. Offspring were phenotypically well-characterized at an average of 32 years old with respect to CMR, and their young age allowed study of CMR without multiple comorbidities that would result in loss from follow-up or medication effects that would otherwise complicate analyses. The candidate SNP selection process allowed us to focus on common variations in specific pathways as well as imprinted genes. Finally, we examined multiple CMR outcomes which provide opportunity to assess pleotropic effects of SNPs.

This study had a number of limitations as well. We analyzed a limited number of candidate SNPs and many of them are tag SNPs rather than known functional variants. While we had good power at some minor allele frequencies, we had less than 80% power to detect interaction associations smaller than 40% of the standard deviation of the outcome of interest. The generalizability of our findings may be limited by specific lifestyle and demographic characteristics of our population. Furthermore, we lack the necessary

data, such as maternal biomarkers measured during gestation or detailed offspring phenotypes at birth, to confirm that our statistical associations are driven by biological changes.

In the maternal-offspring genotype interaction analyses, we report some evidence for maternal-offspring genotype interactions in adult offspring CMR among our candidate genes. Previous literature has found evidence that interactions between maternal and offspring genotypes at the same genetic locus are risk factors for offspring disease (10-14).

Particular attention should be paid to the limitations of the statistical analysis, which was conducted by creating interaction terms as the product of maternal genotype and offspring genotype, assuming an additive genetic model. While there exist models (227) with less restrictive ways of identifying statistical interaction between maternal and offspring genotype, the primary advantage of the one chosen here is a power gain, particularly for loci with allele frequencies at or near 20% with our sample size. It is expected to perform well with respect to power in the situation where each additional minor allele confers additional benefit or risk with respect to the outcome. This type of interaction has been reported previously with respect to preterm birth (228). Antagonistic interactions between maternal and offspring genotype have also been reported (10, 12); while our model may identify the interaction, the mechanism may be misattributed in the absence of additional biological information due to the nature of our chosen interaction term. A more appropriate model in the situation exists (227), but this model does not allow for paternal imprinting and the interaction term has less power in other interaction mechanisms. Interactions between maternal and offspring genotype at different loci have also been reported (11, 229). Due to power constraints we did not test for these, but the possibility should not be discounted. Finally, it must be emphasized that the analysis of maternal-offspring genotype interactions was intended to be exploratory. Results should be interpreted cautiously, with more emphasis on informing future work than explicit interpretation of individual regression results.

In conclusion, we found suggestive evidence for maternal-offspring genetic interactions at multiple SNPs in the MTHFD1L gene with offspring triglycerides, as well as MTRR on systolic and diastolic blood pressure. Besides future replication efforts, larger numbers of mother-offspring dyads with data on genetic variation, maternal intrauterine environment, and long-term follow-up of offspring for CMR

development are needed to examine contributions of maternal-offspring geotype interactions to the developmental origins of CMR.

Chapter 5: Summary and Discussion

In this dissertation, we investigated genetic variants in the immune system and vitamin D metabolism in the context of the developmental origins of adult CMR in mother-offspring dyad participants of the Jerusalem Perinatal Study. In Chapter 2, we identified candidate SNPs in genes related to the immune system and vitamin D metabolism, and found that offspring variation at rs10894157 in CNTN5 was associated with lower BMI among our JPS-1 offspring, and that this relationship did not appear to be mediated by either CMV IgG nor 25[OH]D concentration. Similarly, we found two SNPs in SLC2A1, rs841858 and rs3820548, associated with offspring LDL, and rs841858 associated with triglycerides. We also found suggestion of effect modification of associations between offspring CMR with both CMV IgG and 25[OH]D concentration by offspring genotype. In particular, the associations of rs3771170 in IL-18R1 with LDL and rs1950902 in MTHFD1 with systolic BP were modified by 25[OH]D. In Chapter 3, we analyzed candidate maternal SNPs in genes related to the immune system and vitamin D metabolism and offspring CMR, but did not find significant associations. However, we did find evidence for effect modification of associations between maternal genotype and offspring CMR by offspring CMV IgG and 25[OH]D. In particular, maternal rs4987853 in BCL2 interacted with offspring CMV IgG on offspring HDL cholesterol, and maternal rs4861522 in IL-1R2 and maternal rs13143866 in IL-21, respectively, interacted with offspring 25[OH]D concentration on offspring LDL cholesterol. Finally, in Chapter 4, we investigated maternal-offspring genetic interactions within candidate SNPs in genes related to the immune system and vitamin D metabolism and their associations with offspring CMR. Though only one of the interactions was statistically significant, we found suggestion of such interaction among our study population, including interactions at SNPs in MTRR on systolic and diastolic BP, as well as SNPs in MTHFD1 on triglycerides.

Some observations across the three studies deserve mention. Interactions between offspring 25[OH]D concentrations and offspring IL-18R1 (β_3 = -0.80, SE = 0.21, p = 0.0002), and between offspring 25[OH]D and maternal IL-1R2 (β_3 = -1.11, SE = 0.24, p < 0.0001) were observed. The ligand of IL-18R1, IL-18, is a proinflammatory cytokine within the IL-1 family (133), presenting the possibility that the maternal genetic associations may be mediated through offspring genotype. Inclusion of the offspring genotype as a covariate in the maternal genotype-offspring CMR model did not change the magnitude of the maternal SNP-offspring 25[OH]D interaction substantively (Supplementary Table S3.1). Similarly,

inclusion of the maternal genotype as a covariate in the offspring genotype-offspring CMR model did not change the magnitude of the offspring SNP-offspring 25[OH]D interaction substantively (Supplementary Table S2.1). However, it should be noted that these two genes, IL-18R1 and IL-1R2, are located approximately 100,000 base pairs apart on Chromosome 2 and several other IL-1 family genes are also nearby, including IL-1R1 between them (230). In light of the close physical proximity of these two SNPs, it is our opinion that the possibility of a single variant intermediate between these two candidate SNPs being the true cause of the association should not be discounted. Similar situations may exist for other SNPs evaluated in our project.

In chapters 2 and 3, we identified associations between offspring and maternal SNPs in SLC2A1 with offspring triglycerides, respectively, among our top hits (Tables 2.2, 3.2). In sensitivity analyses presented in Supplementary Table S3.9, we examined the effect of adding the offspring top hit SNPs to models of the maternal genotype associations with offspring triglycerides. While neither maternal SNP was statistically significant to begin with, the association of maternal rs751210 with offspring triglycerides was substantially attenuated by the addition of either offspring rs841858 or rs3820546 to the model. The association between maternal rs12407920 and offspring triglycerides was less statistically significant, and slightly attenuated with the addition of offspring genotype as well (Table S3.9). These nonsignificant maternal genotype associations with offspring triglycerides identified in Chapter 3 may be due to correlation with offspring genotype at nearby loci.

There are multiple mechanisms by which maternal genotype, independently of offspring genotype, can be responsible for offspring phenotype. A study by Zhang, et al., selected maternal genetic variants that had been previously shown to influence serum IgE within an individual (231) and demonstrated associations with offspring IgE in childhood, independent of the offspring's genotype at these loci of interest, and furthermore that the associations differed depending on environmental factors experienced by the offspring (232). Zhang, et al., go on to suggest several possible mechanisms for the influence of maternal genotypes on offspring phenotypes. Due to the close alignment of maternal and fetal immune systems during pregnancy, maternal Th1/Th2 balance may affect that of the fetus. Others have proposed that maternal antibodies and/or cytokines may mediated their effects on the offspring by direct transplacental transport (233, 234). Other early life environmental factors, such as breast feeding

and the nutrient and antibody content of breast milk, or maternal behavior, may be influenced by maternal genetic factors and in turn affect offspring development during the critical period of rapid perinatal and immediate postnatal growth. Thirdly, maternal genotype may alter epigenetic programming during intrauterine or early life development. Given that maternal history of atopy is a stronger predictor of offspring atopy than paternal history of atopy (235), researchers have taken this to mean that epigenetic programming of offspring plays a major role in how maternal factors influence offspring (235, 236), though it is certainly not the only plausible way. Animal studies support the role of epigenetics as well: in rats, maternal nurturing behaviors towards their offspring are associated with differential DNA methylation patterns that arise within the first week of life, are reversible with a change in maternal behavior brought on by cross-fostering, and persist into adulthood (237).

In Chapter 4, we reported evidence for a maternal-offspring genotype interaction in adult offspring diastolic blood pressure at rs1532268 in MTRR. The chosen model, illustrated in Equation 1, is expected to perform well with respect to power in the situation where each additional minor allele confers additional benefit or risk with respect to the outcome (227). While our model may identify an interaction, the mechanism could be misattributed in the absence of additional biological information due to the nature of our chosen interaction term. Finally, it must be emphasized that the analysis of maternal-offspring genotype interactions was intended to be exploratory. Results should be interpreted cautiously, with more emphasis on informing future work than explicit interpretation of individual regression results.

Overall, the present dissertation has a number of strengths. The JPS-1 participants came from a well-characterized cohort that was oversampled for individuals at high risk for CMR from a developmental origins perspective. CMR were measured before most of the participants had developed overt disease, allowing analysis to be performed without complex exclusions or adjustment for multiple comorbidities. The presence of maternal genotype allowed some assessment of a factor that can influence the intrauterine environment on our CMR outcomes. The candidate gene/SNP selection process allowed us to focus on common variations in specific pathways. Finally, we examined multiple CMR outcomes which provided opportunity to assess pleotropic effects of SNPs.

There are limitations to this dissertation as well. Due to reasons of cost, minor allele frequency, and genotyping assay, many of our candidate SNPs were tag SNPs rather than SNPs with known

structural implications on the protein product. This adds additional steps in future confirmatory work to identify the causal polymorphism in replicated findings. The statistical limitations of our interaction model constrain the types of maternal-offspring genotype interactions we are most likely to pick up in the analysis at the expense of other types, as discussed in detail above and in Chapter 4. We had good statistical power at some combinations of minor allele frequency and association magnitude, but less power to detect genetic associations in both offspring and maternal candidate SNPs at minor allele frequencies of less than 20%, regardless of association magnitude, and less than 80% power to assess interaction associations smaller than 40% of the standard deviation of the outcome of interest, regardless of minor allele frequency. Specific lifestyle and demographic characteristics of our population, such as the narrow geographical range and nearly 17% of our participants self-identifying as of the Ultra-Orthodox Jewish religion may limit the generalizability of our findings. All CMR outcomes were measured at the same time, and so a temporal relationship between CMV IgG or 25[OH]D and other CMR cannot be inferred from our data. We also lack data on prenatal maternal biomarkers, which limits our assessment of maternal characteristics influencing the intrauterine environment.

In sum, our findings provide some evidence demonstrating that maternal and offspring immune and vitamin D metabolism related genetic variations, as well as their interactions play a role in the developmental origins of offspring CMR. In addition to replication of our major findings reported here, future studies can augment this area of research with careful design. Use of family triads or extended pedigrees as the study population would improve the ability to detect a wider variety of maternal and paternal genotype effects by allowing for the differentiation of parent-of-origin effects from maternal genotype effects or maternal-offspring genotype interactions in a constrained statistical model, or the use of a more flexible statistical model, though triads are not strictly necessary for investigation of just maternal-offspring interactions. A larger study population could not only improve statistical power to detect associations at small minor allele frequencies, but enable use of a more saturated interaction model to better describe maternal-offspring genotype interactions. Finer mapping of candidate genes, such as with a genome-wide association study, may allow for more precise localization of causal alleles, though potentially at a power loss due to the larger number of comparisons required in analyses. Our SNP-CMV IgG and SNP-25[OH]D interactions could be characterized as gene-gene-environment

interactions. While the environment has an obvious impact on CMV IgG titer and 25[OH]D concentration, genetic variation also plays a key role. Therefore, CMV IgG titer and 25[OH]D concentration, by themselves, represent a confluence of gene-environment interactions. With respect to the maternal SNP analyses in Chapter 3, maternal SNPs, through their effects on maternal health, could represent an environmental exposure, particularly of the intrauterine environment, at the level of the offspring. Assessment of maternal biomarkers during pregnancy would improve characterization of the intrauterine environment and allow for more detailed investigations of gene-gene, gene-environment, and gene-gene-environment interactions acting during that critical period, and prospective follow-up of CMR would establish temporality. Overall, a better understanding of the mechanisms of early life and developmental origins of adult diseases and risk factors, such as CMR, has the potential to help in disease prevention or early identification of susceptible populations.

References

1. Barker DJP. The Developmental Origins of Adult Disease. Journal of the American College of Nutrition. 2004;23(sup6):588S-95S.
2. McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol Rev. 2005;85:571-633.
3. Rinaudo PF, Lamb J. Fetal origins of perinatal morbidity and/or adult disease. Seminars in reproductive medicine. 2008 Sep;26(5):436-45. PubMed PMID: 18825611.
4. Tsatsoulis A, Mantzaris MD, Bellou S, Andrikoula M. Insulin resistance: an adaptive mechanism becomes maladaptive in the current environment - an evolutionary perspective. Metabolism: clinical and experimental. 2013 May;62(5):622-33. PubMed PMID: 23260798.
5. Zhou A, Dekker GA, Lumbers ER, Leemaqz SY, Thompson SD, Heinemann G, et al. The association of maternal ACE A11860G with small for gestational age babies is modulated by the environment and by fetal sex: a multicentre prospective case-control study. Molecular human reproduction. 2013 Sep;19(9):618-27. PubMed PMID: 23615722. Pubmed Central PMCID: 3749805.
6. Freathy RM, Weedon MN, Bennett A, Hypponen E, Relton CL, Knight B, et al. Type 2 diabetes TCF7L2 risk genotypes alter birth weight: a study of 24,053 individuals. American journal of human genetics. 2007 Jun;80(6):1150-61. PubMed PMID: 17503332. Pubmed Central PMCID: 1867102.
7. Wu W, Clark EAS, Stoddard GJ, Watkins WS, Esplin MS, Manuck TA, et al. Effect of interleukin-6 polymorphism on risk of preterm birth within population strata: a meta-analysis. BMC Genetics. 2013;14:30.
8. Sata F, Toya S, Yamada H, Suzuki K, Saijo Y, Yamazaki A, et al. Proinflammatory cytokine polymorphisms and the risk of preterm birth and low birthweight in a Japanese population. Molecular human reproduction. 2009 Feb;15(2):121-30. PubMed PMID: 19141488.
9. Halmoy A, Johansson S, Winge I, McKinney JA, Knappskog PM, Haavik J. Attention-deficit/hyperactivity disorder symptoms in offspring of mothers with impaired serotonin production. Arch Gen Psychiatry. 2010;67(10):1033-43.
10. Reali G. Forty years of anti-D immunoprophylaxis. Blood transfusion = Trasfusione del sangue. 2007 Jan;5(1):3-6. PubMed PMID: 19204744. Pubmed Central PMCID: 2535875.
11. Goddard KA, Tromp G, Romero R, Olson JM, Lu Q, Xu Z, et al. Candidate-gene association study of mothers with pre-eclampsia, and their infants, analyzing 775 SNPs in 190 genes. Human heredity. 2007;63(1):1-16. PubMed PMID: 17179726.
12. Parimi N, Tromp G, Kuivaniemi H, Nien JK, Gomez R, Romero R, et al. Analytical approaches to detect maternal/fetal genotype incompatibilities that increase risk of pre-eclampsia. BMC medical genetics. 2008;9:60. PubMed PMID: 18598365. Pubmed Central PMCID: 2474585.
13. Relton CL. Gene-gene interaction in folate-related genes and risk of neural tube defects in a UK population. Journal of Medical Genetics. 2004;41(4):256-60.
14. Childs EJ, Sobel EM, Palmer CG, Sinsheimer JS. Detection of intergenerational genetic effects with application to HLA-B matching as a risk factor for schizophrenia. Human heredity. 2011;72(3):161-72. PubMed PMID: 22004985. Pubmed Central PMCID: 3721948.
15. Curat CA, Miranville A, Sengenes C, Diehl M, Tonus C, Busse R, et al. From blood monocytes to adipose tissue-resident macrophages: induction of diapedesis by human mature adipocytes. Diabetes 2004;53:1285-92.
16. Ahmad R, Al-Mass A, Atizado V, Al-Hubail A, Al-Ghimlas F, Al-Arouj M, et al. Elevated expression of the toll like receptors 2 and 4 in obese individuals: its significance for obesity-induced inflammation. Journal of inflammation. 2012;9(1):48. PubMed PMID: 23191980. Pubmed Central PMCID: 3542010.

17. Ramseyer VD, Garvin JL. Tumor necrosis factor-a: regulation of renal function and blood pressure. Am J Physiol Renal Physiol. 2013;15(10):F1231-F342.
18. Zhang W, Wang W, Yu H, Zhang Y, Dai Y, Ning C, et al. Interleukin 6 underlies angiotensin II-induced hypertension and chronic renal damage. Hypertension. 2012 Jan;59(1):136-44. PubMed PMID: 22068875. Pubmed Central PMCID: 3842011.
19. Guzik TJ, Hoch NE, Brown KA, McCann LA, Rahman A, Dikalov S, et al. Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. The Journal of experimental medicine. 2007 Oct 1;204(10):2449-60. PubMed PMID: 17875676. Pubmed Central PMCID: 2118469.
20. Crowley SD, Song Y, Lin EE, Griffiths R, Kim H, Ruiz P. Lymphocyte responses exacerbate antiogensin II-dependent hypertension. Am J Physiol Regal Inter Comp Physiol. 2010;298:R1089-R97.
21. Blake GJ, Rifai N, Buring JE, Ridker PM. Blood pressure, C-reactive protein, and risk of future cardiovascular events. Circulation. 2003 Dec 16;108(24):2993-9. PubMed PMID: 14638538. Epub 2003/11/26. eng.
22. Milani RV, Lavie CJ. Modulatory Effect of Inflammation on Blood Pressure Reduction via Therapeutic Lifestyle Change. The Ochsner journal. 2009 Winter;9(4):175-80. PubMed PMID: 21603441. Pubmed Central PMCID: PMC3096294. Epub 2009/01/01. eng.
23. Guerrero-Romero F, Simental-Mendia LE, Rodriguez-Moran M. Association of C-reactive protein levels with fasting and postload glucose levels according to glucose tolerance status. Archives of medical research. 2014 Jan;45(1):70-5. PubMed PMID: 24326321.
24. van Woudenbergh GJ, Theofylaktopoulou D, Kuijsten A, Ferreira I, van Greevenbroek MM, van der Kallen CJ, et al. Adapted dietary inflammatory index and its association with a summary score for low-grade inflammation and markers of glucose metabolism: the Cohort study on Diabetes and Atherosclerosis Maastricht (CODAM) and the Hoorn study. The American journal of clinical nutrition. 2013 Dec;98(6):1533-42. PubMed PMID: 24153342.
25. Cohen JI, Maayan L, Convit A. Preliminary evidence for obesity-associated insulin resistance in adolescents without elevations of inflammatory cytokines. Diabetology & Metabolic Syndrome. 2012;4:26.
26. Lee CC, Lorenzo C, Haffner SM, Wagenknecht LE, Festa A, Goodarzi MO, et al. The association of inflammatory and fibrinolytic proteins with 5 year change in insulin clearance: the Insulin Resistance Atherosclerosis Study (IRAS). Diabetologia. 2013 Jan;56(1):112-20. PubMed PMID: 23052060. Pubmed Central PMCID: 4010386.
27. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. Allergy. 2000;55:688-97.
28. Dietert RR, Zelikoff JT. Early-life environment, developmental immunotoxicology, and the risk of pediatric allergic disease including asthma. Birth defects research Part B, Developmental and reproductive toxicology. 2008 Dec;83(6):547-60. PubMed PMID: 19085948.
29. Mortimer K, Neugebauer R, Lurmann F, Alcorn S, Balmes J, Tager I. Air pollution and pulmonary function in asthmatic children: effects of prenatal and lifetime exposures. Epidemiology (Cambridge, Mass). 2008 Jul;19(4):550-7; discussion 61-2. PubMed PMID: 18520616. Epub 2008/06/04. eng.
30. Pistiner M, Gold DR, Abdulkerim H, Hoffman E, Celedon JC. Birth by cesarean section, allergic rhinitis, and allergic sensitization among children with a parental history of atopy. The Journal of allergy and clinical immunology. 2008 Aug;122(2):274-9. PubMed PMID: 18571710. Epub 2008/06/24. eng.
31. Johansson A, Ludvigsson J, Hermansson G. Adverse health effects related to tobacco smoke exposure in a cohort of three-year olds. Acta paediatrica (Oslo, Norway : 1992). 2008 Mar;97(3):354-7. PubMed PMID: 18241297. Epub 2008/02/05. eng.
32. Raherison C, Penard-Morand C, Moreau D, Caillaud D, Charpin D, Kopfersmitt C, et al. In utero and childhood exposure to parental tobacco smoke, and allergies in schoolchildren. Respiratory medicine. 2007 Jan;101(1):107-17. PubMed PMID: 16735111. Epub 2006/06/01. eng.

