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Effect modification by *Transferrin* C2 polymorphism on lead exposure, hemoglobin levels, and IQ

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Abstract

Background—Iron deficiency and lead exposure remain significant public health issues in many parts of the world and are both independently associated with neurocognitive deficits. Polymorphisms in iron transport pathways have been shown to modify the absorption and toxicity of lead.

Objective—We hypothesized that the transferrin (TF) C2 polymorphism modifies the effects of lead and hemoglobin on intelligence.

Methods—Children aged 3–7 years (N=708) were enrolled from 12 primary schools in Chennai, India. The Binet-Kamat Scale of Intelligence were administered to ascertain intelligence quotient (IQ). Venous blood was analyzed for lead and hemoglobin levels. Genotyping for the TF C2 polymorphism (rs1049296) was carried out using a MassARRAY iPLEXTM platform. Stratified analyses and interaction models, using generalized estimating equations, were examined to explore interactions between lead, hemoglobin, and TF C2 categories.

Results—A one-unit increase in log blood lead and 1 g/dl higher hemoglobin was associated with −7.7 (95% CI: −13.6, −1.8) and 1.7 (95% CI 1.4, 2.1) IQ points, respectively, among children carrying the C2 variant. In comparison, among children who had the homozygous wildtype allele, the same increment of lead and hemoglobin were associated with −2.1(95% CI: −6.5, 2.4) and

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Conflicts of Interest:

The authors declare that there are no conflicts of interest.

2.8(95% CI:1.5, 4.0) IQ points, respectively. There was a significant interaction between lead ($p=0.04$) and hemoglobin ($p=0.07$) with the C2 variant.

Conclusion—Children who carry the TF C2 variant may be more susceptible to the neurotoxic effects of lead exposure and less protected by higher levels of hemoglobin.

Keywords

lead; hemoglobin; iron; transferrin; intelligence quotient (IQ); genotype

INTRODUCTION

Lead exposure is well-documented to be associated with cognitive deficits among children, yet, there is significant variation in effect estimates which may be due to different susceptibility factors, such as nutritional status and genetics. Iron deficiency is the most widespread and common nutritional deficiency, especially among children due to their rapid growth and increased iron requirement (CDC, 1998). It is estimated that the prevalence of iron deficiency anemia is around 46–66% in developing countries (Stoltzfus et al., 2007). In India, 74% of urban children less than 3 years of age are anemic (International Institute for Population Sciences and Macro International, 2007).

Animal and epidemiological studies suggest that iron deficiency is associated with decreased behavioral and cognitive function in children (Congdon et al., 2012; Lozoff, 2011; Kordas, 2010; Osendarp et al., 2010; Stoltzfus et al., 2007). High lead exposure and iron deficiency most often coincide with key periods of accelerated brain development and, unfortunately, are disproportionately higher among disadvantaged populations (Kordas, 2010).

Iron and lead share similar toxicokinetic and toxicodynamic properties. Animal studies show that increased body iron demand or iron deficiency leads to the up-regulation of the divalent metal transporter (DMT) and, thus, increased absorption of lead (Bressler et al., 2004) and epidemiological studies support this association (Kwong et al., 2004). The strongest evidence comes from a longitudinal study by Wright et al. (2003) which demonstrated increased risk of lead poisoning among children with low body iron status. An intervention study in India showed that food iron fortification reduced median blood lead levels among 5–9 year old children (Zimmermann et al., 2006).

Transferrin (TF) is a key protein for iron distribution throughout the body and for transport and distribution of iron in the brain (Moos 2002). Studies in animals have shown up-regulation of TF receptors on the blood brain barrier and of DMT on the olfactory neurons during lowered iron status and as well as increased transport of metals, such as manganese (Moos 2002; Heilig et al., 2005). It is possible that lead transport across the blood brain barrier may also be similarly modified during iron deficiency. Lead has been shown to interfere with TF receptor expression and inhibition of TF endocytosis in rabbit reticulocytes (Qian and Morgan, 1990; Kohno et al., 1993), but little is known whether it also alters TF system in the brain. Once in the brain, lead and iron have been shown to influence both myelination and the dopamine system, leading to altered nerve conduction and changes in cognition and behavior (Kordas, 2010; Brubaker et al 2009; Todorich et al 2008; Beard 2007; Beard et al. 2006; Ortiz et al 2004; Zuch et al. 1998; Cory-Slechta 1995, 1996, 1997).