33. Bolton M, Horvath DJ, Jr, Li B, Cortado H, Newsom D, White P, et al. Intrauterine growth restriction is a direct consequence of localized maternal uropathogenic Escherichia coli cystitis. PloS one. 2012;7(3):e33897.
34. Andrews WW, Hauth JC, Goldenberg RL. Infection and preterm birth. Am J Perinatology. 2000;17(7):357-65.
35. Mazumder B, Almond D, Park K, Crimmins EM, Finch CE. Lingering prenatal effects of the 1918 influenza pandemic on cardiovascular disease. Journal of developmental origins of health and disease. 2010 Feb 1;1(1):26-34. PubMed PMID: 20198106. Pubmed Central PMCID: 2826837.
36. Hurme M, Haanpaa M, Nurmikko T, Wang XY, Virta M, Pessi T, et al. IL-10 gene polymorphism and herpesvirus infections. Journal of medical virology. 2003;70 Suppl 1:S48-50. PubMed PMID: 12627487.
37. Helminen ME, Kilpinen S, Virta M, Hurme M. Susceptibility to primary Epstein-Barr virus infection is associated with interleukin-10 gene promoter polymorphism. The Journal of infectious diseases. 2001;184:777-80.
38. Hurme M, Helminen M. Resistance to human cytomegalovirus infection may be influenced by genetic polymorphisms of the tumor necrosis factor-alpha and interleukin-1 receptor antagonist genes. Scand J Infect Dis. 1998;30:447-.
39. Alonso Arias R, Moro-Garcia MA, Echeverria A, Solano-Jaurrieta JJ, Suarez-Garcia FM, Lopez-Larrea C. Intensity of the humoral response to cytomegalovirus is associated with the phenotypic and functional status of the immune system. Journal of virology. 2013 Apr;87(8):4486-95. PubMed PMID: 23388717. Pubmed Central PMCID: 3624366.
40. Zhu J, Shearer GM, Marincola FM, Norman JE, Rott D, Zou J-P, et al. Discordant cellular and humoral immune responses to cytomegalovirus infection in healthy blood donors: existence of a Th1-type dominant response. International Immunology. 2001;13(6):785-90. .
41. Ben-Smith A, Gorak-Stolinska P, Floyd S, Weir RE, Lalor MK, Mvula H, et al. Differences between naive and memory T cell phenotype in Malawian and UK adolescents: a role for Cytomegalovirus? BMC infectious diseases. 2008;8:139. PubMed PMID: 18922182. Pubmed Central PMCID: 2596152.
42. Olson NC, Doyle MF, Jenny NS, Huber SA, Psaty BM, Kronmal RA, et al. Decreased naive and increased memory CD4(+) T cells are associated with subclinical atherosclerosis: the multi-ethnic study of atherosclerosis. PloS one. 2013;8(8):e71498. PubMed PMID: 24009662. Pubmed Central PMCID: 3751895.
43. Tracy RP, Doyle MF, Olson NC, Huber SA, Jenny NS, Sallam R, et al. T-helper type 1 bias in healthy people is associated with cytomegalovirus serology and atherosclerosis: the Multi-Ethnic Study of Atherosclerosis. Journal of the American Heart Association. 2013 Jun;2(3):e000117. PubMed PMID: 23688675. Pubmed Central PMCID: 3698770.
44. Roberts ET, Haan MN, Dowd JB, Aiello AE. Cytomegalovirus antibody levels, inflammation, and mortality among elderly Latinos over 9 years of follow-up. Am J Epidemiol. 2010 Aug 15;172(4):363-71. PubMed PMID: 20660122. Pubmed Central PMCID: 2950794.
45. Marashi SM, Raeiszadeh M, Enright V, Tahami F, Workman S, Chee R, et al. Influence of cytomegalovirus infection on immune cell phenotypes in patients with common variable immunodeficiency. The Journal of allergy and clinical immunology. 2012 May;129(5):1349-56 e3. PubMed PMID: 22444511.
46. Nazmi A, Diez-Roux AV, Jenny NS, Tsai MY, Szklo M, Aiello AE. The influence of persistent pathogens on circulating levels of inflammatory markers: a cross-sectional analysis from the Multi-Ethnic Study of Atherosclerosis. BMC public health. 2010;10:706. PubMed PMID: 21083905. Pubmed Central PMCID: 2996373.
47. Miller MS, Gardner TJ, Krammer F, Aguado LC, Tortorella D, Basler CF, et al. Neutralizing antibodies against previously encountered influenza virus strains increase over time: a longitudinal

analysis. Science translational medicine. 2013 Aug 14;5(198):198ra07. PubMed PMID: 23946196. Pubmed Central PMCID: 4091683.
48. Zanghellini F, Boppana SB, Emery VC, Griffiths PD, Pass RF. Asymptomatic primary cytomegalovirus infection: virologic and immunologic features. The Journal of infectious diseases. 1999 Sep;180(3):702-7. PubMed PMID: 10438357. Epub 1999/08/07. eng.
49. Moro-Garcia MA, Alonso-Arias R, Lopez-Vazquez A, Suarez-Garcia FM, Solano-Jaurrieta JJ, Baltar J, et al. Relationship between functional ability in older people, immune system status, and intensity of response to CMV. Age. 2012 Apr;34(2):479-95. PubMed PMID: 21487706. Pubmed Central PMCID: 3312637.
50. Harkins L, Volk AL, Samanta M, Mikolaenko I, Britt WJ, Bland KI, et al. Specific localization of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. Lancet. 2002;360:1557-63.
51. Soderberg-Naucler C. Does cytomegalovirus play a causative role in the development of various inflammatory diseases and cancer? Journal of internal medicine. 2006 Mar;259(3):219-46. PubMed PMID: 16476101.
52. Aiello AE, Diez-Roux A, Noone AM, Ranjit N, Cushman M, Tsai MY, et al. Socioeconomic and psychosocial gradients in cardiovascular pathogen burden and immune response: the multi-ethnic study of atherosclerosis. Brain, behavior, and immunity. 2009 Jul;23(5):663-71. PubMed PMID: 19150399. Pubmed Central PMCID: 2694852.
53. Huang ZR, Yu LP, Yang XC, Zhang F, Chen YR, Feng F, et al. Human cytomegalovirus linked to stroke in a Chinese population. CNS neuroscience & therapeutics. 2012 Jun;18(6):457-60. PubMed PMID: 22672297.
54. Mundkur LA, Shivanandan H, Hebbagudi S, Endresz V, Varma M, Rao V, et al. Human cytomegalovirus neutralising antibodies and increased risk of coronary artery disease in Indian population. Heart. 2012 Jul;98(13):982-7. PubMed PMID: 22668865.
55. Haarala A, Kahonen M, Lehtimaki T, Aittoniemi J, Jylhava J, Hutri-Kahonen N, et al. Relation of high cytomegalovirus antibody titres to blood pressure and brachial artery flow-mediated dilation in young men: the Cardiovascular Risk in Young Finns Study. Clinical and experimental immunology. 2012 Feb;167(2):309-16. PubMed PMID: 22236008. Pubmed Central PMCID: 3278698.
56. Schimanski S, Linnemann B, Luxembourg B, Seifried E, Jilg W, Lindhoff-Last E, et al. Cytomegalovirus infection is associated with venous thromboembolism of immunocompetent adults--a case-control study. Annals of hematology. 2012 Apr;91(4):597-604. PubMed PMID: 21913128.
57. Smieja M. Multiple Infections and Subsequent Cardiovascular Events in the Heart Outcomes Prevention Evaluation (HOPE) Study. Circulation. 2002;107(2):251-7.
58. Sorlie PD, Nieto J, Adam E, Folsom AR, Shahar E, Massing M. A prospective study of cytomegalovirus, herpes simplex virus 1, and coronary heart disase. Arch Intern Med. 2000;160:2027-32.
59. Blum A, Giladi M, Weinberg M, Kaplan G, Pasternack H, Laniado S, et al. High anti-cytomegalovirus (CMV) IgG antibody titer is associated with coronary artery disease and may predict post-coronary balloon angioplasty restenosis. Am J Cardiol. 1998;81:866-8.
60. Cheng J, Ke Q, Jin Z, Wang H, Kocher O, Morgan JP, et al. Cytomegalovirus infection causes an increase of arterial blood pressure. PLoS pathogens. 2009 May;5(5):e1000427. PubMed PMID: 19436702. Pubmed Central PMCID: 2673691.
61. Khoretonenko MV, Leskov IL, Jennings SR, Yurochko AD, Stokes KY. Cytomegalovirus infection leads to microvascular dysfunction and exacerbates hypercholesterolemia-induced responses. The American journal of pathology. 2010 Oct;177(4):2134-44. PubMed PMID: 20802174. Pubmed Central PMCID: 2947306.

62. Li C, Samaranayake NR, Ong KL, Wong HK, Cheung BMY. Is human cytomegalovirus infection associated with hypertension? The United States National Health and Nutrition Examination Survey 1999-2002. PloS one. 2012;7(7):e39760.
63. Wall NA, Chue CD, Edwards NC, Pankhurst T, Harper L, Steeds RP, et al. Cytomegalovirus seropositivity is associated with increased arterial stiffness in patients with chronic kidney disease. PloS one. 2013;8(2):e55686. PubMed PMID: 23451030. Pubmed Central PMCID: 3581505.
64. Li S, Zhu J, Zhang W, Chen Y, Zhang K, Popescu LM, et al. Signature microRNA expression profile of essential hypertension and its novel link to human cytomegalovirus infection. Circulation. 2011 Jul 12;124(2):175-84. PubMed PMID: 21690488.
65. Chen S, de Craen AJ, Raz Y, Derhovanessian E, Vossen AC, Westendorp RG, et al. Cytomegalovirus seropositivity is associated with glucose regulation in the oldest old. Results from the Leiden 85-plus Study. Immunity & ageing : I & A. 2012;9(1):18. PubMed PMID: 22929089. Pubmed Central PMCID: 3478991.
66. Haeseker MB, Pijpers E, Dukers-Muijrers NH, Nelemans P, Hoebe CJ, Bruggeman CA, et al. Association of cytomegalovirus and other pathogens with frailty and diabetes mellitus, but not with cardiovascular disease and mortality in psycho-geriatric patients; a prospective cohort study. Immunity & ageing : I & A. 2013;10(1):30. PubMed PMID: 23880245. Pubmed Central PMCID: 3726516.
67. Adler SP, Hur JK, Wang JB, Vetrovec GW. Prior infection with cytomegalovirus is not a major risk factor for angiographically demonstrated coronary artery atherosclerosis. The Journal of infectious diseases. 1998;177:209-12.
68. Haider AW, Wilson PW, Larson MG, Evans JC, Michelson EL, Wolf PA, et al. The association of seropositivity to Helicobacter pylori, Chlamydia pneumoniae, and cytomegalovirus with risk of cardiovascular disease: a prospective study. Journal of the American College of Cardiology. 2002;40(8):1408-13.
69. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. The American journal of clinical nutrition. 2004;80(suppl):1678S-88S.
70. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. The American journal of clinical nutrition. 2008;87(suppl):1080S-6S.
71. De Boer IH, Levin G, Robinson-Cohen C, Biggs ML, Hoofnagle AN, Siscovick DS, et al. Serum 25-hydroxyvitamin D concentration and risk for major clinical disease events in a community-based population of older adults. Ann Intern Med. 2012;156(9):627-34.
72. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. Am J Physiol Renal Physiol. 2005;289:F8-F28.
73. Cantorna MT, Zhu Y, Froicu M, Wittke A. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system

The American journal of clinical nutrition. 2004;80(suppl):1717S-20S.
74. Mora JR, Iwata M, von Andrian UH. Vitamin effects on the immune system: vitamins A and D take centre stage. Nature reviews Immunology. 2008 Sep;8(9):685-98. PubMed PMID: 19172691. Pubmed Central PMCID: 2906676.
75. Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. Arch Intern Med. 2008;168(11):1174-80.
76. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, et al. Vitamin D deficiency and risk of cardiovascular disease. Circulation. 2008 Jan 29;117(4):503-11. PubMed PMID: 18180395. Pubmed Central PMCID: 2726624.
77. George PS, Pearson ER, Witham MD. Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. Diabetic medicine : a journal of the British Diabetic Association. 2012 Aug;29(8):e142-50. PubMed PMID: 22486204.

78. Jorde R, Strand Hutchinson M, Kjaergaard M, Sneve M, Grimnes G. Supplementation with High Doses of Vitamin D to Subjects without Vitamin D Deficiency May Have Negative Effects: Pooled Data from Four Intervention Trials in Tromso. ISRN endocrinology. 2013;2013:348705. PubMed PMID: 23577264. Pubmed Central PMCID: 3612485.
79. Schnatz PF, Jiang X, Vila-Wright S, Aragaki AK, Nudy M, O'Sullivan DM, et al. Calcium/vitamin D supplementation, serum 25-hydroxyvitamin D concentrations, and cholesterol profiles in the Women's Health Initiative calcium/vitamin D randomized trial. Menopause. 2014 Mar 3. PubMed PMID: 24594863.
80. Wood AD, Secombes KR, Thies F, Aucott L, Black AJ, Mavroeidi A, et al. Vitamin D3 supplementation has no effect on conventional cardiovascular risk factors: a parallel-group, double-blind, placebo-controlled RCT. The Journal of clinical endocrinology and metabolism. 2012 Oct;97(10):3557-68. PubMed PMID: 22865902.
81. Kane L, Moore K, Lutjohann D, Bikle D, Schwartz JB. Vitamin D3 effects on lipids differ in statin and non-statin-treated humans: superiority of free 25-OH D levels in detecting relationships. The Journal of clinical endocrinology and metabolism. 2013 Nov;98(11):4400-9. PubMed PMID: 24030939. Pubmed Central PMCID: 3816263.
82. Forman JP, Curhan GC, Taylor EN. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension among young women. Hypertension. 2008 Nov;52(5):828-32. PubMed PMID: 18838623. Pubmed Central PMCID: 2747298.
83. Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, et al. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension. 2007 May;49(5):1063-9. PubMed PMID: 17372031.
84. Witham MD, Price RJ, Struthers AD, Donnan PT, Messow CM, Ford I, et al. Cholecalciferol treatment to reduce blood pressure in older patients with isolated systolic hypertension: the VitDISH randomized controlled trial. JAMA internal medicine. 2013 Oct 14;173(18):1672-9. PubMed PMID: 23939263.
85. Scragg R, Slow S, Stewart AW, Jennings LC, Chambers ST, Priest PC, et al. Long-Term High-Dose Vitamin D3 Supplementation and Blood Pressure in Healthy Adults: A Randomized Controlled Trial. Hypertension. 2014 Jun 30. PubMed PMID: 24980662.
86. Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. The Journal of clinical endocrinology and metabolism. 2007 Sep;92(9):3517-22. PubMed PMID: 17535985.
87. Zhang C, Qiu C, Hu FB, David RM, van Dam RM, Bralley A, et al. Maternal plasma 25-hydroxyvitamin D concentrations and the risk for gestational diabetes mellitus. PloS one. 2008;3(11):e3753. PubMed PMID: 19015731. Pubmed Central PMCID: 2582131.
88. Bodnar LM, Catov JM, Zmuda JM, Cooper ME, Parrott MS, Roberts JM, et al. Maternal serum 25-hydroxyvitamin D concentrations are associated with small-for-gestational age births in white women. The Journal of nutrition. 2010 May;140(5):999-1006. PubMed PMID: 20200114. Pubmed Central PMCID: 2855265.
89. Javaid MK, Crozier SR, Harvey NC, Gale CR, Dennison EM, Boucher BJ, et al. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. The Lancet. 2006;367(9504):36-43.
90. Hypponen E, Berry DJ, Wjst M, Power C. Serum 25-hydroxyvitamin D and IgE - a significant but nonlinear relationship. Allergy. 2009 Apr;64(4):613-20. PubMed PMID: 19154546.
91. Hypponen E, Sovio U, Wjst M, Patel S, Pekkanen J, Hartikainen AL, et al. Infant vitamin d supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. Annals of the New York Academy of Sciences. 2004 Dec;1037:84-95. PubMed PMID: 15699498.

92. Chawes BL, Bonnelykke K, Jensen PF, Schoos AM, Heickendorff L, Bisgaard H. Cord Blood 25(OH)-Vitamin D Deficiency and Childhood Asthma, Allergy and Eczema: The COPSAC2000 Birth Cohort Study. PloS one. 2014;9(6):e99856. PubMed PMID: 24925304. Pubmed Central PMCID: 4055727.
93. Maslova E, Hansen S, Jensen CB, Thorne-Lyman AL, Strom M, Olsen SF. Vitamin D intake in mid-pregnancy and child allergic disease - a prospective study in 44,825 Danish mother-child pairs. BMC pregnancy and childbirth. 2013;13:199. PubMed PMID: 24176134. Pubmed Central PMCID: 3871013.
94. Swamy GK, Garrett ME, Miranda ML, Ashley-Koch AE. Maternal vitamin D receptor genetic variation contributes to infant birthweight among black mothers. American journal of medical genetics Part A. 2011 Jun;155A(6):1264-71. PubMed PMID: 21548019. Pubmed Central PMCID: 3100406.
95. Morley R, Carlin JB, Pasco JA, Wark JD, Ponsonby AL. Maternal 25-hydroxyvitamin D concentration and offspring birth size: effect modification by infant VDR genotype. European journal of clinical nutrition. 2009 Jun;63(6):802-4. PubMed PMID: 19018272.
96. Mattson DL, Lund H, Guo C, Rudemiller N, Geurts AM, Jacob H. Genetic mutation of recombination activating gene 1 in Dahl salt-sensitive rats attenuates hypertension and renal damage. Am J Physiol Regal Inter Comp Physiol. 2013;304:R407-R14.
97. Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, et al. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. The Lancet. 1999;353(9165):1649-52.
98. Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes. 2004;53:693-700.
99. Cheung BM, Ong KL, Tso AW, Leung RY, Xu A, Cherny SS, et al. C-reactive protein as a predictor of hypertension in the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS) cohort. Journal of human hypertension. 2012 Feb;26(2):108-16. PubMed PMID: 21270838.
100. Feitosa MF, Wojczynski MK, Straka R, Kammerer CM, Lee JH, Kraja AT, et al. Genetic analysis of long-lived families reveals novel variants influencing high density-lipoprotein cholesterol. Frontiers in genetics. 2014;5:159. PubMed PMID: 24917880. Pubmed Central PMCID: 4042684.
101. Lo JC, Wang Y, Tumanov AV, Bamji M, Yao Z, Reardon CA, et al. Lymphotoxin beta receptor-dependent control of lipid homeostasis. Science. 2007 Apr 13;316(5822):285-8. PubMed PMID: 17431181.
102. Cheung BM, Ong KL, Tso AW, Leung RY, Cherny SS, Sham PC, et al. Relationship of plasma interleukin-6 and its genetic variants with hypertension in Hong Kong Chinese. American journal of hypertension. 2011 Dec;24(12):1331-7. PubMed PMID: 21833041.
103. Humphries SE, Luong LA, Ogg MS, Hawe E, Miller GJ. The interleukin-6-174 G/C promoter polymorphism is associated with risk of coronary heart disease and systolic blood pressure in healthy men. european Heart Journal. 2001;22:2243-52.
104. Ke L, Graubard BI, Albanes D, Fraser DR, Weinstein SJ, Virtamo J, et al. Hypertension, pulse, and other cardiovascular risk factors and vitamin D status in Finnish men. American journal of hypertension. 2013 Aug;26(8):951-6. PubMed PMID: 23598420. Pubmed Central PMCID: 3816321.
105. van Ballegooijen AJ, Kestenbaum B, Sachs MC, de Boer IH, Siscovick DS, Hoofnagle AN, et al. Association of 25-hydroxyvitamin D and parathyroid hormone with incident hypertension: MESA (Multi-Ethnic Study of Atherosclerosis). Journal of the American College of Cardiology. 2014 Apr 1;63(12):1214-22. PubMed PMID: 24480627. Pubmed Central PMCID: 3999436.
106. Margolis KL, Martin LW, Ray RM, Kerby TJ, Allison MA, Curb JD, et al. A prospective study of serum 25-hydroxyvitamin D levels, blood pressure, and incident hypertension in postmenopausal women. American journal of epidemiology. 2012 Jan 1;175(1):22-32. PubMed PMID: 22127681. Pubmed Central PMCID: 3291161.

107. Peeters ACTM, Netea MG, Janssen MCH, Kullberg BJ, Van der Meer WM, Thien T. Pro-inflammatory cytokines in patients with essential hypertension. European J Clinical Investigation. 2001;2011:31-6.
108. Kunutsor SK, Apekey TA, Steur M. Vitamin D and risk of future hypertension: meta-analysis of 283,537 participants. European journal of epidemiology. 2013 Mar;28(3):205-21. PubMed PMID: 23456138.
109. Chai W, Cooney RV, Franke AA, Bostick RM. Effects of calcium and vitamin D supplementation on blood pressure and serum lipids and carotenoids: a randomized, double-blind, placebo-controlled, clinical trial. Annals of epidemiology. 2013 Sep;23(9):564-70. PubMed PMID: 23958407. Epub 2013/08/21. eng.
110. Pan WH, Wang CY, Li LA, Kao LS, Yeh SH. No significant effect of calcium and vitamin D supplementation on blood pressure and calcium metabolism in elderly Chinese. The Chinese journal of physiology. 1993;36(2):85-94. PubMed PMID: 8198625. Epub 1993/01/01. eng.
111. Sollid ST, Hutchinson MY, Fuskevag OM, Figenschau Y, Joakimsen RM, Schirmer H, et al. No effect of high-dose vitamin D supplementation on glycemic status or cardiovascular risk factors in subjects with prediabetes. Diabetes care. 2014. Epub 2014 Jun 19.
112. Miettinen ME, Kinnunen L, Leiviska J, Keinanen-Kiukaanniemi S, Korpi-Hyovalti E, Hiskanen L, et al. Association of serum 25-hydroxyvitamin D with lifestyle factors and metabolic cardiovascular disease markers: population-based cross-sectional study (FIN-D2D). PloS one. 2014;9(7):e100235.
113. Jorde R, Figenschau Y, Hutchinson M, Emaus N, Grimnes G. High serum 25-hydroxyvitamin D concentrations are associated with a favorable serum lipid profile. European journal of clinical nutrition. 2010 Dec;64(12):1457-64. PubMed PMID: 20823896.
114. Cutillas-Marco E, Prosper AF, Grant WB, Morales-Suarez-Varela MM. Vitamin D status and hypercholesterolemia in Spanish general population. Dermato-endocrinology. 2013 Jun 1;5(3):358-62. PubMed PMID: 24516690. Pubmed Central PMCID: 3908966.
115. Wang H, Xia N, Yang Y, Peng DQ. Influence of vitamin D supplementation on plasma lipid profiles: a meta-analysis of randomized controlled trials. Lipids in health and disease. 2012;11:42. PubMed PMID: 22433171. Pubmed Central PMCID: 3325888.
116. Tashiro K, Abe T, Oue N, Yasui W, Ryoji M. Characterization of vitamin D-mediated induction of the CYP 24 transcription. Molecular and cellular endocrinology. 2004 Oct 29;226(1-2):27-32. PubMed PMID: 15489002.
117. Harlap S, Davies AM, Deutsch L, Calderon-Margalit R, Manor O, Paltiel O, et al. The Jerusalem Perinatal Study cohort, 1964-2005: methods and a review of the main results. Paediatric and perinatal epidemiology. 2007 May;21(3):256-73. PubMed PMID: 17439536. Pubmed Central PMCID: 2993014.
118. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behavioural Brain Research. 2001;125:279-84.
119. Sobel ME. Some new results on indirect effects and their standard errors in covariance structure models. Sociological Methodology. 1986;16:159-86.
120. Mo X, Xu L, Yang Q, Feng H, Peng J, Zhang Y, et al. Microarray profiling analysis uncovers common molecular mechanisms of rubella virus, human cytomegalovirus, and herpes simplex virus type 2 infections in ECV304 cells. Curr Mol Med. 2011;11(6):481-8.
121. Kamei Y, Takeda Y, Teramoto K, Tsutsumi O, Taketani Y, Watanabe K. Human NB-2 of the contactin subgroup molecules: chromosomal localization of the gene (CNTN5) and distinct expression pattern from other subgroup members. Genomics. 2000 Oct 1;69(1):113-9. PubMed PMID: 11013081.
122. Zuko A, Kleijer KT, Oguro-Ando A, Kas MJ, van Daalen E, van der Zwaag B, et al. Contactins in the neurobiology of autism. European journal of pharmacology. 2013 Nov 5;719(1-3):63-74. PubMed PMID: 23872404.

123. Nakabayashi K, Komaki G, Tajima A, Ando T, Ishikawa M, Nomoto J, et al. Identification of novel candidate loci for anorexia nervosa at 1q41 and 11q22 in Japanese by a genome-wide association analysis with microsatellite markers. Journal of human genetics. 2009 Sep;54(9):531-7. PubMed PMID: 19680270.
124. Global Lipids Genetics C, Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, et al. Discovery and refinement of loci associated with lipid levels. Nature genetics. 2013 Nov;45(11):1274-83. PubMed PMID: 24097068. Pubmed Central PMCID: 3838666.
125. Mueckler M, Caruso C, Baldwin SA, Panico M, Blench I, Morris HR, et al. Sequence and structure of a human glucose transporter. Science. 1985;229(4717):941-5.
126. Olson AL, Pessin JE. Structure, function, and regulation of the mammalian facilitative glucose transporter gene family. Annu Rev Nutr. 1996;16:235-56.
127. Seidner G, Garcia Alvarez M, Yeh J, O'Driscoll KR, Klepper J, Stump T, et al. GLUT-1 deficiency syndrome caused by haploinsufficiency of the blood-brain barrier hexose carrier. Nature genetics. 1998;18:188-91.
128. Hildebrand MS, Damiano JA, Mullen SA, Bellows ST, Oliver KL, Dahl HH, et al. Glucose metabolism transporters and epilepsy: only GLUT1 has an established role. Epilepsia. 2014 Feb;55(2):e18-21. PubMed PMID: 24483274.
129. Vazquez-Chantada M, Gonzalez-Lahera A, Martinez-Arranz I, Garcia-Monzon C, Regueiro MM, Garcia-Rodriguez JL, et al. Solute carrier family 2 member 1 is involved in the development of nonalcoholic fatty liver disease. Hepatology. 2013 Feb;57(2):505-14. PubMed PMID: 22961556.
130. Kawano Y, Cohen DE. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. Journal of gastroenterology. 2013 Apr;48(4):434-41. PubMed PMID: 23397118. Pubmed Central PMCID: 3633701.
131. Cardoso SP, Keen L, Bidwell J. Identification of two novel single nucleotide polymorphisms in the promoter of the human interleukin-18 recepter alpha. European Journal of Immunogenetics. 2004;31:27-9.
132. Ovsyannikova IG, Haralambieva IH, Kennedy RB, O'Byrne MM, Pankratz VS, Poland GA. Genetic variation in IL18R1 and IL18 genes and Inteferon gamma ELISPOT response to smallpox vaccination: an unexpected relationship. The Journal of infectious diseases. 2013 Nov 1;208(9):1422-30. PubMed PMID: 23901078. Pubmed Central PMCID: 3789570.
133. Biet F, Locht C, Kremer L. Immunoregulatory functions of interleukin 18 and its role in defense against bacterial pathogens. Journal of molecular medicine. 2002 Mar;80(3):147-62. PubMed PMID: 11894141.
134. Tajik N, Keshavarz SA, Masoudkabir F, Djalali M, Sadrzadeh-Yeganeh HH, Eshraghian MR, et al. Effect of diet-induced weight loss on inflammatory cytokines in obese women. Journal of endocrinological investigation. 2013 Apr;36(4):211-5. PubMed PMID: 22733212.
135. Dezayee ZM. Interleukin-18 can predict pre-clinical atherosclerosis and poor glycemic control in type 2 diabetes mellitus. International journal of applied & basic medical research. 2011 Jul;1(2):109-12. PubMed PMID: 23776788. Pubmed Central PMCID: 3657961.
136. Imai T, Oikawa Y, Shimada A, Oguchi S, Takamiya Y, Katsuki T, et al. Proatherogenic effect of interleukin-18 is exerted with high-fat diet, but not with normal diet in spontaneously hyperlipidemic mice. J Atheroscler Thromb. 2011;18:1090-101.
137. Kong J, Grando SA, Li YC. Regulation of IL-1 Family Cytokines IL-1 , IL-1 Receptor Antagonist, and IL-18 by 1,25-Dihydroxyvitamin D3 in Primary Keratinocytes. The Journal of Immunology. 2006;176(6):3780-7.
138. Liu NQ, Kaplan AT, Lagishetty V, Ouyang YB, Ouyang Y, Simmons CF, et al. Vitamin D and the regulation of placental inflammation. Journal of immunology. 2011 May 15;186(10):5968-74. PubMed PMID: 21482732.

139. Vilarrasa N, Vendrell J, Maravall J, Elio I, Solano E, San Jose P, et al. Is plasma 25(OH) D related to adipokines, inflammatory cytokines and insulin resistance in both a healthy and morbidly obese population? Endocrine. 2010 Oct;38(2):235-42. PubMed PMID: 21046484. Epub 2010/11/04. eng.
140. Hum DW, Bell AW, Rozen R, MacKenzie RE. Primary structure of a human trifunctional enzyme. Isolation of a cDNA encoding methylenetetrahydrofolate dehydrogenase-methenyltetrahydrofolate cyclohydrolase-formyltetrahydrofolate synthetase. The Journal of biological chemistry. 1988 Nov 5;263(31):15946-50. PubMed PMID: 3053686. Epub 1988/11/05. eng.
141. MacFarlane AJ, Perry CA, Girnary HH, Gao D, Allen RH, Stabler SP, et al. Mthfd1 is an essential gene in mice and alters biomarkers of impaired one-carbon metabolism. The Journal of biological chemistry. 2009 Jan 16;284(3):1533-9. PubMed PMID: 19033438. Pubmed Central PMCID: 2615521.
142. Sutherland HG, Hermile H, Sanche R, Menon S, Lea RA, Haupt LM, et al. Association Study of MTHFD1 Coding Polymorphisms R134K and R653Q With Migraine Susceptibility. Headache. 2014 Jul 18. PubMed PMID: 25039261. Epub 2014/07/22. Eng.
143. Huang J, Mei J, Jiang L, Jiang Z, Liu H, Ding F. rs1801133 C>T polymorphism is associated with an increased risk of tetralogy of Fallot. Biomedical reports. 2014 Mar;2(2):172-6. PubMed PMID: 24649091. Pubmed Central PMCID: PMC3917760. Epub 2014/03/22. Eng.
144. Kelemen LE, Goodman MT, McGuire V, Rossing MA, Webb PM, Kobel M, et al. Genetic variation in TYMS in the one-carbon transfer pathway is associated with ovarian carcinoma types in the Ovarian Cancer Association Consortium. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2010 Jul;19(7):1822-30. PubMed PMID: 20570913. Pubmed Central PMCID: PMC3013232. Epub 2010/06/24. eng.
145. Jin G, Huang J, Hu Z, Dai J, Tang R, Chen Y, et al. Genetic variants in one-carbon metabolism-related genes contribute to NSCLC prognosis in a Chinese population. Cancer. 2010 Dec 15;116(24):5700-9. PubMed PMID: 20737570. Epub 2010/08/26. eng.
146. Yang B, Fan S, Zhi X, Li Y, Liu Y, Wang D, et al. Associations of MTHFR gene polymorphisms with hypertension and hypertension in pregnancy: a meta-analysis from 114 studies with 15411 cases and 21970 controls. PloS one. 2014;9(2):e87497. PubMed PMID: 24505291. Pubmed Central PMCID: 3914818.
147. Ng X, Boyd L, Dufficy L, Naumovski N, Blades B, Travers C, et al. Folate nutritional genetics and risk for hypertension in an elderly population sample. Journal of nutrigenetics and nutrigenomics. 2009;2(1):1-8. PubMed PMID: 19776634. Epub 2009/09/25. eng.
148. El-Saie LT, Rabie AR, Kamel MI, Seddeik AK, Elsaie ML. Effect of narrowband ultraviolet B phototherapy on serum folic acid levels in patients with psoriasis. Lasers in medical science. 2011 Jul;26(4):481-5. PubMed PMID: 21344249. Epub 2011/02/24. eng.
149. Juzeniene A, Stokke KT, Thune P, Moan J. Pilot study of folate status in healthy volunteers and in patients with psoriasis before and after UV exposure. Journal of photochemistry and photobiology B, Biology. 2010 Nov 3;101(2):111-6. PubMed PMID: 20207157. Epub 2010/03/09. eng.
150. Haziot A, Hijiya N, Gangloff SC, Silver J, Goyert SM. Induction of a novel mechanism of accelerated bacterial clearance by lipopolysaccharide in CD14-deficient and Toll-like receptor 4-deficient mice. Journal of immunology. 2001 Jan 15;166(2):1075-8. PubMed PMID: 11145687. Epub 2001/01/06. eng.
151. Soderberg C, Larsson S, Bergstedt-Lindqvist S, Moller E. Definition of a subset of human peripheral blood mononuclear cells that are permissive to human cytomegalovirus infection. Journal of virology. 1993 Jun;67(6):3166-75. PubMed PMID: 7684461. Pubmed Central PMCID: PMC237655. Epub 1993/06/01. eng.

152. Compton T, Kurt-Jones EA, Boehme KW, Belko J, Latz E, Golenbock DT, et al. Human Cytomegalovirus Activates Inflammatory Cytokine Responses via CD14 and Toll-Like Receptor 2. Journal of virology. 2003;77(8):4588-96.
153. Krichen H, Gorgi Y, Dhaouadi T, Mecheri Y, Sfar I, Bardi R, et al. Toll-like receptor 4 and CD14 gene polymorphisms in Tunisian kidney transplantation. Transplantation proceedings. 2013;45(10):3472-7. PubMed PMID: 24314935.
154. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. The Lancet. 2010;376(9736):180-8.
155. Wang W, Ingles SA, Torres-Mejia G, Stern MC, Stanczyk FZ, Schwartz GG, et al. Genetic variants and non-genetic factors predict circulating vitamin D levels in Hispanic and non-Hispanic white women: the Breast Cancer Health Disparities Study. Int J Mol Epidemiol Genet. 2014;5(1):31-46.
156. Li L, Yin X, Wu X, Zhang L, Pan S, Zheng Z, et al. Serum 25(OH)D and vitamin D status in relation to VDR, GC, and CYP2R1 variants in Chinese. endocrine Journal. 2014;61(2):133-41.
157. Nissen J, Rasmussen LB, Ravn-Haren G, Andersen EW, Hansen B, Andersen R, et al. Common variants in CYP2R1 and GC genes predict vitamin D concentrations in healthy Danish children and adults. PloS one. 2014;9(2):e89907. PubMed PMID: 24587115. Pubmed Central PMCID: 3937412.
158. Foucan L, Velayoudom-Cephise FL, Larifla L, Armand C, Deloumeaux J, Fagour C, et al. Polymorphisms in GC and NADSYN1 Genes are associated with vitamin D status and metabolic profile in Non-diabetic adults. BMC endocrine disorders. 2013;13(1):36. PubMed PMID: 24073860. Pubmed Central PMCID: 3849583.
159. Zhang Y, Wang X, Liu Y, Qu H, Qu S, Wang W, et al. The GC, CYP2R1 and DHCR7 genes are associated with vitamin D levels in northeastern Han Chinese children. Swiss medical weekly. 2012;142:w13636. PubMed PMID: 22801813.
160. Zhang Z, He JW, Fu WZ, Zhang CQ, Zhang ZL. An analysis of the association between the vitamin D pathway and serum 25-hydroxyvitamin D levels in a healthy Chinese population. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2013 Aug;28(8):1784-92. PubMed PMID: 23505139.
161. Buyske S. Maternal genotype effects can alias case genotype effects in case-control studies. European journal of human genetics : EJHG. 2008 Jul;16(7):783-5. PubMed PMID: 18398431.
162. Etheredge AJ, Finnell RH, Carmichael SL, Lammer EJ, Zhu H, Mitchell LE, et al. Maternal and infant gene-folate interactions and the risk of neural tube defects. American journal of medical genetics Part A. 2012 Oct;158A(10):2439-46. PubMed PMID: 22903727. Pubmed Central PMCID: 3448841.
163. Pfeifer K. Mechanisms of genomic imprinting. American journal of human genetics. 2000;67:777-87.
164. Shaw GM, Wasserman CR, Lammer EJ, O'Malley CD, Murray JC, Basart AM, et al. Orofacial clefts, parental cigarette smoking, and transforming growth factor-alpha gene variants. American journal of human genetics. 1996;58(3):551-61.
165. Jarvis JP, Kenney-Hunt J, Ehrich TH, Pletscher LS, Semenkovich CF, Cheverud JM. Maternal genotype affects adult offspring lipid, obesity, and diabetes phenotypes in LGXSM recombinant inbred strains. Journal of lipid research. 2005 Aug;46(8):1692-702. PubMed PMID: 15897602.
166. Wolf JB, Vaughn TT, Pletscher LS, Cheverud JM. Contribution of maternal effect QTL to genetic architecture of early growth in mice. Heredity. 2002 Oct;89(4):300-10. PubMed PMID: 12242647.
167. Casellas J, Farber CR, Gularte RJ, Haus KA, Warden CH, Medrano JF. Evidence of maternal QTL affecting growth and obesity in adult mice. Mammalian genome : official journal of the International Mammalian Genome Society. 2009 May;20(5):269-80. PubMed PMID: 19399551. Pubmed Central PMCID: 2690847.