Previous studies have shown that polymorphisms in the *HFE* gene, encoding for the human hemochromatosis protein which regulates iron homeostasis through its interaction with the TF receptor, is associated with higher levels of lead in children (Hopkins et al. 2008) and increased susceptibility to the adverse health effects of lead exposure (Cantonwine et al., 2010; Zhang et al., 2010; Park et al., 2009; Wang et al., 2007). It is not known if

polymorphisms in the *TF* gene, encoding for TF protein, modify the effects of lead and iron on cognition among children.

In this study, we explored if the *TFC2* polymorphism modifies the effects of lead exposure and hemoglobin (as a marker of iron status) on IQ among school-aged children in Chennai, India.

MATERIALS AND METHODS

Study population and design

The study population has been described previously (Palaniappan et al., 2011; Roy et al., 2011; Roy et al., 2009; Roy et al., 2009). In brief, 756 children 3–7 years of age were enrolled from 12 schools, in four different zones in the city of Chennai. Parents of the children were invited to group discussions with the research team at the schools and informed consent was acquired from the primary caregiver of each child.

Cognitive testing and blood collection for lead, hemoglobin, and genotype analyses were completed on the same day. A trained interviewer administered a questionnaire in Tamil (the regional language) to the primary caregiver of the child to collect demographic information and other characteristics. Hemoglobin levels were missing, due to laboratory error, for three study participants and 45 participants had missing genotype information due to DNA degradation. There were 708 study participants with complete information.

This collaborative study on lead, genetics, and neurotoxicity was conducted by the Harvard School of Public Health (HSPH; Boston, MA, USA) and Sri Ramachandra Medical College and Research Institute (SRMC; Chennai, India). The study was approved by institutional review boards at HSPH, SRMC, and the Indian Council for Medical Research (New Delhi, India) and complied with all federal U.S. and Indian regulations and laws regarding research in human subjects.

Assessment of cognitive function

The IQ of each child was derived using the standardized Tamil-translated Binet-Kamat Scales of Intelligence (Kamat, 1967), which was administered in a separate, quiet room on the school premises by trained research personnel who were blinded to all aspects of the children's lead exposure, hemoglobin status, and developmental history. This amended and validated version of the 1934 Stanford-Binet Scales of Intelligence is a culture-specific scale that uses Indian pictorial scenes and concepts. The test is designed to measure aptitude and intellectual capacity through assessment of verbal reasoning, comprehension, quantitative reasoning, vocabulary, abstract reasoning, similarities, differences, visual reasoning, and short-term memory. The child's mental age (in months) was calculated based on his or her performance on the Binet-Kamath IQ Test. The mental age was then divided by the chronological age (in months) and multiplied by 100 to obtain the child's IQ.

Assessment of blood lead and hemoglobin

Whole blood (10 mL) was collected into lead-free tubes (Becton-Dickinson blue-top tubes; Becton-Dickinson, Franklin Lakes, NJ USA) from the cubital vein of each child by a trained phlebotomist during a school day. Blood lead was measured at SRMC, using a LeadCare[®] Analyzer instrument (ESA Laboratories, Chelmsford, MA, USA), which is a well-validated field instrument (Shannon and Rifai 1997) with limit of detection of 1.4 µg/dL blood lead (range of 1.4–65 µg/dL) and resolution of 0.1 µg/dL. Duplicates and ESA company controls were run every 20 samples and with every new batch and kit lot. The instrument was calibrated every time a new test kit batch or control batch was used.

Blood hemoglobin concentrations (g/dL) were measured using standard clinical methods on a BC-3000 Plus Auto Hematology Analyzer (MINDRAY; Bio-Medical Electronics Co., Ltd., Shenzhen, China) at the Pathology and Hematology Laboratory in SRMC. The auto analyzer has a range of 1–25 g/dL for hemoglobin.

Genotyping

DNA was extracted from an aliquot of whole blood from each child and analyzed for the *TF* C2 polymorphism (rs1049296). The sequence, including 600 bp upstream and downstream of the SNP, was ascertained from Genewindow (<http://genewindow.nci.nih.gov/Welcome>). All polymerase chain reaction (PCR) and extension primers were synthesized at BioServe Biotechnologies, Ltd. (Beltsville, MD, USA). Genotyping was performed by BioServe at their Hyderabad, India facility using a MassARRAY iPLEX[®] platform (Sequenom, San Diego, CA, USA), which is a PCR-based process and mass spectrometry-based system.

To ensure that the samples were processed correctly and without external contamination, quality control checks involved four positive controls for each assay and four negative controls (no template controls) per plate. The positive controls were 24 DNA samples, in duplicate, from the National Institutes of Health Polymorphism Discovery Panel (Collins et al., 1998). The controls were selected to cover all of the possible genotypes available for the multiplex assay panel developed. The peaks for individual assays were checked manually in addition to using Sequenom's SpectroTYPER[®] software automated detection.

The full data set was anonymized after genotyping to protect the confidentiality of our study participants and to conform to current institutional review board policies.

Statistical analysis

Genotype frequencies were calculated and the observed frequencies were tested against expected according to principles of Hardy-Weinberg equilibrium using the chi-square test statistic. Univariate distributions of all variables of interest were explored across *TFC2* polymorphism category. Bivariate analysis of variance tests were conducted to ascertain if the distributions of covariates differed among the *TFC2* genotypes. In all multivariable analyses, outliers in lead (N=7) and IQ (N=1) identified using the generalized extreme studentized deviate method (Rosner, 1983) were removed.

Blood lead levels were natural log-transformed to maintain a normal distribution and because the lead- IQ relationship in most published papers uses log blood lead as the unit, allowing for comparison across studies and populations. Further, an international pooled analysis of lead and IQ suggests that the relationship is best fit using a log-linear model (Lanphear et al. 2005). Hemoglobin levels (continuous measure) was used as our measure of iron status rather than anemia status (hemoglobin < 11.5) as only 18% of our population was anemic and categorization reduced the power to detect any effect on IQ or interaction with the transferrin polymorphism. Multivariable linear regression models were developed using ordinary least squares regression to estimate associations between IQ and blood lead and/or hemoglobin. Covariates [age + age² (months), sex, mid-arm circumference (cm), average monthly family income (<2,000, 2,000–4,000, 4,000–6,500, >6,500 Indian rupees), and family size] were retained in the model if the main effect estimate of lead or hemoglobin changed by >10% or if it increased the *R*² by >10%. Parental education (both mother and father: illiterate/primary school, middle school, high school, college) was included in multivariable models in order to maintain consistency with other studies that have evaluated the impacts of lead and hemoglobin on cognition (Roy et al, 2011).

The study used a multi-level sampling approach, so correlation among levels of sampling (classroom, school, and zone) were calculated using intraclass correlations in a random

effects model. The distributions of the residuals were not strictly normal; therefore, generalized estimating equations (GEEs) were used to account for clustering at the school and classroom levels. The GEE models use quasi-likelihood methods to model the marginal associations after accounting for clustering (Qaqish and Liang, 1992).

First, multivariable main effects models were created to estimate the effects of lead, hemoglobin, and *TFC2* genotypes [categorized as C/C (wild-type, reference), C/T, or T/T] on IQ, adjusted for age (age and age², in months), sex, mid-arm circumference (cm), average monthly family income (four categories), parental education (mother and father: illiterate/primary school, middle school, high school, college), and family size (model 1). Next, effects of lead and hemoglobin on IQ were estimated according to genotype (three categories) using stratified models adjusted for the covariates above (model 2).

We then combined the *TFC2* genotype categories into two categories based on the presence or absence of the variant “T” allele (C/C vs. C/T and T/T combined) to ensure sufficient power since the homozygous variant (T/T) only contained 33 subjects. In model 3, the effects of lead and hemoglobin on IQ were estimated stratified across these two categories and, in model 4, the interaction between lead and hemoglobin with each other and with the C2 polymorphism were evaluated. The interaction terms (hemoglobin × C2 category and lead × C2 category) were modeled first in separate models, each while adjusting for the other exposure, and then jointly, to assess joint interactions, after controlling for other potential confounders. A three-way interaction (hemoglobin × lead × C2) was not assessed because no interaction between lead and hemoglobin was observed. All statistical tests were two-sided and assessed at α -level of 5%. Statistical analyses were performed using SAS[®] for Windows, version 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Mean blood lead and hemoglobin (Hb) levels, respectively, were 11.5 ± 5.4 (range: 2.9–40.5) $\mu\text{g/dl}$ and 11.9 ± 1.2 (range: 5.2–14.5) g/dL ; 18.4% of the children were anemic (Hb < 11.5 g/dl). There were no non-detects of lead or hemoglobin levels. In this population, only 4.5% (N=33) were homozygous for the *TFC2* variant polymorphism (T/T), while 29.5% (N=209) were heterozygous (C/T) and 65.7% were homozygous wildtype (C/C) (Table 1).