168. Santos MS, Joles JA. Early determinants of cardiovascular disease. Best practice & research Clinical endocrinology & metabolism. 2012 Oct;26(5):581-97. PubMed PMID: 22980042.
169. Wander PL, Hochner H, Sitlani CM, Enquobahrie DA, Lumley T, Lawrence GM, et al. Maternal genetic variation accounts in part for the associations of maternal size during pregnancy with offspring cardiometabolic risk in adulthood. PloS one. 2014;9(3):e91835. PubMed PMID: 24670385. Pubmed Central PMCID: 3966761.
170. Asher MI, Montefort S, Björkstén B, Lai CKW, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. The Lancet. 2006;368(9537):733-43.
171. Moore SE, Jalil F, Ashraf R, Szu SC, Prentice AM, Hanson LA. Birth weight predicts response to vaccination in adults born in an urban slum in Lahore, Pakistan. The American journal of clinical nutrition. 2004;80:453-9.
172. Bottomly D, Ferris MT, Aicher LD, Rosenzweig E, Whitmore A, Aylor JT, et al. Expression quantitative trait loci for extreme host response to influenza A in pre-collaborative cross mice. G3. 2012;2(2):213-21.
173. Franco LM, Bucasas KL, Wells JM, Nino D, Wang X, Zapata GE, et al. Integrative genomic analysis of the human immune response to influenza vaccination. eLife. 2013;2:e00299.
174. Brannon PM. Vitamin D and adverse pregnancy outcomes: beyond bone health and growth. The Proceedings of the Nutrition Society. 2012 May;71(2):205-12. PubMed PMID: 22260841.
175. Belderbos ME, Houben ML, Wilbrink B, Lentjes E, Bloemen EM, Kimpen JL, et al. Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. Pediatrics. 2011 Jun;127(6):e1513-20. PubMed PMID: 21555499. Epub 2011/05/11. eng.
176. Luczynska A, Logan C, Nieters A, Elgizouli M, Schottker B, Brenner H, et al. Cord blood 25(OH)D levels and the subsequent risk of lower respiratory tract infections in early childhood: the Ulm birth cohort. European journal of epidemiology. 2014 May 25. PubMed PMID: 24859820. Epub 2014/05/27. Eng.
177. Devereux G, Litonjua AA, Turner SW, Craig LCA, McNeill G, Martindale S, et al. Maternal vitamin D intake during pregnancy and early childhood wheezing. The American journal of clinical nutrition. 2007;85:853-9.
178. Miyake Y, Sasaki S, Tanaka K, Hirota Y. Dairy food, calcium and vitamin D intake in pregnancy, and wheeze and eczema in infants. The European respiratory journal. 2010 Jun;35(6):1228-34. PubMed PMID: 19840962.
179. Nwaru BI, Ahonen S, Kaila M, Erkkola M, Haapala AM, Kronberg-Kippila C, et al. Maternal diet during pregnancy and allergic sensitization in the offspring by 5 yrs of age: a prospective cohort study. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2010 Feb;21(1 Pt 1):29-37. PubMed PMID: 20003068. Epub 2009/12/17. eng.
180. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell. 1993 Aug 27;74(4):609-19. PubMed PMID: 8358790. Epub 1993/08/27. eng.
181. Boumela I, Assou S, Aouacheria A, Haouzi D, Dechaud H, De Vos J, et al. Involvement of BCL2 family members in the regulation of human oocyte and early embryo survival and death: gene expression and beyond. Reproduction. 2011 May;141(5):549-61. PubMed PMID: 21339285.
182. Santner-Nanan B, Straubinger K, Hsu P, Parnell G, Tang B, Xu B, et al. Fetal-maternal alignment of regulatory T cells correlates with IL-10 and Bcl-2 upregulation in pregnancy. Journal of immunology. 2013 Jul 1;191(1):145-53. PubMed PMID: 23733877. Pubmed Central PMCID: PMC3690326. Epub 2013/06/05. eng.

183. Klingenberg R, Gerdes N, Badeau RM, Gistera A, Strodthoff D, Ketelhuth DF, et al. Depletion of FOXP3+ regulatory T cells promotes hypercholesterolemia and atherosclerosis. J Clin Invest. 2013 Mar 1;123(3):1323-34. PubMed PMID: 23426179. Pubmed Central PMCID: PMC3582120. Epub 2013/02/22. eng.
184. Xie JJ, Wang J, Tang TT, Chen J, Gao XL, Yuan J, et al. The Th17/Treg functional imbalance during atherogenesis in ApoE(-/-) mice. Cytokine. 2010 Feb;49(2):185-93. PubMed PMID: 19836260. Epub 2009/10/20. eng.
185. Barg E, Szopa J, Pesz KA, Gasiorowski K. Indices of insulin resistance and dyslipidemia are correlated with lymphocyte proneness to apoptosis in obese or overweight low birth weight children. Hormone research in paediatrics. 2013;79(5):293-9. PubMed PMID: 23689125.
186. Zhang A, Hildreth RL, Colberg-Poley AM. Human cytomegalovirus inhibits apoptosis by proteasome-mediated degradation of Bax at endoplasmic reticulum-mitochondrion contacts. Journal of virology. 2013 May;87(10):5657-68. PubMed PMID: 23487455. Pubmed Central PMCID: 3648137.
187. Pou J, Martinez-Gonzalez J, Rebollo A, Rodriguez C, Rodriguez-Calvo R, Martin-Fuentes P, et al. Type II interleukin-1 receptor expression is reduced in monocytes/macrophages and atherosclerotic lesions. Biochimica et biophysica acta. 2011 Sep;1811(9):556-63. PubMed PMID: 21683158.
188. Froicu M, Weaver V, Wynn TA, McDowell MA, Welsh JE, Cantorna MT. A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. Molecular endocrinology. 2003 Dec;17(12):2386-92. PubMed PMID: 14500760.
189. Verway M, Bouttier M, Wang TT, Carrier M, Calderon M, An BS, et al. Vitamin D induces interleukin-1beta expression: paracrine macrophage epithelial signaling controls M. tuberculosis infection. PLoS pathogens. 2013;9(6):e1003407. PubMed PMID: 23762029. Pubmed Central PMCID: 3675149.
190. Mahon BD, Wittke A, Weaver V, Cantorna MT. The targets of vitamin D depend on the differentiation and activation status of CD4 positive T cells. Journal of cellular biochemistry. 2003 Aug 1;89(5):922-32. PubMed PMID: 12874827.
191. Minambres I, Sanchez-Quesada JL, Sanchez-Hernandez J, Rodriguez J, de Leiva A, Perez A. Vitamin D concentrations in familial combined hyperlipidemia: effects of lipid lowering treatment. Diabetol Metab Syndr. 2014;6(1):7. PubMed PMID: 24450309. Pubmed Central PMCID: PMC3904931. Epub 2014/01/24. eng.
192. Kumar DV, Prasad BV, Vishwanth HL, Kamath V. A study on interleukin -1beta and lipid profile as markers of cardiovascular risk in rheumatoid arthritis. Journal of clinical and diagnostic research : JCDR. 2013 Jul;7(7):1298-302. PubMed PMID: 23998050. Pubmed Central PMCID: PMC3749620. Epub 2013/09/03. eng.
193. Park YB, Choi HK, Kim MY, Lee WK, Song J, Kim DK, et al. Effects of antirheumatic therapy on serum lipid levels in patients with rheumatoid arthritis: a prospective study. The American journal of medicine. 2002 Aug 15;113(3):188-93. PubMed PMID: 12208376. Epub 2002/09/05. eng.
194. Khoo AL, Chai LY, Koenen HJ, Sweep FC, Joosten I, Netea MG, et al. Regulation of cytokine responses by seasonality of vitamin D status in healthy individuals. Clinical and experimental immunology. 2011 Apr;164(1):72-9. PubMed PMID: 21323660. Pubmed Central PMCID: PMC3074219. Epub 2011/02/18. eng.
195. Villaggio B, Soldano S, Cutolo M. 1,25-dihydroxyvitamin D3 downregulates aromatase expression and inflammatory cytokines in human macrophages. Clinical and experimental rheumatology. 2012 Nov-Dec;30(6):934-8. PubMed PMID: 23253631. Epub 2012/12/21. eng.
196. Cantorna MT, Yu S, Bruce D. The paradoxical effects of vitamin D on type 1 mediated immunity. Molecular aspects of medicine. 2008 Dec;29(6):369-75. PubMed PMID: 18561994. Pubmed Central PMCID: PMC2633636. Epub 2008/06/20. eng.

197. Wurster AL, Rodgers VL, Satoskar AR, Whitters MJ, Young DA, Collins M, et al. Interleukin 21 Is a T Helper (Th) Cell 2 Cytokine that Specifically Inhibits the Differentiation of Naive Th Cells into Interferon -producing Th1 Cells. Journal of Experimental Medicine. 2002;196(7):969-77.
198. Palmer MT, Lee YK, Maynard CL, Oliver JR, Bikle DD, Jetten AM, et al. Lineage-specific effects of 1,25-dihydroxyvitamin D(3) on the development of effector CD4 T cells. The Journal of biological chemistry. 2011 Jan 14;286(2):997-1004. PubMed PMID: 21047796. Pubmed Central PMCID: PMC3020784. Epub 2010/11/05. eng.
199. Attridge K, Wang CJ, Wardzinski L, Kenefeck R, Chamberlain JL, Manzotti C, et al. IL-21 inhibits T cell IL-2 production and impairs Treg homeostasis. Blood. 2012 May 17;119(20):4656-64. PubMed PMID: 22442347. Epub 2012/03/24. eng.
200. Jia HY, Zhang ZG, Gu XJ, Guo T, Cui B, Ning G, et al. Association between interleukin 21 and Graves' disease. Genetics and molecular research : GMR. 2011;10(4):3338-46. PubMed PMID: 22057994. Epub 2011/11/08. eng.
201. Messaoudi S, Al-Khateeb GM, Dendana M, Sater MS, Jazia KB, Nouira M, et al. Genetic variations in the interleukin-21 gene and the risk of recurrent idiopathic spontaneous miscarriage. European cytokine network. 2011 Jun;22(2):123-6. PubMed PMID: 21768062. Epub 2011/07/20. eng.
202. Thompson SD, Sudman M, Ramos PS, Marion MC, Ryan M, Tsoras M, et al. The susceptibility loci juvenile idiopathic arthritis shares with other autoimmune diseases extend to PTPN2, COG6, and ANGPT1. Arthritis and rheumatism. 2010 Nov;62(11):3265-76. PubMed PMID: 20722033. Pubmed Central PMCID: PMC2970764. Epub 2010/08/20. eng.
203. Laurat E, Poirier B, Tupin E, Caligiuri G, Hansson GK, Bariety J, et al. In Vivo Downregulation of T Helper Cell 1 Immune Responses Reduces Atherogenesis in Apolipoprotein E-Knockout Mice. Circulation. 2001;104(2):197-202.
204. Mascarenhas-Melo F, Sereno J, Teixeira-Lemos E, Marado D, Palavra F, Pinto R, et al. Implication of low HDL-c levels in patients with average LDL-c levels: a focus on oxidized LDL, large HDL subpopulation, and adiponectin. Mediators of inflammation. 2013;2013:612038. PubMed PMID: 24282340. Pubmed Central PMCID: 3824339.
205. Green RF, Stoler JM. Alcohol dehydrogenase 1B genotype and fetal alcohol syndrome: a HuGE minireview. American journal of obstetrics and gynecology. 2007 Jul;197(1):12-25. PubMed PMID: 17618743.
206. Priest NK, Wade MJ. Maternal-zygotic epistasis and the evolution of genetic diseases. Journal of biomedicine & biotechnology. 2010;2010:478732. PubMed PMID: 20467476. Pubmed Central PMCID: 2867001.
207. White CA, Johansson M, Roberts CT, Ramsay AJ, Robertson SA. Effect of interleukin-10 null mutation on maternal immune response and reproductive outcome in mice. Biology of reproduction. 2004 Jan;70(1):123-31. PubMed PMID: 13679317.
208. Joerink M, Oortveld MAW, Stenius F, Rindsjo E, Alm J, Scheynius A. Lifestyle and parental allergen sensitization are reflected in the intrauterine environment at gene expression level. Allergy. 2010;65:1282-9.
209. Lokossou AG, Dechavanne C, Bouraima A, Courtin D, Le Port A, Ladekpo R, et al. Association of IL-4 and IL-10 maternal haplotypes with immune responses to P. falciparum in mothers and newborns. BMC infectious diseases. 2013;13:215. PubMed PMID: 23668806. Pubmed Central PMCID: 3679728.
210. Velez DR, Fortunato SJ, Williams SM, Menon R. Interleukin-6 (IL-6) and receptor (IL6-R) gene haplotypes associate with amniotic fluid protein concentrations in preterm birth. Human molecular genetics. 2008 Jun 1;17(11):1619-30. PubMed PMID: 18276608.
211. Menon R, Velez DR, Simhan H, Ryckman K, Jiang L, Thorsen P, et al. Multilocus interactions at maternal tumor necrosis factor-alpha, tumor necrosis factor receptors, interleukin-6 and interleukin-6

receptor genes predict spontaneous preterm labor in European-American women. American journal of obstetrics and gynecology. 2006 Jun;194(6):1616-24. PubMed PMID: 16731080.
212. Evans J, Collins M, Jennings C, van der Merwe L, Soderstrom I, Olsson T, et al. The association of interleukin-18 genotype and serum levels with metabolic risk factors for cardiovascular disease. European journal of endocrinology / European Federation of Endocrine Societies. 2007 Nov;157(5):633-40. PubMed PMID: 17984243.
213. Penas-Steinhardt A, Barcos LS, Belforte FS, de Sereday M, Vilarino J, Gonzalez CD, et al. Functional characterization of TLR4 +3725 G/C polymorphism and association with protection against overweight. PloS one. 2012;7(12):e50992. PubMed PMID: 23239997. Pubmed Central PMCID: 3519812.
214. Steinhardt AP, Aranguren F, Tellechea ML, Gomez Rosso LA, Brites FD, Martinez-Larrad MT, et al. A functional nonsynonymous toll-like receptor 4 gene polymorphism is associated with metabolic syndrome, surrogates of insulin resistance, and syndromes of lipid accumulation. Metabolism: clinical and experimental. 2010 May;59(5):711-7. PubMed PMID: 19922963.
215. Arora P, Garcia-Bailo B, Dastani Z, Brenner D, Villegas A, Malik S, et al. Genetic polymorphisms of innate immunity-related inflammatory pathways and their association with factors related to type 2 diabetes. BMC medical genetics. 2011;12:95.
216. Vimaleswaran KS, Cavadino A, Berry DJ, Jorde R, Dieffenbach AK, Lu C, et al. Association of vitamin D status with arterial blood pressure and hypertension risk: a mendelian randomisation study. The lancet Diabetes & endocrinology. 2014 Jun 25. PubMed PMID: 24974252. Epub 2014/06/30. Eng.
217. Schuch NJ, Garcia VC, Vivolo SR, Martini LA. Relationship between Vitamin D Receptor gene polymorphisms and the components of metabolic syndrome. Nutrition journal. 2013;12:96. PubMed PMID: 23855914. Pubmed Central PMCID: 3726454.
218. Olteanu H, Munson T, Banerjee R. Differences in the efficiency of reductive activation of methionine synthase and exogenous electron acceptors between the common polymorphic variants of human methionine synthase reductase. Biochemistry. 2002;41:13378-85.
219. Pushpakumar SB, Kundu S, Metreveli N, Sen U. Folic acid mitigates antiotensin-II-induced blood pressure and renal remodeling. PloS one. 2013;8(12):e83813.
220. Fowdar JY, Lason MV, Szvetko AL, Lea RA, Griffiths LR. Investigation of homocysteine-pathway-related variants in essential hypertension. International journal of hypertension. 2012;2012:190923. PubMed PMID: 23133742. Pubmed Central PMCID: 3485977.
221. Jiang S, Zhao R, Pan M, Venners SA, Zhong G, Hsu YH. Associations of MTHFR and MTRR polymorphisms with serum lipid levels in Chinese hypertensive patients. Clinical and applied thrombosis/hemostasis : official journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis. 2014 May;20(4):400-10. PubMed PMID: 23188888.
222. Parle-McDermott A, Pangilinan F, O'Brien KK, Mills JL, Magee AM, Troendle J, et al. A common variant in MTHFD1L is associated with neural tube defects and mRNA splicing efficiency. Human mutation. 2009 Dec;30(12):1650-6. PubMed PMID: 19777576. Pubmed Central PMCID: 2787683.
223. Steegers-Theunissen RP, Obermann-Borst SA, Kremer D, Lindemans J, Siebel C, Steegers EA, et al. Periconceptional maternal folic acid use of 400 ug per day is related to increased methylation of the IGF2 gene in the very young child. PloS one. 2009;4(11):e7845.
224. Hollingsworth JW, Maruoka S, Boon K, Garantziotis S, Li Z, Tomfohr J, et al. In utero supplementation with methyl donors enhances allergic airway disease in mice. J Clin Invest. 2008;118:3462-9.
225. Franceschini N, Carty C, Buzkova P, Reiner AP, Garrett T, Lin Y, et al. Association of genetic variants and incident coronary heart disease in multiethnic cohorts: the PAGE study. Circulation Cardiovascular genetics. 2011 Dec;4(6):661-72. PubMed PMID: 22042884. Pubmed Central PMCID: 3293207.

226. Angelakopoulou A, Shah T, Sofat R, Shah S, Berry DJ, Cooper J, et al. Comparative analysis of genome-wide association studies signals for lipids, diabetes, and coronary heart disease: Cardiovascular Biomarker Genetics Collaboration. European Heart Journal. 2012;33:393-407.
227. Ainsworth HF, Unwin J, Jamison DL, Cordell HJ. Investigation of maternal effects, maternal-fetal interactions and parent-of-origin effects (imprinting), using mothers and their offspring. Genetic epidemiology. 2011 Jan;35(1):19-45. PubMed PMID: 21181895. Pubmed Central PMCID: 3025173.
228. Jones NM, Holzman C, Tian Y, Witkin SS, Genc M, Friderici K, et al. Innate immune system gene polymorphisms in maternal and child genotype and risk of preterm delivery. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2012 Mar;25(3):240-7. PubMed PMID: 21627550.
229. Hilby SE, Apps R, Sharkey AM, Farrell LE, Gardner L, Mulder A, et al. Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2. J Clin Invest. 2010;120(11):4102-10.
230. Dale M, Nicklin MJH. Interleukin-1 receptor cluster: gene organization of IL1R2, IL1R1, IL1RL2 (IL-1Rrp2), IL1RL1 (T1/ST2), and IL18R1 (IL-1Rrp) on human chromosome 2q. Genomics. 1999;57:177-9.
231. Zhang G, Candelaria P, Makela JM, Khoo SK, Hayden MC, von Hertzen L, et al. Disparity of innate immunity-related gene effects on asthma and allergy on Karelia. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2011 Sep;22(6):621-30. PubMed PMID: 21749458. Epub 2011/07/14. eng.
232. Zhang G, Khoo SK, Makela MJ, Candelaria P, Hayden CM, von Hertzen L, et al. Maternal Genetic Variants of IL4/IL13 Pathway Genes on IgE With "Western or Eastern Environments/Lifestyles". Allergy, asthma & immunology research. 2014 Jul;6(4):350-6. PubMed PMID: 24991459. Pubmed Central PMCID: PMC4077962. Epub 2014/07/06. eng.
233. Deverman BE, Patterson PH. Cytokines and CNS development. Neuron. 2009 Oct 15;64(1):61-78. PubMed PMID: 19840550. Epub 2009/10/21. eng.
234. Bilenberg N, Hougaard D, Norgaard-Pedersen B, Nordenbaek CM, Olsen J. Twin study on transplacental-acquired antibodies and attention deficit/hyperactivity disorder--a pilot study. Journal of neuroimmunology. 2011 Jul;236(1-2):72-5. PubMed PMID: 21601295. Epub 2011/05/24. eng.
235. Litonjua AA, Carey VJ, Burge HA, Weiss ST, Gold DR. Parental history and the risk for childhood asthma. Does mother confer more risk than father? American journal of respiratory and critical care medicine. 1998 Jul;158(1):176-81. PubMed PMID: 9655726. Epub 1998/07/09. eng.
236. Fedulov AV, Kobzik L. Allergy risk is mediated by dendritic cells with congenital epigenetic changes. American journal of respiratory cell and molecular biology. 2011 Mar;44(3):285-92. PubMed PMID: 20118218. Pubmed Central PMCID: PMC3095930. Epub 2010/02/02. eng.
237. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, et al. Epigenetic programming by maternal behavior. Nature neuroscience. 2004 Aug;7(8):847-54. PubMed PMID: 15220929. Epub 2004/06/29. eng.

Appendix A: List of Candidate SNPs

Table S1. Candidate SNPs.

Gene Name	SNP	Type	Offspring Minor Allele Frquency	Offspring Hardy-Weinberg Equilibrium p-value		Maternal Minor Allele Frquency	Maternal Hardy-Weinberg Equilibrium p-value
ABCA1	rs1883025	intron	0.27	0.5148		0.28	0.8031
APH1B[1]	rs17184382	intergenic	0.45	0.3024		0.44	0.4187
BCL2[2]	rs1531697	intron	0.17	0.4351		0.18	0.3341
BCL2	rs1542578	intron	0.42	0.1609		0.41	0.6990
BCL2	rs1564483	3'UTR	0.21	0.8535		0.23	1.0000
BCL2	rs1801018	synonymous	0.39	0.7944		0.39	0.2112
BCL2	rs2046136	intron	0.33	0.6725		0.34	0.8354
BCL2[4]	rs2850761	intron	0.48	0.5761		0.49	0.0001
BCL2	rs4940574	intron	0.21	0.9256		0.20	0.3240
BCL2	rs4940576	intron	0.30	1.0000		0.29	0.9400
BCL2	rs4987736	intron	0.43	1.0000		0.43	0.7035
BCL2	rs4987853	3'UTR	0.25	0.6237		0.24	0.7959
BCL2[2]	rs7242542	intron	0.11	0.7456		0.12	0.4681
C8orf82/ZNF34[1]	rs2294120	intron	0.39	0.2262		0.40	0.2943
CCR5	rs2734648	intron	0.44	0.0079		0.45	0.2572
CD14	rs2563298	3'UTR	0.32	0.1713		0.32	0.1951
CD14	rs2569190	intron	0.47	0.7087		0.46	0.1352
CD14[2]	rs5744455	upstream	0.19	0.6898		0.21	0.7031
CD36	rs1537593	intron	0.09	1.0000		0.10	1.0000
CD36	rs1953299	intron	0.48	0.1339		0.45	0.0227
CD36	rs3173804	intron	0.47	0.4540		0.46	0.4518
CD36	rs997906	intron	0.39	0.3261		0.37	0.0152
CD53	rs2885805	intron	0.45	0.6051		0.45	0.4216
CD53[2,3]	rs2966952		0.10	< 0.0001		0.20	0.1372
CNTN5	rs10894157	intron	0.24	0.2861		0.26	0.7257
CRP	rs1130864	3'UTR	0.28	0.9363		0.29	1.0000
CUBN	rs1801222	missense	0.16	0.6412		0.18	0.8199
CUBN[2]	rs1801231	missense	0.17	0.1076		0.16	0.1818
CYP24A1	rs6013897	intergenic	0.26	0.4521		0.26	0.4926
CYP27B1	rs4646536	intron	0.22	0.2400		0.01	0.5848
CYP2R1	rs10741657	upstream	0.29	0.2756		0.28	0.2777
CYP2R1[4]	rs12794714	synonymous	0.48	0.0008		0.48	< 0.0001
CYP2R1	rs1993116	intron	0.30	0.3200		0.29	0.6256
FCGR2A	rs1801274	missense	0.44	0.8963		0.42	0.2186
FOXP3[3]	rs3761548	intron	0.50	< 0.0001		0.50	0.3761
FOXP3[2,3]	rs3761549	intron	0.10	< 0.0001		0.09	0.1322
GATA-3[2,3]	rs1058240	3'UTR	0.12	0.0001		0.14	0.8894
GATA-3	rs3802604	intron	0.45	0.6974		0.43	1.0000
GC	rs2282679	intron	0.27	0.1403		0.29	0.8724
IL-10	rs3024496	3'UTR	0.37	0.1705		0.37	0.3510
IL-10	rs3024498	3'UTR	0.15	1.0000		0.16	0.0019
IL-12(B)	rs3212227	3'UTR	0.24	0.2180		0.24	0.2696

Table S1. Candidate SNPs. (continued)

Gene Name	SNP	Type	Offspring Minor Allele Frquency	Offspring Hardy-Weinberg Equilibrium p-value		Maternal Minor Allele Frquency	Maternal Hardy-Weinberg Equilibrium p-value
IL-18[2]	rs1834481	intron	0.12	0.4515		0.13	0.3982
IL-18	rs549908	synonymous	0.27	1.0000		0.27	0.6940
IL-18	rs5744280	intron	0.43	0.2804		0.41	0.1773
IL-18R1[2]	rs11465596	intron	0.09	0.7187		0.10	0.7356
IL-18R1[2]	rs2080289	intron	0.19	0.3580		0.18	0.3459
IL-18R1	rs2287033	intron	0.43	1.0000		0.42	0.7489
IL-18R1	rs3732127	3'UTR	0.22	0.9270		0.22	0.7181
IL-18R1	rs3771170	intron	0.24	0.0896		0.24	0.4887
IL-18R1[2]	rs7558013	intron	0.15	0.6276		0.16	0.9093
IL-18R1	rs7579737	intron	0.39	0.4748		0.40	0.7458
IL-1A	rs2856836	3'UTR	0.37	0.8420		0.38	0.8948
IL-1A	rs3783546	intron	0.26	0.5736		0.24	0.3548
IL-1B	rs1143634	synonymous	0.31	0.5116		0.31	0.1897
IL-1R2	rs2110562	intron	0.47	0.5743		0.49	1.0000
IL-1R2	rs3218984	intron	0.23	0.4334		0.22	0.7161
IL-1R2[2]	rs4851522	intron	0.17	0.1772		0.16	0.4258
IL-2	rs2069772	intron	0.20	0.0127		0.21	0.3582
IL-21	rs2221903	intron	0.25	0.1947		0.24	0.0772
IL-21	rs13143866	intron	0.29	0.0555		0.29	0.0361
IL-21	rs2055979	intron	0.38	0.2940		0.38	0.2130
IL-4[2,3,4]	rs2070874	5'UTR	0.14	0.0004		0.17	0.0001
IL-6	rs1800797	intergenic	0.22	0.6425		0.45	0.2413
IL-6	rs1554606	upstream	0.28	0.8165		0.28	0.2191
IL-6	rs2069840	upstream	0.27	0.1835		0.27	0.0396
IL-6R[2]	rs1386821	intron	0.14	0.1519		0.14	1.0000
IL-6R	rs2229238	3'UTR	0.18	0.5321		0.18	0.3430
IL-6R	rs4075015	intron	0.50	0.8521		0.49	0.2913
IL-6R	rs4553185	downstream	0.45	0.0327		0.43	0.4093
IL-6R[2]	rs4845374	intron	0.16	0.8416		0.16	0.0273
IL-6R	rs4845623	intron	0.39	0.0315		0.42	0.1400
KCNK9[1]	rs2468677	intergenic	0.46	0.6526		0.46	0.3851
KCNQ1[1]	rs231362	intron	0.39	0.1761		0.38	0.1022
KLF13[1]	rs4779526	intergenic	0.24	0.0215		0.25	0.1091
KLF13[1]	rs8034505	intergenic	0.34	0.7233		0.36	0.0025
KLF14[1]	rs4731702	intergenic	0.37	0.5772		0.37	0.0049
LILRA3[2,4]	rs386000	intergenic	0.10	0.0024		0.11	< 0.0001
LRP2	rs2075252	missense	0.21	0.5582		0.23	0.7731
LRP2	rs2229263	missense	0.31	0.0716		0.31	0.8754
LRP2[2]	rs4667591	missense	0.18	0.9150		0.20	0.0887

Table S1. Candidate SNPs. (continued)

Gene Name	SNP	Type	Offspring Minor Allele Frquency	Offspring Hardy-Weinberg Equilibrium p-value		Maternal Minor Allele Frquency	Maternal Hardy-Weinberg Equilibrium p-value
LTA	rs1799964	intergenic	0.20	0.2848		0.21	0.2195
LTA[2]	rs1800630	intergenic	0.14	1.0000		0.14	0.1983
LTA	rs1041981	missense	0.26	0.9329		0.25	1.0000
LTA	rs2229094	missense	0.26	0.0065		0.30	0.0069
MTHFD1	rs1076991	5'UTR	0.47	0.2094		0.48	0.0144
MTHFD1[2]	rs1950902	missense	0.12	0.5512		0.12	0.3266
MTHFD1L	rs1555179	intron	0.29	0.2268		0.30	0.8815
MTHFD1L	rs175866	intron	0.45	0.0085		0.45	0.8504
MTHFD1L	rs3818056	intron	0.39	0.5559		0.39	0.0132
MTHFD1L	rs4341013	intron	0.20	0.0822		0.22	0.7118
MTHFD1L	rs4869713	intron	0.33	0.2052		0.34	0.5805
MTHFD1L	rs4869959	intron	0.20	0.6278		0.21	0.7761
MTHFD1L	rs572522	intron	0.44	0.7530		0.45	0.7057
MTHFD1L	rs6940322	intron	0.35	0.3401		0.36	0.0103
MTHFD1L	rs803471	intron	0.30	0.0474		0.30	0.3387
MTHFD1L	rs9478878	intron	0.30	0.5563		0.29	0.6521
MTHFR	rs1801131	missense	0.34	0.6290		0.35	0.3711
MTHFR	rs1801133	missense	0.38	0.5512		0.37	0.8940
MTR[2]	rs1805087	missense	0.18	0.4441		0.19	0.0802
MTRR[2]	rs10380	missense	0.13	0.0281		0.15	0.4940
MTRR	rs1532268	missense	0.39	0.0580		0.40	0.3978
MTRR[2]	rs162036	missense	0.16	0.3278		0.17	0.8084
MTRR	rs1801394	missense	0.44	0.5550		0.43	0.1267
RXRA	rs1045570	3'UTR	0.21	0.2835		0.22	0.6927
SLC2A1[2]	rs12407920	intron	0.10	0.0195		0.11	0.3299
SLC2A1	rs3754219	intron	0.42	0.4076		0.41	0.5198
SLC2A1	rs3820546	intron	0.47	0.4544		0.46	0.4160
SLC2A1	rs3820548	intron	0.30	1.0000		0.31	0.1881
SLC2A1	rs710221	intron	0.43	0.5688		0.43	0.7994
SLC2A1	rs751210	intron	0.37	0.0821		0.36	0.1376
SLC2A1	rs841853	intron	0.33	0.1404		0.34	0.7830
SLC2A1[2]	rs841858	intron	0.16	0.1945		0.16	0.2860
SLC7A10/PEPD[1]	rs8182584	intron	0.47	0.3030		0.48	0.5913

Table S1. Candidate SNPs. (continued)

Gene Name	SNP	Type	Offspring Minor Allele Frquency	Offspring Hardy-Weinberg Equilibrium p-value		Maternal Minor Allele Frquency	Maternal Hardy-Weinberg Equilibrium p-value
TLR2	rs3804099	synonymous	0.43	0.7945		0.41	0.4099
TLR3	rs3775291	missense	0.28	0.9366		0.30	0.0053
TLR4[2]	rs11536889	3'UTR	0.11	0.0234		0.11	0.2290
TLR4[2]	rs7873784	3'UTR	0.18	0.1537		0.20	0.3408
TNF[2]	rs3093664	intron	0.09	1.0000		0.09	0.1340
TNFRSF1A	rs1800693	intron	0.43	0.6561		0.44	0.2055
TNFRSF1A[2]	rs4149578	intron	0.08	0.3904		0.08	0.0397

[1]Only included in Chapter 4 analysis (imprinted)

[2]Not included in Chapter 4 analysis due to MAF < 0.2

[3]Not included in Chapter 2 or 4 analysis due to violation of offspring Hardy-Weinberg Equilibrium ($p < 0.00044$)

[4]Not included in Chapter 3 or 4 analysis due to violation of maternal Hardy-Weinberg Equilibrium ($p < 0.00044$)

Appendix B: Supplementary Tables for Chapter 2

Table S2.1. Sensitivity analyses of significant results.

Association	Model	Coefficient	Robust SE	p-value
Association of rs10894157 with BMI	Original model	-1.31	0.31	0.000018*
	Without eight covariates describing ethnicity	-1.35	0.30	0.000008
	With sex as only covariate	-1.44	0.28	0.000000
	With mppbmi, gwg	-1.14	0.28	0.000048
	with missing fasting	-1.31	0.31	0.000020
Association of rs841858 with LDL	Original model	-7.33	2.10	0.0005*
	without bmi	-7.02	2.20	0.001400
	Without eight covariates describing ethnicity	-6.90	2.12	0.001200
	With sex as only covariate	-4.50	1.82	0.014000
	With mppbmi, gwg	-5.89	1.78	0.000999
	with missing fasting	-7.33	2.10	0.000500
Association of rs3820548 with LDL	Original model	-5.84	1.75	0.0009*
	without bmi	-5.56	1.83	0.002500
	Without eight covariates describing ethnicity	-5.65	1.76	0.001400
	With sex as only covariate	-4.50	1.82	0.014000
	With mppbmi, gwg	-5.89	1.78	0.000999
	with missing fasting	-5.84	1.75	0.000904
Association of rs841858 with triglycerides (natural log-transformed)	Original model	-0.12	0.03	0.0004*
	without bmi	-0.11	0.03	0.001300
	Without eight covariates describing ethnicity	-0.11	0.03	0.000680
	With sex as only covariate	-0.10	0.03	0.002600
	With mppbmi, gwg	-0.12	0.03	0.000247
	with missing fasting	-0.12	0.03	0.000350

Table S2.2. The interaction between offspring rs3771170 and 25[OH]D on LDL is robust to covariate inclusion/exclusion.

	SNP association			25[OH]D association			SNP X 25[OH]D Interaction		
	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
Original Model	16.75	5.34	0.0034	0.67	0.23	0.0030	-0.87	0.22	0.0001
Without BMI	17.19	5.72	0.0030	0.60	0.24	0.0130	-0.88	0.23	0.0016
Without eight covariates describing ethnicity	15.36	5.23	0.0030	0.60	0.22	0.0070	-0.80	0.21	0.0008
With mppbmi, gwg	17.06	5.52	0.0020	0.72	0.24	0.0030	-0.90	0.23	0.0001
With sex, bmi, season of blood draw only	13.74	0.51	0.0070	0.57	0.21	0.0060	-0.72	0.20	0.0004
with missing fasting	16.75	5.34	0.0018	0.67	0.23	0.0033	-0.87	0.22	0.0001
With maternal rs4851522 genotype	16.72	5.34	0.0020	0.67	0.23	0.0040	-0.86	0.22	0.0001
With offspring rs4851522 genotype	16.75	5.36	0.0020	0.67	0.23	0.0030	-0.87	0.22	0.0001
With offspring rs4851522 genotype-25[OH]D interaction	16.45	5.26	0.0020	0.69	0.23	0.0030	-0.85	0.22	0.0001
With maternal rs4851522 genotype-offspring 25[OH]D interaction*	13.66	5.00	0.0060	0.99	0.23	0.0000	-0.74	0.21	0.0004
*maternal interaction p < 0.0005									

Table S2.3. The interaction between offspring rs1950902 and 25[OH]D on Systolic BP is robust to covariate inclusion/exclusion.

	SNP association			25[OH]D association			SNP X 25[OH]D Interaction		
	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
Original Model	5.85	2.03	0.0041	0.08	0.06	0.1721	-0.28	0.08	0.0004*
Without BMI	6.15	2.13	0.0040	0.03	0.06	0.6550	-0.30	0.08	0.0004
Without eight covariates describing ethnicity	5.78	2.01	0.0040	0.10	0.06	0.0890	-0.28	0.08	0.0005
With mppbmi, gwg	5.90	2.05	0.0040	0.08	0.06	0.1720	-0.29	0.08	0.0003
With sex, bmi, season of blood draw only	5.53	1.94	0.0050	0.07	0.05	0.1550	-0.26	0.08	0.0119
with missing fasting	5.85	2.03	0.0040	0.08	0.06	0.1721	-0.28	0.08	0.0004

Table S2.4. Sensitivity analysis of CMV positivity and offspring genotype.

Gene	SNP	Odds Ratio	Std Err	p-value
CD14	rs5744455	1.04	0.26	0.0001*
BCL2	rs4940576	0.56	0.19	0.0030
TLR3	rs3775291	0.51	0.23	0.0245
MTRR	rs1801394	0.50	0.23	0.0296
BCL2	rs4940574	0.48	0.24	0.0472

*Met BH cutoff for statistical significance.

Table S2.5. Combination genotype analysis of SLC2A1 SNPs rs841858 and rs3820548 on LDL cholesterol. Zero minor alleles at each locus used as baseline, minimum 10 participants required in each stratum.

number of rs841858 minor alleles	number of rs3820548 minor alleles	Coefficient	Std Err	p-value
0	1	-2.89	3.28	0.3790
0	2	-8.50	4.91	0.0840
1	1	-7.89	3.01	0.0090
2	1	-11.72	5.58	0.0360
2	2	-15.61	6.54	0.0170

Table S2.6. Combination genotype analysis of SLC2A1 SNPs rs841858 and rs3820548 on triglycerides (natural log-transformed). Zero minor alleles at each locus used as baseline, minimum 10 participants required in each stratum.

number of rs841858 minor alleles	number of rs3820546 minor alleles	Coefficient	Std Err	p-value
0	1	0.09	0.06	0.1420
0	2	0.10	0.07	0.1660
1	1	-0.01	0.07	0.8690
1	0	-0.13	0.08	0.1040
2	0	-0.10	0.09	0.2730

Table S2.7. Comparison of additive, dominant, and recessive genotype models in the interaction between rs3771170 in MTHFD1 and 25[OH]D concentration on LDL.

Model	SNP association			25[OH]D Association			SNP X 25[OH]D Interaction		
	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
Additive	16.75	5.34	0.0018	0.67	0.23	0.0033	-0.87	0.22	0.0001
Dominant	-3.16	2.67	0.2370	0.14	0.20	0.4830	0.11	0.14	0.4390
Recessive	11.06	11.29	0.3270	0.26	0.18	0.1640	-0.98	0.41	0.0180

Table S2.8. Comparison of additive, dominant, and recessive genotype models in the interaction between rs1950902 in MTHFD1 and 25[OH]D concentration on systolic BP.

Model	SNP association			25[OH]D Association			SNP X 25[OH]D Interaction		
	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
Additive	5.85	2.03	0.0041	0.08	0.06	0.1721	-0.28	0.08	0.0004
Dominant	5.58	1.74	0.0010	0.07	0.06	0.2040	-0.26	0.06	< 0.0005
Recessive	9.42	4.17	0.0240	0.00	0.06	0.9930	-0.60	0.15	< 0.0005

Appendix C: Supplementary figures S2.1-S2.9: Graphical representations of the results of candidate SNP analyses on nine CMR-related outcomes.

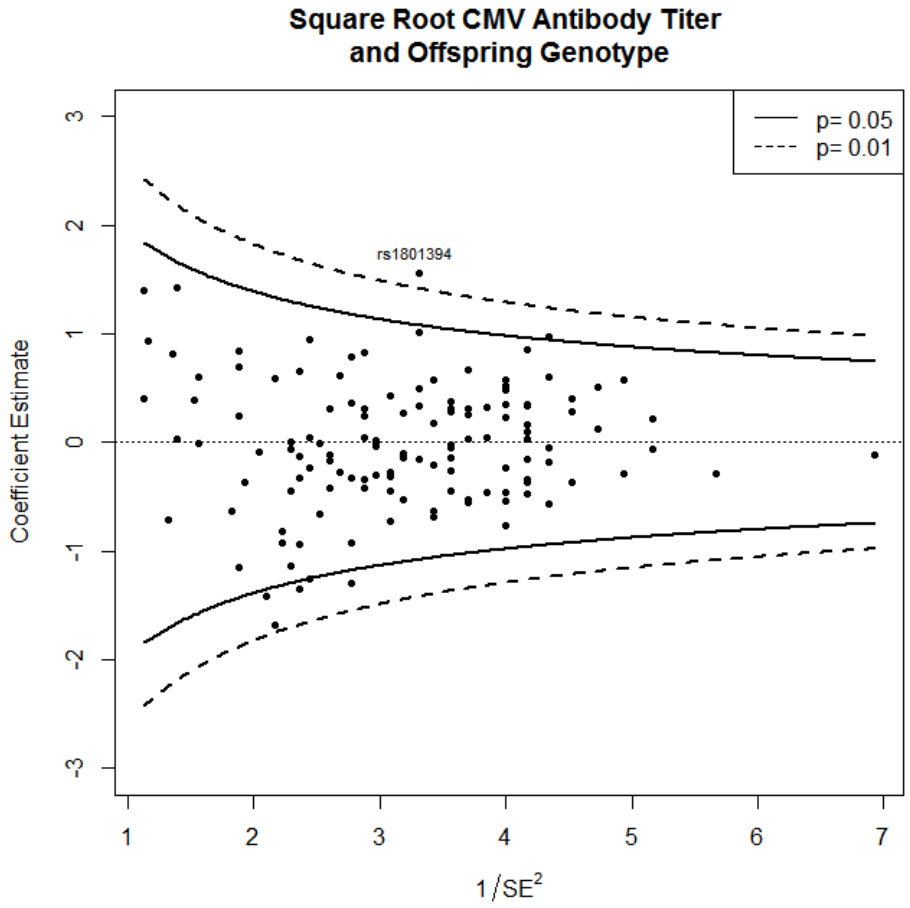


Figure S2.1: Results of candidate SNP – CMV IgG association analyses.