Hemoglobin was lower by 0.6 g/dl (95% CI $-0.99, -0.18$; $p=0.005$) in the T/T variant carriers in comparison to the wildtype group. No other significant differences between the variant carriers and the homozygous wildtype children were noted (Table 2). In bivariate analyses, higher lead levels were associated with lower IQ and higher hemoglobin levels with higher IQ (both $p<0.0001$).

The results of the multivariable GEE models are presented in Table 3. All models were adjusted for: age, age², sex, parental education, family income, mid-arm circumference, and clustering by school. In the analysis of main effects (model 1), a one-unit increase log blood lead was associated with a decrease of 4.7 (95% CI 1.4, 7.9; $p=0.005$) IQ points. An increase in 1 g/dl of hemoglobin was associated with an increase in 2.2 (95% CI 1.2, 3.1; $p<0.0001$) IQ points. There was no evidence of interaction between lead and hemoglobin ($p>0.05$). The *TFC2* genotype was not a significant predictor of IQ ($p=0.17$). In model 2, the effects of lead and hemoglobin on IQ were larger among the variant polymorphism carrier groups (C/T and T/T) than in the homozygous wildtype group. However, the homozygous variant (T/T) only contained 33 subjects and the estimates were unstable, when trying to assess the interaction. Thus, to ensure sufficient power, we combined the heterozygous (C/T) and homozygous variant (T/T) groups, shown as results from Models 3 and 4. In the stratified analyses, a one-unit increase in log blood lead was associated with -9.8 IQ points

($p < 0.0001$) in the variant carriers group in comparison to -1.9 IQ points ($p = 0.37$) in the wildtype group, as seen in Figure 1. In the interaction model (lead* C2), a one-unit increase in log blood lead was associated with -7.7 (95% CI: $-13.6, -1.8$) IQ points among children carrying the variant allele in comparison to -2.1 (95% CI: $-6.5, 2.4$) IQ points among children homozygous for the wildtype allele. Among children carrying the C2 variant every one-unit increase in log blood lead was associated with 5.9 IQ points lower compared to the wildtype children ($p = 0.047$). Similarly, for hemoglobin, among children carrying the C2 variant, every 1 g/dl increase in hemoglobin was associated with 1.1 IQ points lower than observed among the wildtype children, though this association was not significant in the interaction model ($p = 0.07$).

DISCUSSION

The results of this study suggest that children carrying the variant allele of the *TFC2* polymorphism in this population are significantly more susceptible to the effects of lead and gain less beneficial effects of higher hemoglobin levels. This is evidenced by the higher deficits in IQ per unit-increase in blood lead levels and lower gains in IQ per unit-increase in hemoglobin among children carrying the variant allele in comparison to wildtype children.

The C2 variant has been found to be associated with increased Alzheimer's disease risk in several human genomewide association studies and has also been found to interact with HFE (Kauwe et al., 2010; Lehmann et al., 2012; Robson et al., 2004) suggesting that iron regulation plays an important role in neurodegeneration among the elderly. However, the mechanisms underlying the neurotoxic effects of common variants in *TF* gene, such as the C2 polymorphism, remain unclear. The C2 variant could play a role in increased oxidative stress due to changes in iron loading, in the brain (Beckman and Beckman, 1986). *In vitro* studies (Landeghem et al., 1998; Zatta et al., 2005) have shown no differences in the iron-binding properties of the variant, yet, in men with the C2 variant, higher brain levels of ferritin have been observed (Bartzokis et al., 2010). The C2 polymorphism has been found to affect transferrin glycosylation in a genomewide association study (Kutalik et al., 2011; van Rensburg et al., 2004), thus, glycosylation patterns could also be relevant. Although partial changes in TF glycosylation do not affect its receptor binding, they may speed its degradation (van Rensburg et al., 2004; Hoefkens et al., 1997). Total iron-binding capacity in blood may be marginally lower in C2 homozygotes, although a significant difference was observed in only one study (Cleve et al., 1988; Sikström et al., 1993; Wong and Saha, 1986). In a subset of subjects in the OPTIMA study, TF saturation was higher in non-demented elderly with both *HFE* 282Y and *TFC2*, although neither variant alone had any effect (Lehmann et al., 2006). The C2 variant may also have a role in β -amyloid metabolism (Kauwe et al., 2010).

Though we do not find a direct impact of the C2 polymorphism on IQ directly, this is the first study to demonstrate the role of the *TFC2* polymorphism in modifying the effect of lead exposure and hemoglobin levels on child neurocognition. This is consistent with another study in which the carriers of the C2 variant in the NAS were also found to have increased susceptibility to lead-induced effects on electrocardiographic conduction (Park et al., 2009), which also suggests that *TF* may modify the other health effects of lead.

Other studies have documented *TF* polymorphism-associated changes in susceptibility to lead exposure. Carriers of the variant *TF* allele were found to have increased susceptibility to the effects of lead on electrocardiographic conduction among elderly veterans in Boston (Park et al., 2009). *TF* variants were associated with higher blood lead levels among young children in Mexico (Hopkins et al., 2008). However, *TF* did not alter the lead associated

decrease in birth weight among mother infant pairs in the same study (Cantonwine et al., 2010).

These results are consistent with other studies which suggest that genetic polymorphisms associated with the hemochromatosis gene (*HFE*) are associated with differences in lead susceptibility across different health outcomes. Variants of the *HFE* genes were associated with higher blood lead levels (Hopkins et al., 2008) and lower birth weight (Cantonwine et al., 2010) among children in Mexico. *HFE* variants were also associated with measures of bone lead in the elderly (Wright et al., 2004) and modified bone lead associated changes in pulse pressure and cognitive decline among the elderly in the NAS (Zhang et al., 2010; Wang et al., 2007). We did not measure *HFE* gene variants in this study because the prevalence of known polymorphisms in the *HFE* gene is low among the Indian population (Hanson et al. 2001).

This study has several limitations. The cross-sectional observational design does not allow us to determine temporality of the associations between lead, hemoglobin and cognition. However, the modification of these effects by genotype is unaffected by the design since genotype does not change over time. Also, we note that for both lead and hemoglobin, multiple prospective studies have demonstrated that these exposures (high lead and low iron status) precede decrements in neurological functions tested, and these associations are widely accepted. We were unable to differentiate between iron deficiency and other reasons of lower hemoglobin as we did not measure total iron binding capacity. This could lead to misclassification of iron status due to other nutritional deficiencies. However, a meta analysis of several studies suggests that hemoglobin, as a marker of iron, is associated with cognition (Stoltzfus et al., 2007).

We were limited to assessing the effect on only the full scale IQ score and could not break it down to the verbal and non verbal scores, because the Binet- Kamat scale is an old instrument of IQ assessment (Kamat 1967) and only the full scale IQ has been validated in the Indian population. Thus we do not have age standardized subtest values. Further, parental IQ was not measured and thus we could not control for it and we did not assess the quantity or quality of emotional and cognitive stimulation in the home environment using an instrument, such as the Home Observation for Measurement of the Environment (HOME) Inventory (Bradley & Caldwell, 1984), because a similar culturally-appropriate instrument for the Indian sub-context does not exist. However, we did control for socioeconomic status (income) and parental education (both maternal and paternal), which have been shown to be moderately correlated with the stimulation provided to a child in the home and parental IQ (Seifer, 2001). The instruments used to measure blood lead and hemoglobin levels have been documented to have good field accuracy and precision, reducing the likelihood that substantial measurement error is present. Selection bias in this study is unlikely because 93% of the enrolled subjects participated in the full study and, moreover, comparison of the descriptive characteristics of those who provided genetic information and those who did not did not show any marked differences.

Since this study explores the effect of a single genetic polymorphism, it is possible that our findings could be a result of linkage disequilibrium with other functional genetic polymorphisms or be a false positive due to population admixture. However, it was not possible to explore and correct for population structure in our study, as an in-depth genetic characterization of this population has not yet been carried out. Our results, if confirmed in other studies, have important implications for developing countries, such as India, which have both a high burden of anemia and continue lead exposure among children.