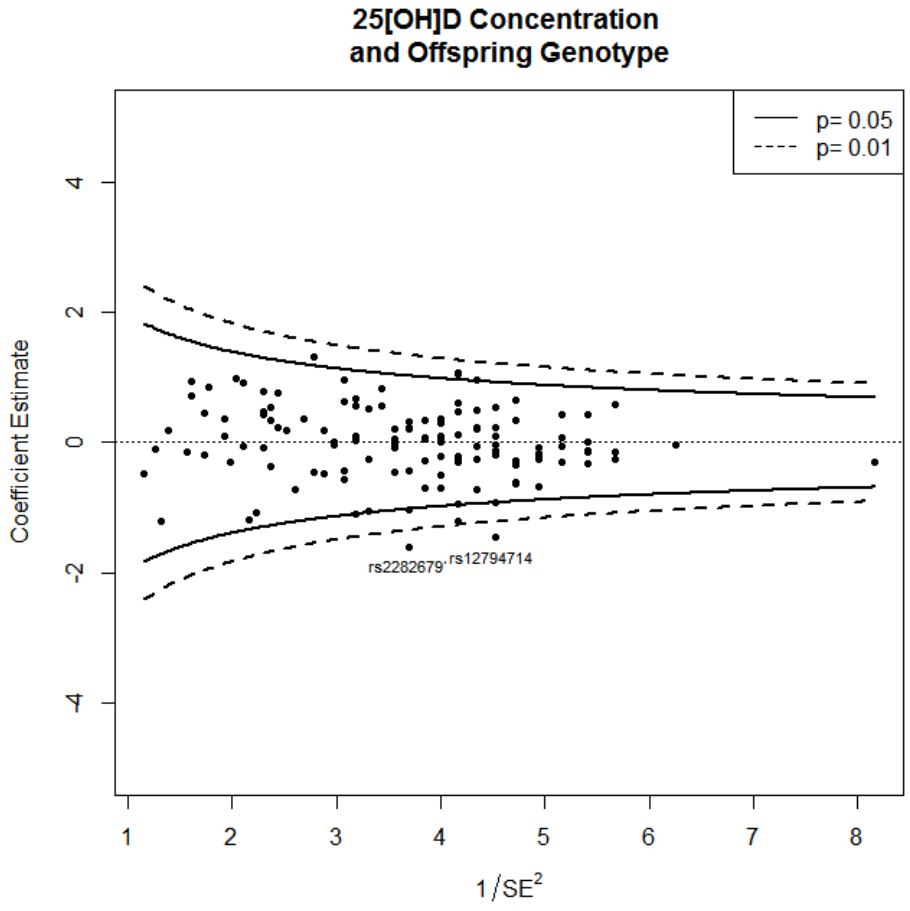


Figure S2.2: Results of candidate SNP – 25[OH]D association analyses.

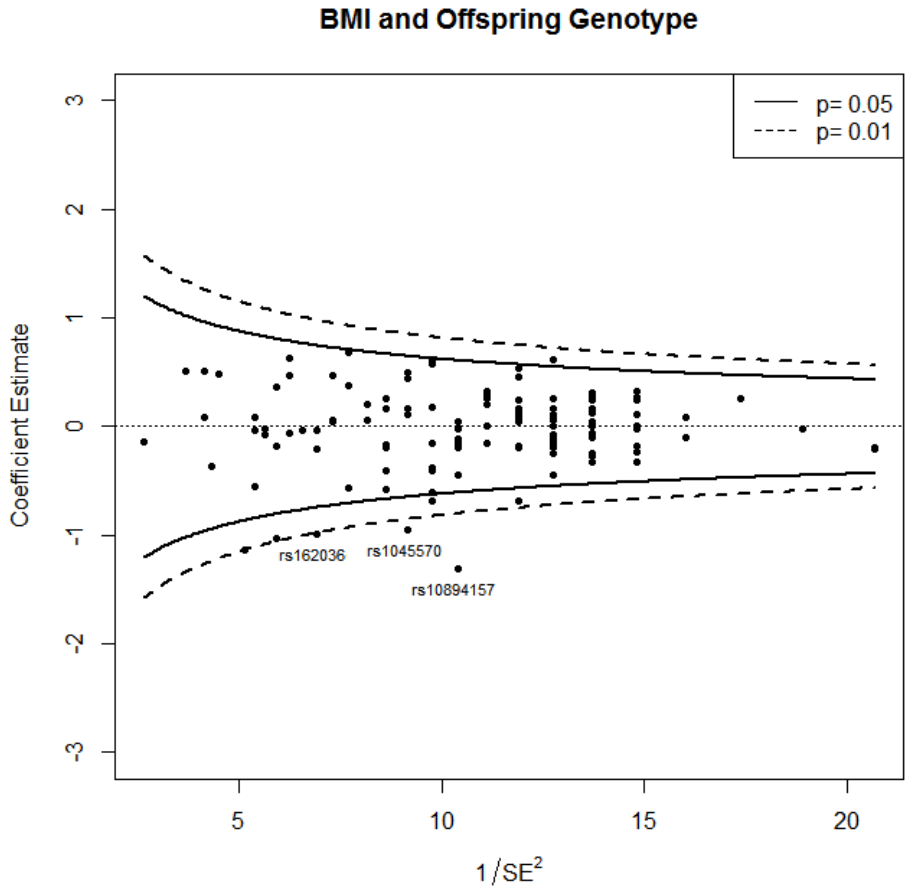


Figure S2.3: Results of candidate SNP – BMI association analyses.

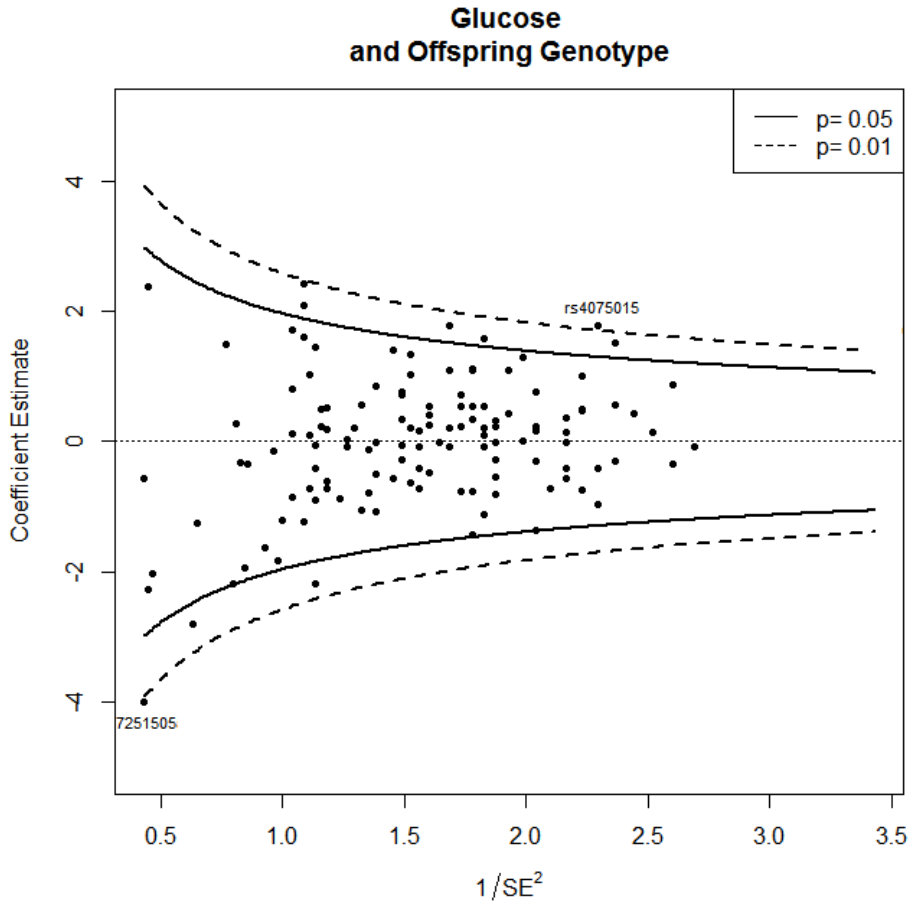


Figure S2.4: Results of candidate SNP – Fasting Glucose association analyses.

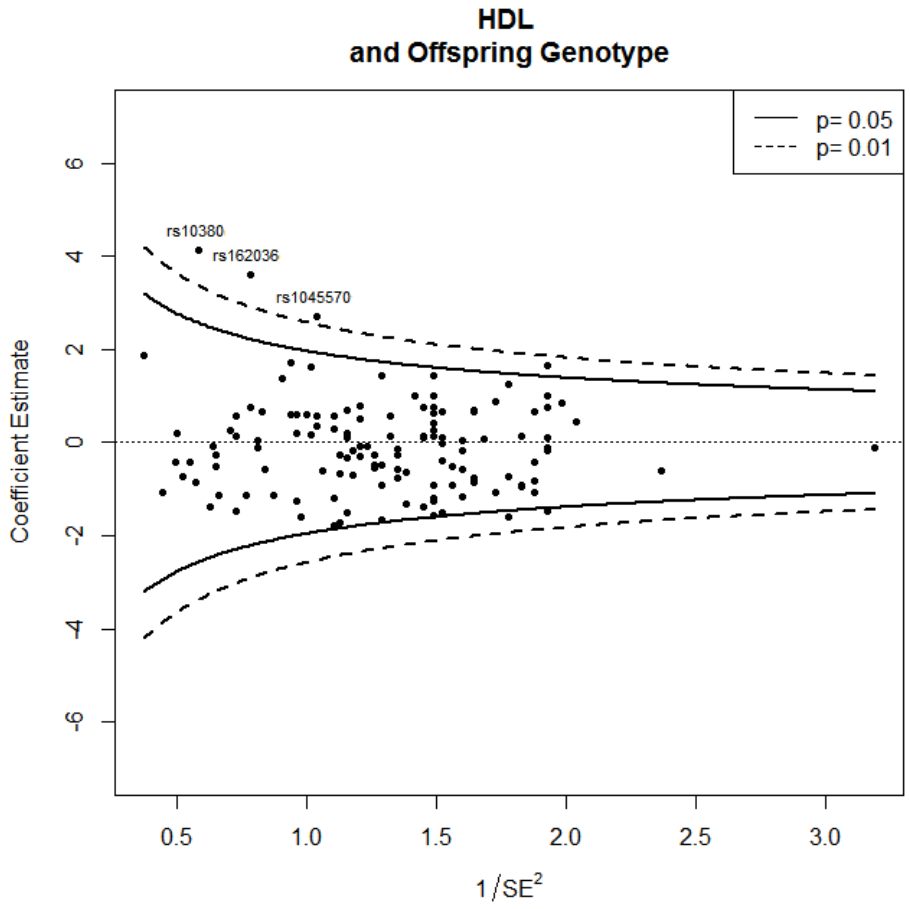


Figure S2.5: Results of candidate SNP – HDL association analyses.

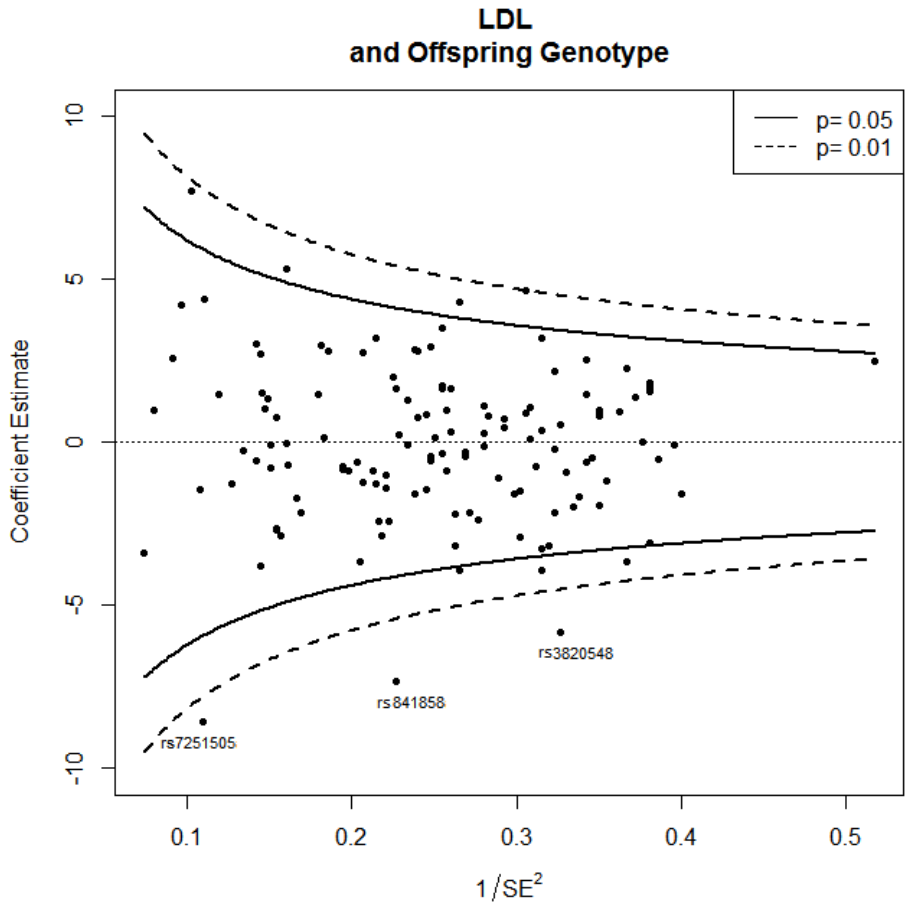


Figure S2.6: Results of candidate SNP – LDL association analyses.

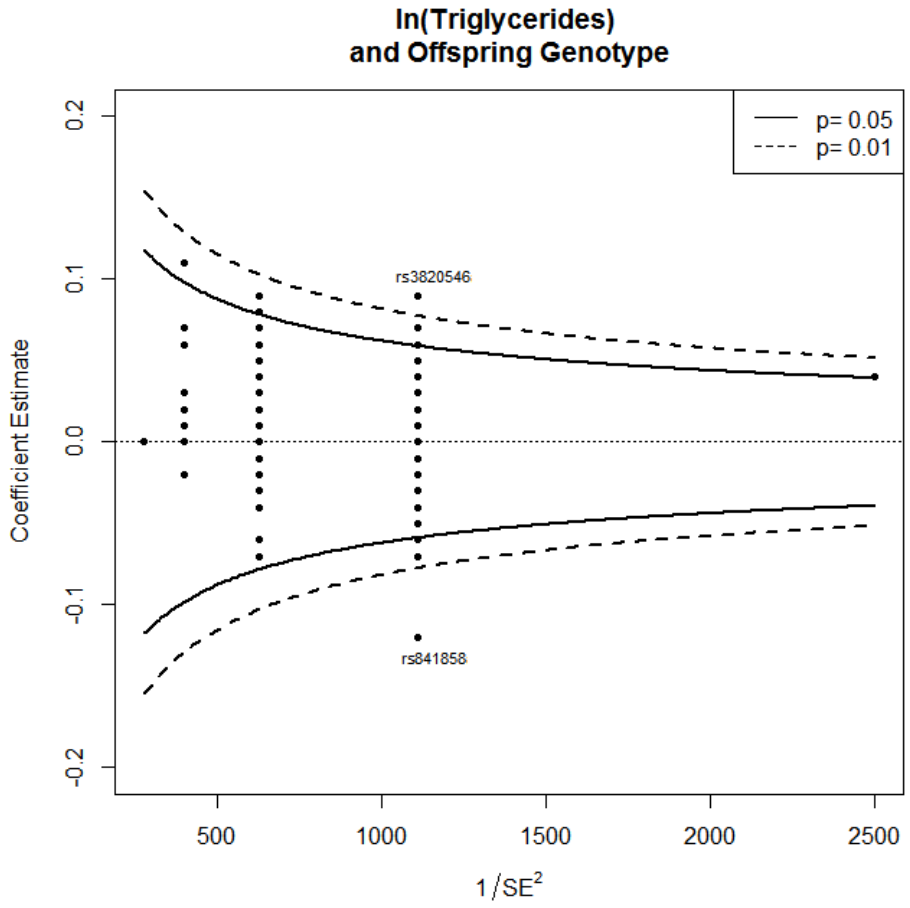


Figure S2.7: Results of candidate SNP – natural logarithm-transformed triglycerides association analyses.

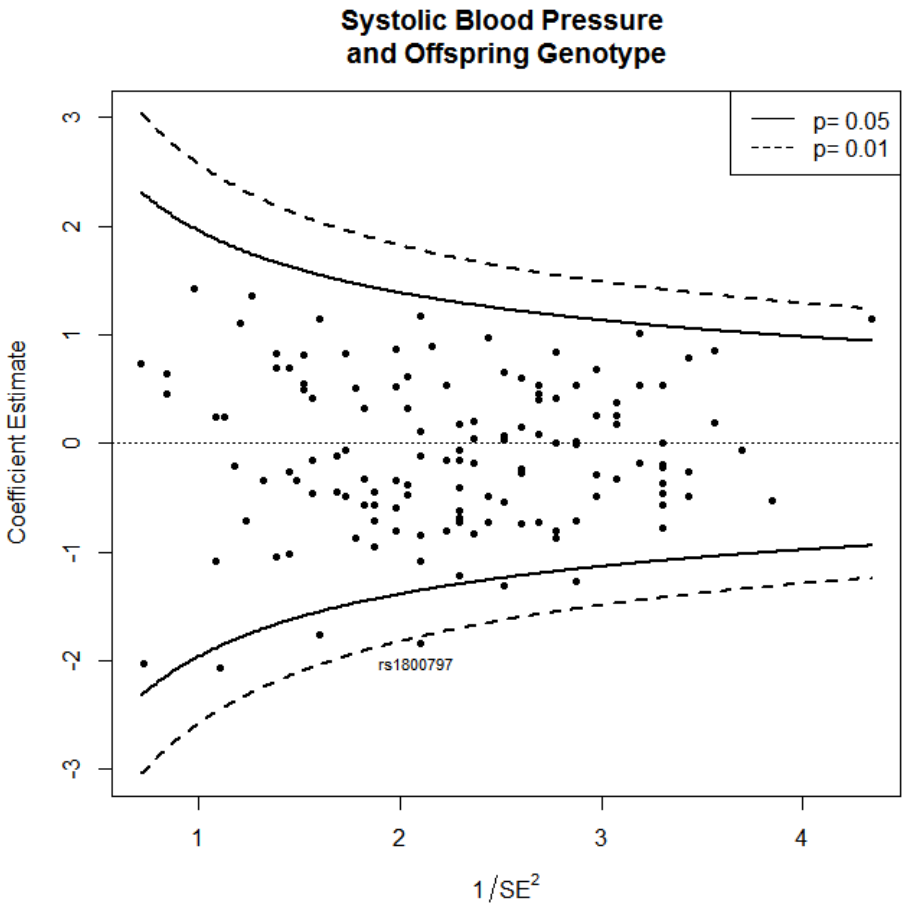


Figure S2.8: Results of candidate SNP – Systolic Blood Pressure association analyses.