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HIGHLIGHTS

- !! Concomitant lead exposure and anemia remains high among children in India
- !! Lead exposure and low hemoglobin levels are associated with deficits in IQ
- !! Iron and lead share several toxicokinetic and toxicodynamic pathways
- !! Children with *TFC2* variant allele may be more susceptible to neurotoxic effects of lead and less protected by hemoglobin

Figure 1.

Blood lead and IQ dose-response relationship among children with *Transferrin C2* (a) wildtype polymorphism (C/C, n=466) and (b) variant polymorphism (C/T + T/T, n= 242). Smooth functional term relating IQ to log blood lead in an adjusted generalized additive mixed model, adjusting for age, sex, hemoglobin, average monthly income, maternal and paternal education, and mid arm circumference accounting for clustering at school level. (Effective degrees of freedom for both graphs = 1.00)

Table 1Distribution^a of *transferrin* C2 polymorphism among 708 children in Chennai, India

Genotype	N	Percent
CC	466	65.8
CT	209	29.6
TT	33	4.7

^aHardy-Weinberg Equilibrium $\chi^2=2.29$, $p=0.15$

Table 2Description of the study population by *transferrin* C2 genotype

	<i>Transferrin</i> genotype	
	C/C (N=466)	C/T + T/T (N=242)
Child Characteristics (Mean $\pm$ SD, or N (%))		
IQ	106.5 $\pm$ 17.3	107.7 $\pm$ 16.3 *
Blood lead (μ g/dl)	11.6 $\pm$ 5.5	11.3 $\pm$ 5.2
Hemoglobin (g/dl)	12.0 $\pm$ 1.2	11.8 $\pm$ 1.2
Mid-arm circumference (cm)	16.0 $\pm$ 1.5	16.1 $\pm$ 1.5
Age (years)	5.0 $\pm$ 0.9	5.0 $\pm$ 0.9
Male	252 (54.1)	128 (52.9)
Family Characteristics (N (%))		
Average monthly income (Indian rupees)		
<2000	79 (17.0)	30 (12.4)
2,000–4000	203 (43.6)	108 (44.6)
4,000–6,500	108 (23.2)	66 (27.3)
>6,500	76 (16.3)	38 (15.7)
Number of children		
0	99 (21.2)	56 (23.1)
1	298 (64.0)	156 (64.5)
2	69 (14.8)	30 (12.4)
Maternal education		
Illiterate/primary school	88 (18.9)	44 (18.2)
Middle school	150 (32.2)	88 (36.4)
High school	121 (26.0)	52 (21.5)
College	107 (23.0)	58 (24.0)
Paternal education		
Illiterate/primary school	53 (11.4)	32 (13.2)
Middle school	132 (28.3)	68 (28.1)
High school	132 (28.3)	69 (28.5)
College	149 (32.0)	73 (30.2)

*
p-value < 0.05

Table 3Effect of lead and hemoglobin levels on IQ by *Transferrin C2* polymorphism*

Model	N	Blood lead [ln($\mu\text{g/dl}$)]		Hemoglobin (g/dl)	
		(95% CI)	p-value	(95% CI)	p-value
1: Main effects:	708	-4.7 (-7.9, -1.4)	0.005	2.2 (1.2, 3.1)	<0.0001
2: Stratified model:					
C/C	466	-1.9 (-6.1, 2.3)	0.4	2.7 (1.4, 3.9)	<0.0001
C/T	209	-9.5 (-13.8, -5.3)	<0.0001	2.4 (0.7, 4.0)	0.005
T/T	33	-5.5 (-16.4, 5.3)	0.3	0.005 0.8 (-0.8, 2.3)	0.3
3: Stratified model:					
C/C	466	-1.9 (-6.1, 2.3)	0.4	2.7 (1.4, 3.9)	<0.0001
C/T+T/T	242	-9.8 (-14.5, -5.2)	<0.0001	1.8 (0.5, 3.1)	0.007
4: Interaction model:	708				
C/C		-2.1 (-6.5, 2.4)	0.047 ϵ	2.8 (1.5, 4.0)	0.07 ϵ
C/T+T/T		-7.7 (-13.6, -1.8)		1.7 (1.4, 2.1)	

* Adjusting for: age, age², sex, parental education, average family income, mid-arm circumference and clustering by school. ϵ : interaction term p values