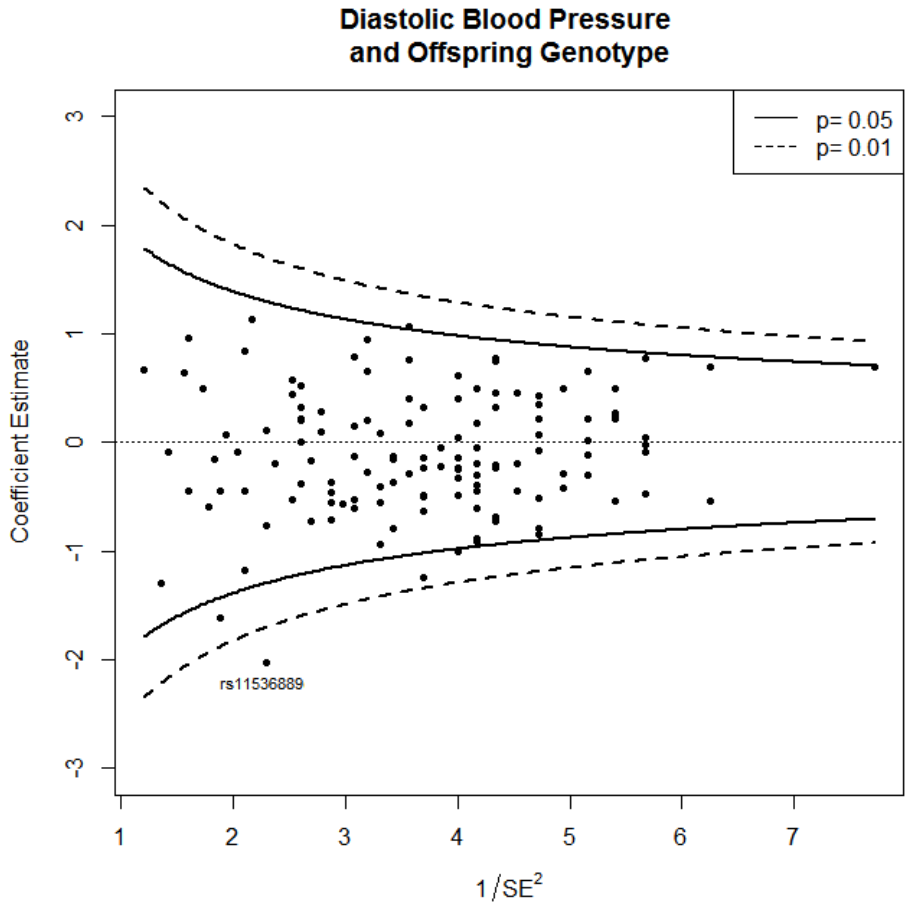


Figure S2.9: Results of candidate SNP – Diastolic Blood Pressure association analyses

Appendix D: Supplementary Tables for Chapter 3.

Table S3.1. The interaction between maternal rs4851522 and offspring 25[OH]D on offspring LDL is robust to covariate

	SNP association			vitd association			SNP X vitdInteraction		
	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
Original Model	26.22	5.89	0.00001	0.61	0.19	0.0017	-1.12	0.24	0.000004*
Without BMI	26.63	6.37	0.0000	0.54	0.20	0.0080	-1.16	0.26	0.0000
Without eight covariates describing offspring ethnicity	26.20	5.94	0.0000	0.57	0.19	0.0030	-1.11	0.24	0.0000
With mppbmi, gwg	27.50	6.11	0.0000	0.65	0.20	0.0010	-1.18	0.25	0.0000
with missing fasting	26.22	5.89	0.0000	0.61	0.19	0.0017	-1.12	0.24	0.0000
With sex, bmi, season of blood draw only	25.09	5.84	0.0000	0.61	0.17	0.0005	-1.05	0.24	0.0000
With offspring rs4851522 genotype	27.23	5.15	0.0000	0.61	0.19	0.0016	-1.13	0.24	0.0000
Including eight covariates describing maternal ethnicity	27.21	5.92	0.0000	0.62	0.19	0.0012	-1.14	0.24	0.0000
With maternal IL-18R1 rs3771170 genotype	26.18	5.99	0.0000	0.57	0.19	0.0020	-1.12	0.25	0.0000
With maternal IL-18R1 rs3771170-offspring 25[OH]D interaction	23.92	6.11	0.0001	0.80	0.23	0.0005	-1.02	0.26	0.0001
With offspring IL-18R1 rs3771170-offspring 25[OH]D interaction	23.84	5.84	0.0000	0.98	0.23	0.0000	-1.03	0.24	0.0000

Table S3.2. The interaction between maternal BCL2 rs4987853 and offspring CMV IgG on offspring HDL is robust to covariate inclusion/exclusion.

	SNP association			vitd association			SNP X vitdInteraction		
	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
Original Model	-3.34	1.48	0.0241	-0.16	0.08	0.0430	0.40	0.11	0.0003*
Without BMI	-3.81	1.53	0.0130	-0.17	0.09	0.0460	0.41	0.12	0.0005
Without eight covariates describing offspring ethnicity	-3.06	1.42	0.0320	-0.17	0.08	0.0310	0.39	0.11	0.0007
With mppbmi, gwg	-3.38	1.51	0.0260	-0.18	0.08	0.0290	0.41	0.11	0.0003
with missing fasting	-3.34	1.78	0.0241	-0.16	0.08	0.0430	0.40	0.11	0.0003
With sex, bmi, season of blood draw only	-2.86	1.42	0.0450	-0.23	0.07	0.0020	0.38	0.11	0.0007
with offspring rs4987853	-3.19	1.50	0.0340	-0.16	0.08	0.0450	0.40	0.11	0.0003
with offspring interaction*	-2.40	1.61	0.1380	-0.21	0.09	0.0230	0.33	0.13	0.0150

*offspring interaction not statistically significant

Table S3.3. The interaction between maternal IL-21 rs13143866 and offspring 25[OH]D on offspring HDL is robust to covariate inclusion/exclusion.

	SNP association			vitd association			SNP X vitdInteraction		
	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
Original Model	8.90	2.45	0.0003	0.26	0.09	0.0034	-0.35	0.09	0.0002*
Without BMI	8.18	2.53	0.0013	0.29	0.09	0.0017	-0.31	0.10	0.0011
Without eight covariates describing offspring ethnicity	8.72	2.43	0.0004	0.28	0.09	0.0017	-0.34	0.10	0.0005
With mppbmi, gwg	8.94	2.89	0.0003	0.25	0.09	0.0050	-0.34	0.10	0.0005
with missing fasting	8.90	2.45	0.0003	0.26	0.09	0.0034	-0.35	0.09	0.0002
With sex, bmi, season of blood draw only	7.49	2.46	0.0025	0.24	0.08	0.0044	-0.28	0.10	0.0047
with offspring rs13143866	10.40	2.56	0.0001	0.28	0.11	0.0092	-0.40	0.10	0.0001
with offspring interaction*	9.77	2.97	0.0011	0.31	0.13	0.0186	-0.37	0.12	0.0021

*offspring interaction not statistically significant

Table S3.4. Sensitivity analysis of CMV positivity and offspring genotype.

Gene	SNP	Coefficient	Std Err	p-value
CYP24A1	rs6013897	-0.10	0.04	0.0056
IL-10	rs3024496	-0.08	0.03	0.0093
SLC2A1	rs751210	-0.07	0.03	0.0098

Table S3.5. Combination genotype analysis of maternal SLC2A1 SNPs rs751210 and rs12407920 on offspring triglycerides (natural log-transformed). Zero minor alleles at each locus used as baseline, minimum 10 participants required in each stratum.

number of rs751210 minor alleles	number of rs12407920 minor alleles	Coefficient	Std Err	p-value
1	0	-0.05	0.05	0.2990
2	0	-0.15	0.09	0.0920
1	1	-0.10	0.07	0.1350
2	1	-0.15	0.07	0.0270

Table S3.6. Comparison of additive, dominant, and recessive genotype models in the interaction between maternal rs4987853 in BCL2 and offspring CMV IgG on offspring systolic BP.

Model	SNP association			CMV IgG association			SNP X CMV IgG Interaction		
	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
Additive	-3.34	1.48	0.0241	-0.16	0.08	0.0430	0.40	0.11	0.0003
Dominant	2.23	1.36	0.1030	0.13	0.07	0.0500	-0.23	0.11	0.0270
Recessive	2.05	3.50	0.5590	0.05	0.05	0.3680	-0.28	0.21	0.1890

Table S3.7. Comparison of additive, dominant, and recessive genotype models in the interaction between maternal rs4851522 in IL-1R2 and offspring 25[OH]D concentration on offspring LDL.

Model	SNP association			25[OH]D Association			SNP X 25[OH]D Interaction		
	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
Additive	26.22	5.89	0.0000	0.61	0.19	0.0017	-1.12	0.24	< 0.00005
Dominant	31.87	7.61	< 0.0005	0.64	0.19	0.0010	-1.36	0.32	< 0.0005
Recessive	22.00	12.31	0.0750	0.23	0.18	0.2120	-1.14	0.42	0.0070

Table S3.8. Comparison of additive, dominant, and recessive genotype models in the interaction between maternal rs13143866 in IL-21 and offspring 25[OH]D concentration on offspring HDL.

Model	SNP association			25[OH]D Association			SNP X 25[OH]D Interaction		
	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
Additive	8.90	2.45	0.0003	0.26	0.09	0.0034	-0.35	0.09	0.0002
Dominant	10.06	3.88	0.0100	0.23	0.09	0.0120	-0.35	0.15	0.0230
Recessive	15.68	2.20	0.0030	0.14	0.09	0.1340	-0.71	0.19	< 0.0005

Table S3.9. Top hit associations between maternal SLC2A1 SNPs and offspring triglycerides (natural log-transformed) are attenuated with addition of offspring top hit SLC2A1 SNPs to models.

Maternal SNP	Model	Coefficient	Robust SE	p-value
rs751210	Original Model	-0.08	0.03	0.0075
	with offspring rs841858	-0.06	0.03	0.0480
	with offspring rs3820546	-0.06	0.03	0.0340
rs12407920	Original Model	-0.10	0.04	0.0124
	with offspring rs841858	-0.09	0.04	0.0150
	with offspring rs3820546	-0.08	0.04	0.0560

Appendix E: Supplementary figures S3.1-S3.9: Graphical representations of the results of candidate maternal SNP analyses on nine offspring CMR-related outcomes.

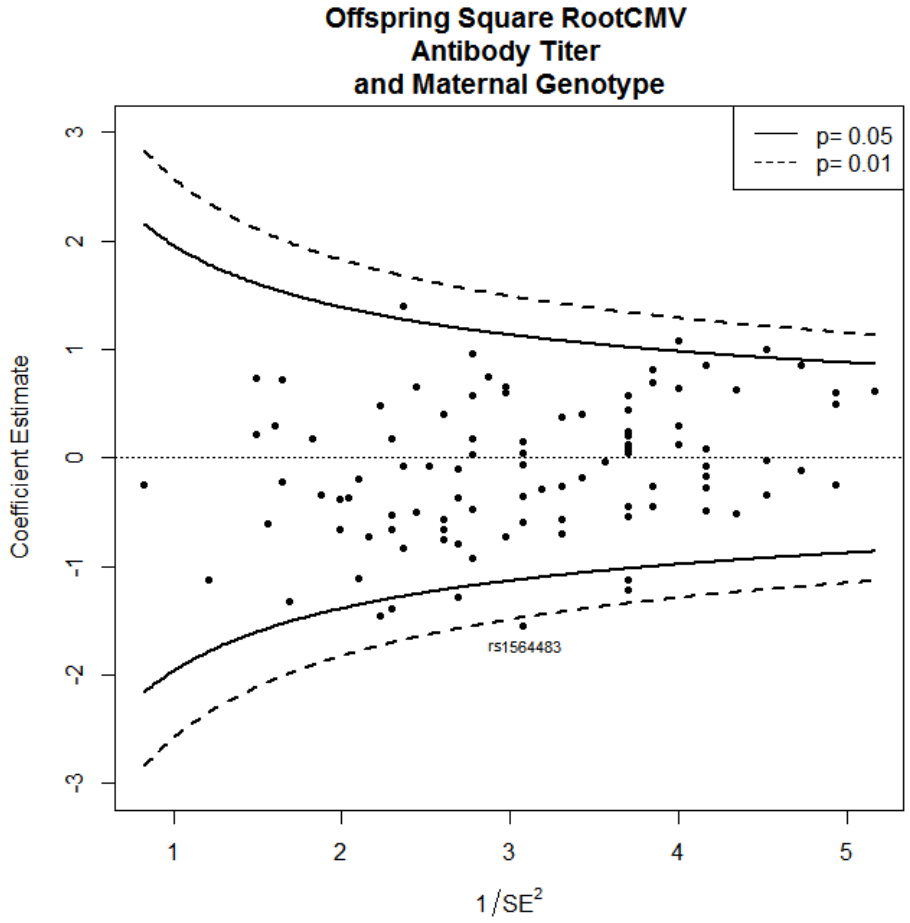


Figure S3.1: Results of candidate maternal SNP – offspring CMV IgG association analyses.

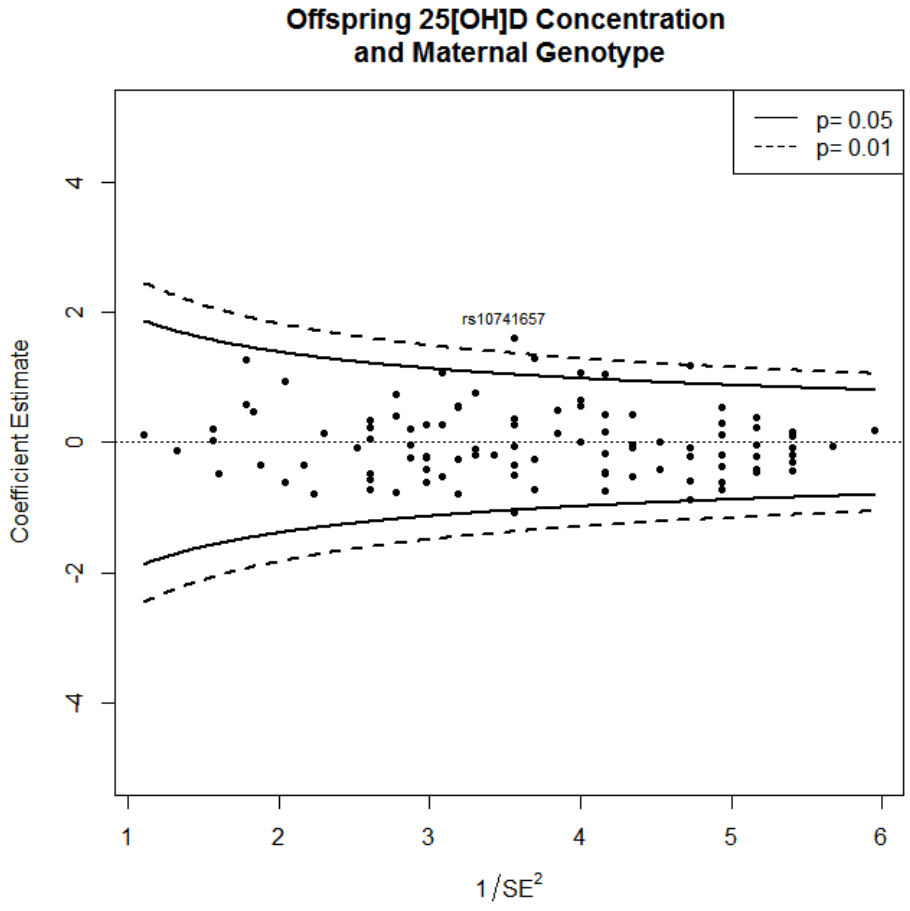


Figure S3.2: Results of candidate maternal SNP – offspring 25[OH]D association analyses.

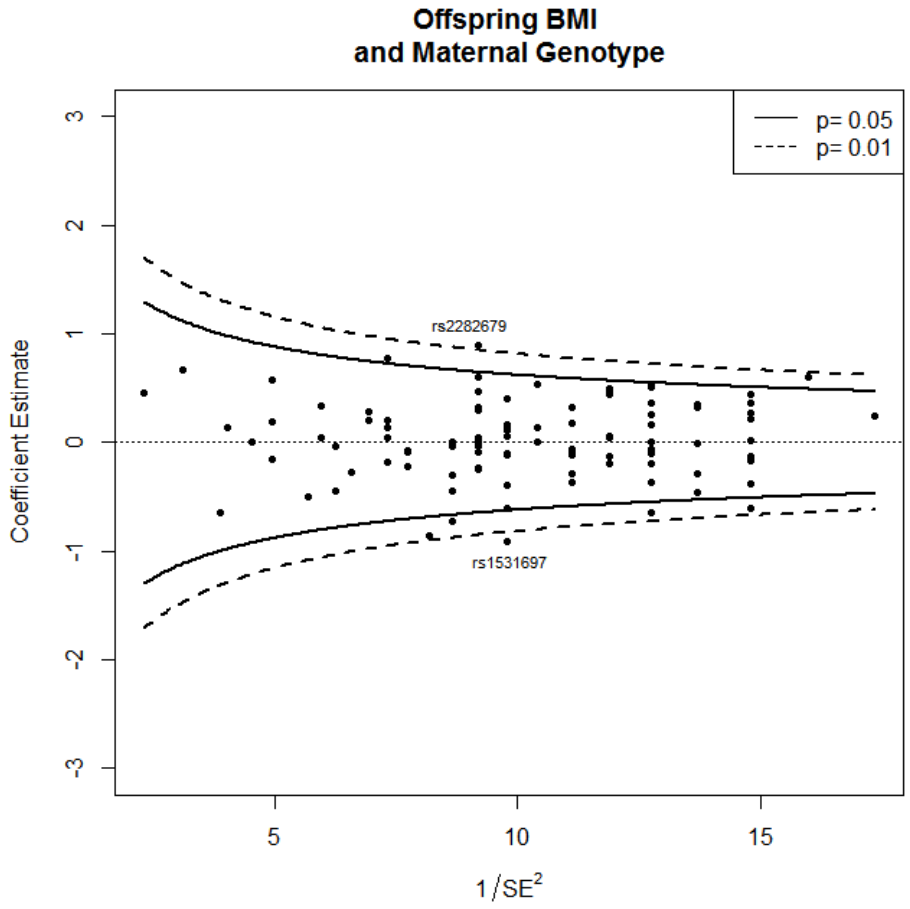


Figure S3.3: Results of candidate maternal SNP – offspring BMI association analyses.

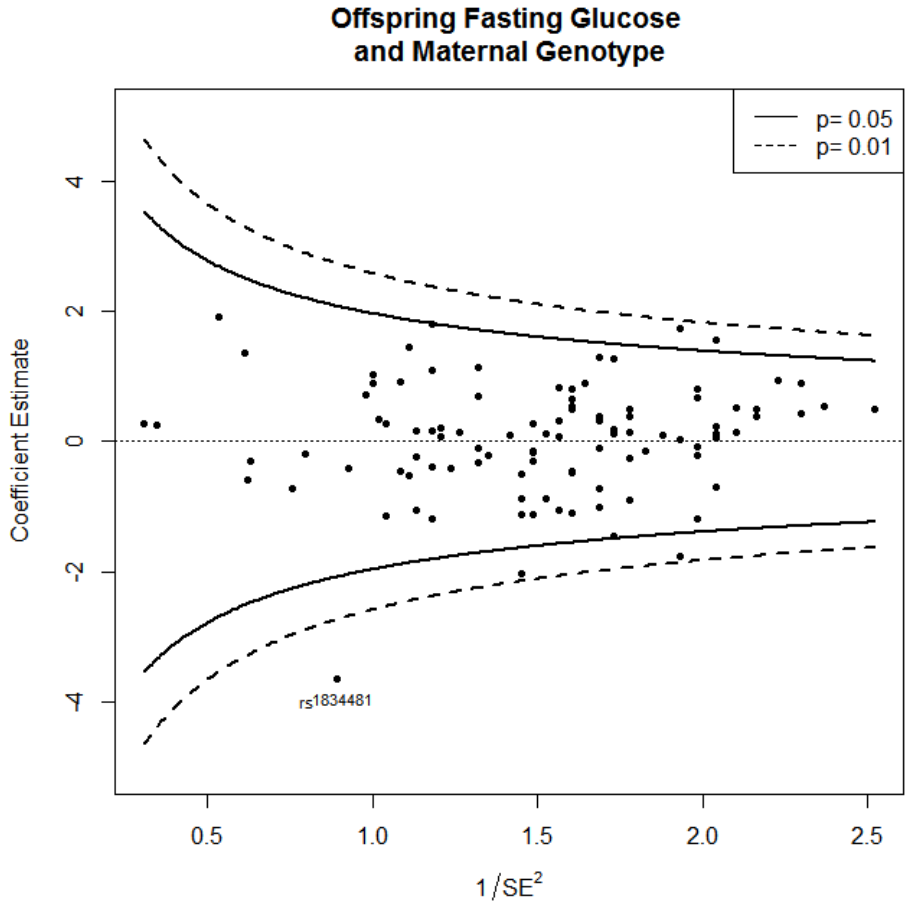


Figure S3.4: Results of candidate maternal SNP – offspring glucose association analyses.

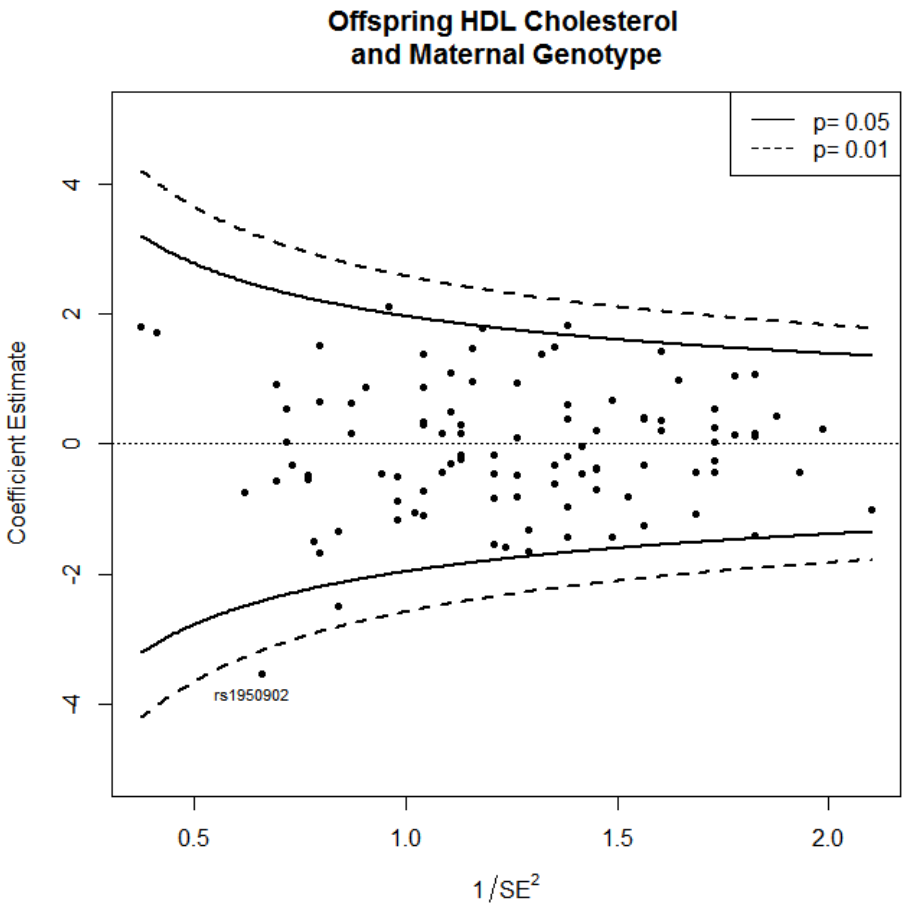


Figure S3.5: Results of candidate maternal SNP – offspring HDL association analyses.

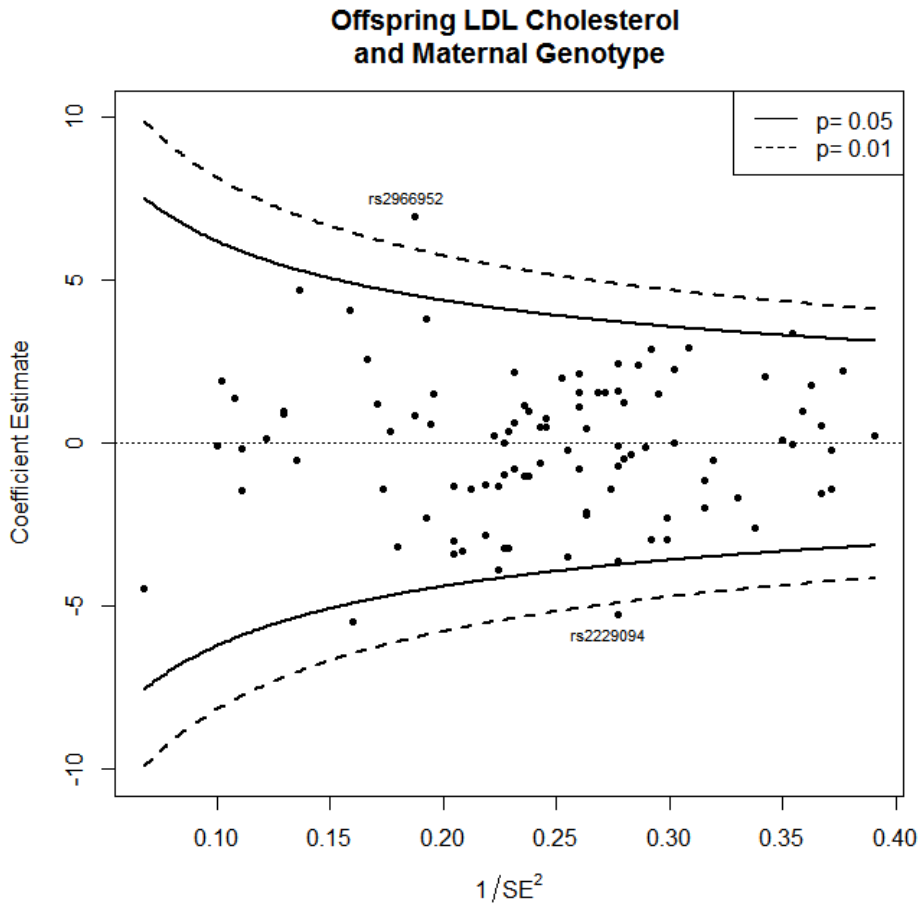


Figure S3.6: Results of candidate maternal SNP – offspring LDL association analyses.

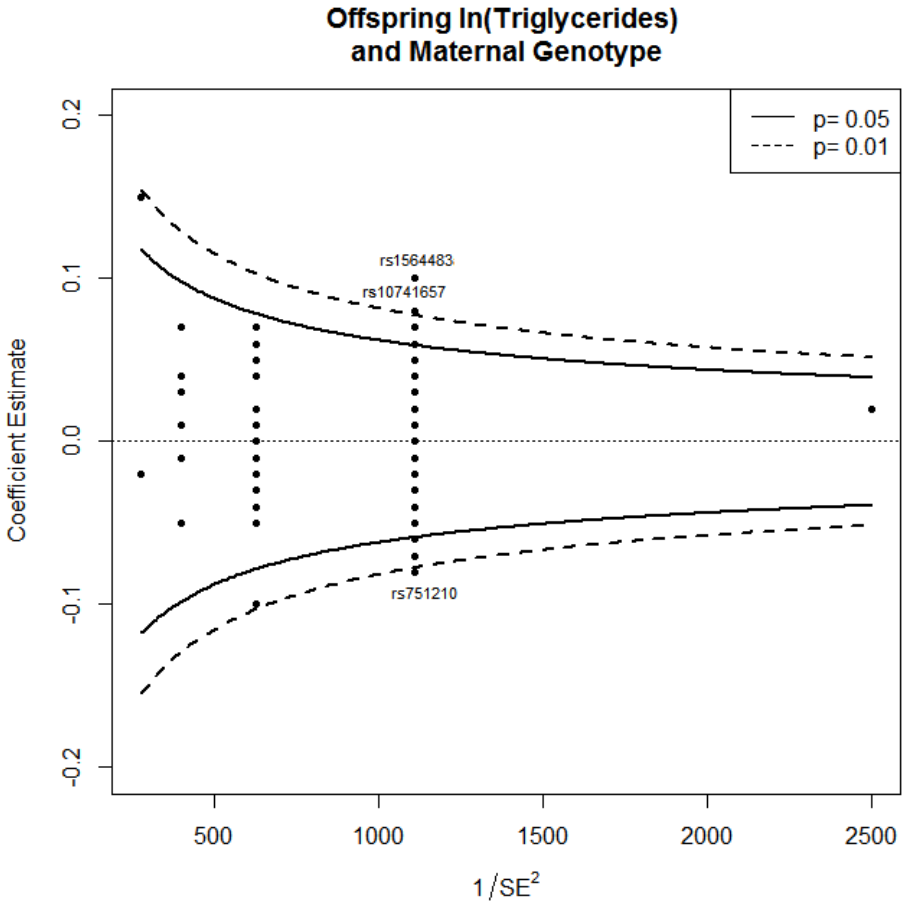


Figure S3.7: Results of candidate maternal SNP – offspring triglyceride association analyses.

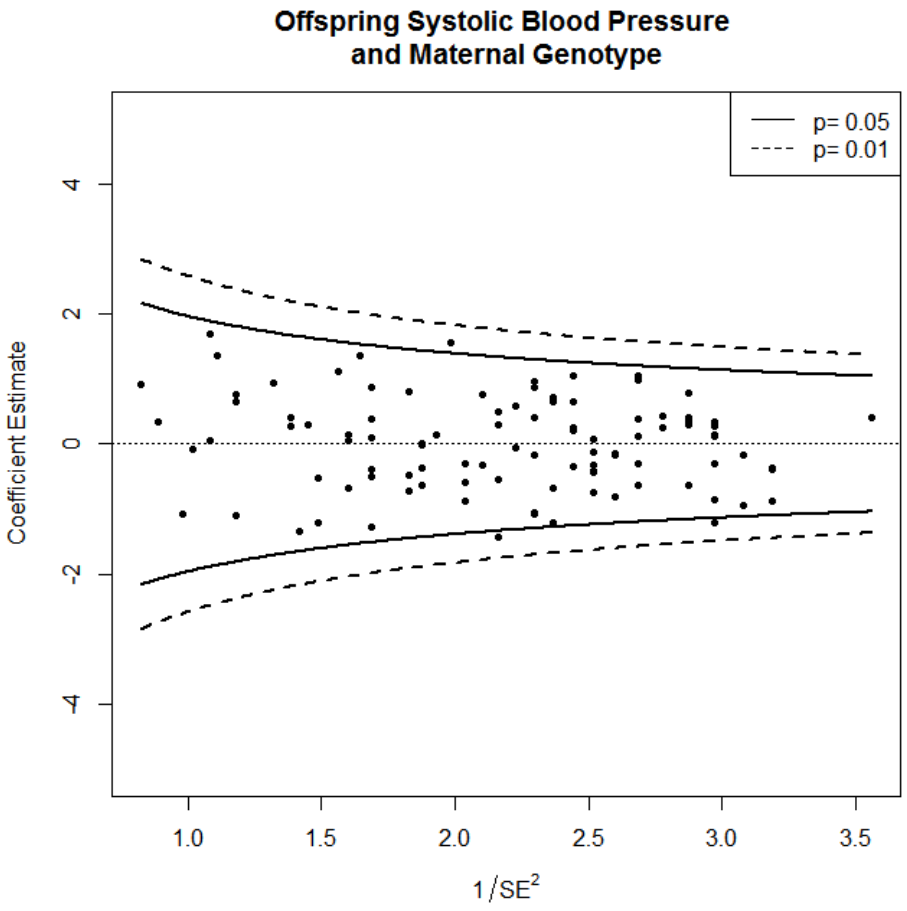


Figure S3.8: Results of candidate maternal SNP – offspring systolic blood pressure association analyses.

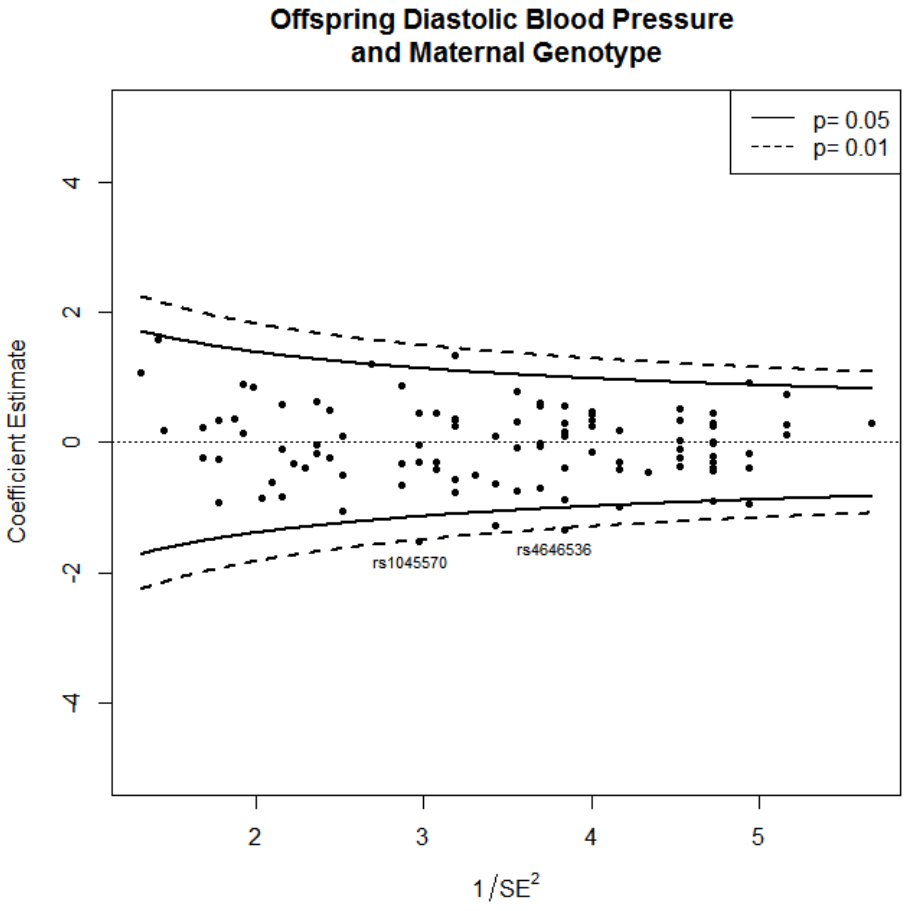


Figure S3.9: Results of candidate maternal SNP – offspring diastolic blood pressure association analyses

Appendix F: Supplementary Tables for Chapter 4.

Table S4.1. Results of maternal-offspring genotype interaction analysis are robust to lack of adjustment for BMI.

			Offspring SNP association			Maternal SNP association			Maternal-Offspring Genotype Interaction		
Outcome	Gene	SNP	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
25[OH]D (ng/mL)	TLR3	rs3775291	-0.60	0.96	0.5330	-2.29	0.80	0.0040	2.16	0.79	0.0060
25[OH]D (ng/mL)	IL-1R2	rs3218984	-2.05	0.84	0.0140	-0.94	0.90	0.2990	2.75	1.00	0.0060
HDL (mg/dL)	IL-10	rs3024498	2.98	2.42	0.2180	1.15	1.57	0.4630	-4.39	2.14	0.0410
LDL (mg/dL)	BCL2	rs4940576	6.69	3.30	0.0430	6.99	3.33	0.0360	-9.94	3.25	0.0020
ln(Triglycerides)	MTHFD1L	rs572522	0.20	0.06	< 0.0005	0.07	0.06	0.2160	-0.14	0.05	0.0030
ln(Triglycerides)	MTHFD1L	rs4869959	0.16	0.06	0.0120	0.05	0.05	0.3310	-0.17	0.06	0.0040
Systolic BP (mmHg)	MTRR	rs1532268	-1.77	1.25	0.1580	-3.62	1.25	0.0040	3.31	1.09	0.0030
Systolic BP (mmHg)	BCL2	rs2046136	2.74	1.10	0.0130	2.33	1.06	0.0280	-3.44	1.12	0.0020
Diastolic BP (mmHg)	MTRR	rs1532268	-1.78	1.05	0.0900	-3.13	0.98	0.0010	2.85	0.87	0.0010
Diastolic BP (mmHg)	KCNQ1	rs231362	-2.23	1.05	0.0340	-2.49	0.96	0.0090	2.51	0.96	0.0090

Supplementary Table S4.2. Results of maternal-offspring genotype interaction analysis are somewhat robust to covariate exclusion - sex and season adjustment only.

			Offspring SNP association			Maternal SNP association			Maternal-Offspring Genotype Interaction		
Outcome	Gene	SNP	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value	Coefficient	Robust SE	p-value
25[OH]D (ng/mL)	TLR3	rs3775291	0.75	1.12	0.5080	-1.90	1.08	0.0790	1.26	1.12	0.2610
25[OH]D (ng/mL)	IL-1R2	rs3218984	-1.60	0.89	0.1460	-0.97	0.96	0.3150	2.14	1.05	0.0420
HDL (mg/dL)	IL-10	rs3024498	2.92	2.40	0.2250	1.72	1.60	0.2830	-4.21	2.20	0.0560
LDL (mg/dL)	BCL2	rs4940576	4.49	3.18	0.1590	6.05	3.36	0.0720	-7.85	3.08	0.0110
ln(Triglycerides)	MTHFD1L	rs572522	0.17	0.06	0.0030	0.05	0.06	0.4130	-0.12	0.05	0.0140
ln(Triglycerides)	MTHFD1L	rs4869959	0.15	0.06	0.0230	0.08	0.53	0.1360	-0.17	0.06	0.0050
Systolic BP (mmHg)	MTRR	rs1532268	-1.85	1.25	0.1400	-3.54	1.25	0.0050	3.02	1.05	0.0040
Systolic BP (mmHg)	BCL2	rs2046136	2.79	1.08	0.0100	2.66	1.05	0.0120	-3.51	1.12	0.0020
Diastolic BP (mmHg)	MTRR	rs1532268	-2.11	1.04	0.0440	-3.59	0.96	< 0.0005	2.91	0.88	0.0010
Diastolic BP (mmHg)	KCNQ1	rs231362	-2.38	0.10	0.0160	-2.46	0.94	0.0090	2.42	0.90	0.0070

Table S4.3. Sensitivity analysis of offspring CMV seropositivity and maternal-offspring genotype interaction.

Gene	SNP	Offspring			Maternal			Interaction		
		Coefficient	Std Err	p-value	Coefficient	Std Err	p-value	Coefficient	Std Err	p-value
IL-6	rs1554606	0.09	0.31	0.7661	1.04	0.35	0.0032	-0.88	0.29	0.0022
SLC7A10/PEPD	rs8182584	-1.27	0.43	0.0030	-0.96	0.39	0.0136	0.98	0.35	0.0046
LTA	rs1799964	-0.67	0.36	0.0630	-0.32	0.34	0.3496	0.90	0.40	0.0259
BCL2	rs2850761	-0.70	0.34	0.0406	-0.59	0.34	0.0806	0.58	0.28	0.0405
LTA	rs1800630	-0.24	0.36	0.5009	-0.75	0.34	0.0262	0.79	0.39	0.0444
ABCA1	rs1883025	-0.64	0.39	0.0989	-0.62	0.44	0.1603	0.90	0.45	0.0485