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THE ASSOCIATION OF GENES AND SOME ENVIRONMENTAL FACTORS WITH DYSLIPIDEMIA IN 6-11 YEAR-OLD CHILDREN IN HANOI

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INTRODUCTION

1. Research rationale

Dyslipidemia, abnormal of lipid and lipoproteins levels in the blood, is the risk of atherosclerosis which leading risk factor for coronary artery disease, hypertension, and stroke - the main cause of mortality globally. Dyslipidemia is the cause of death for 4.4 million people over the world.

Nowadays, dyslipidemia is a great challenge for public health not only in developed countries but also in developing countries. In Vietnam, in 2008, the results of research according to people at age of 25-74 years in Hanoi showed that the incidence of disorder at least one lipid component was 59.8%; hypercholesterolemia - high total cholesterol (TC) level prevalence was 47.2%; hypertriglyceridemia - elevated triglyceride (TG) level prevalence was 38.4%, 25.9% people had both hypercholesterolemia and hypertriglyceridemia. It is alarming that the rate of dyslipidemia in children has been rising in recent years. This is proportional to the percentage of overweight-obesity children. The rate of dyslipidemia in overweight-obesity children was up to 85.3%. Dyslipidemia in childhood can progress in adult stage and cause premature damage to children such as fatty liver, cirrhosis, atherosclerosis... Therefore, early detection of dyslipidemia in children will help prevent export complications and improve cardiovascular risk in the future. However, problems in of dyslipidemia receives children little investment and research interests.

Dyslipidemia is well-known as a multifactorial disease which is influenced by gen-gen and gen-environment interactions. There are many environmental factors related to the plasma lipid profile, such as socioeconomic status, diet and physical activity, and especially obesity. In addition, genetic factors also play an important role in dyslipidemia, especially in children. According to Genome-wide association studies (GWAS), there are 95 locus related to blood lipid values in adults. Some genes such as *CETP, CLIP, LPL, APOA1, APOC3, APOA5, APOB* were detected that they are related to dyslipidemia in many different populations in the world. Among them, *APOE, APOA5* and *APOC3* genes - coded for apolipopretein (apo) E, ApoAI-V and ApoC-III - have an important role in regulating lipid and lipoprotein levels in plasma.

Some studies have shown that the *APOE, APOA5* and *APOC3* genes closely related to dyslipidemia in children. However, studies of the relationship of these genes in determining the amount of lipoprotein children and young teenagers are still limited.

Besides, the genetic characteristics of ethnic, differences in nutritional factors, physical activity, socio-economic factors have different impacts on the relationship of genes and disease in different ethnic groups. Until now, in Vietnam, there is not any work that simultaneously analyzes the relationship of environmental factors and genes for dyslipidemia in children. Therefore, we focus on three research questions (1) which environmental factors related to dyslipidemia in Vietnamese children? (2) Are *APOE, APOA5, APOC3* genes, previously identified related to dyslipidemia and its components in non-Asian children, also in Vietnamese children? (3) How environmental factors affect to this relationship?

To answer these questions, we studied "**The association of gene and some environmental factors with dyslipidemia in 6-11 years old children in Hanoi".**

2. Objectives

This study was conducted to achieve the following objectives:

The first objective: The association of some single nucleotide polymorphism (SNP) with dyslipidemia in 6-11 years old children in Hanoi.

The second objective: The association of some environmental factors with dyslipidemia in 6-11 years old children in Hanoi.

The third objective: The combined analysis of the association of some environmental factors and gene with dyslipidemia in 6-11 years old children in Hanoi.

3. The scientific and practical effective

3.1. The scientific effective

The genotyping methods of the SNP in this study can be widely applied in the research facility. The proportion of alleles in this study is the basis for calculating the sample size for the next study and to estimate the distribution of alleles in the population.

3.2. The practical effective

- This study provide predictive models of the association of some environmental factors and some SNP to dyslipidemia in primary school children in Hanoi.

- The results of this study is a scientific basis for further studies as the long-term cohort studies of the role of genetic and lifestyle changes for dyslipidemia to build predictive models dyslipidemia based on genetic analysis and preventive lifestyle diseases contribute actively, improve the quality of life and longevity for Vietnamese.

- The organizations can be applied to the results of this research is The prevention of overweight-obesity and non-communicable chronic diseases national program and The school nutrition program.

4. New contributions

- The study reports the optimal protocol for genotyping *APOE*, *APOA5* and *APOC3* genes using restriction fragment length polymorphism method in Vietnamese samples. It is necessary to apply it for genotyping these polymorphism for large-scale studies in Vietnam laboratory. This is one of the first studies of *APOE* genotyping using restriction fragment length polymorphism method and polyacrylamide electrophoresis method in Vietnam.

- This is one of the first studies provide the data on association of *APOE* (rs429358 và rs7412), *APOA5* (rs662799) and *APOC3* (rs2854116) polymorphism with dyslipidemia in 6-11 years old children in Hanoi.

CHAPTER 2. SUBJECTS AND METHODS

2.1. Research Design

This study was supported by grant No. 01C-08/05-2011-2 from Hanoi Department of Science and Technology. The study was conducted from 9/2011 to 8/2015 month in two stages:

- Stage 1 - Cross-sectional study: The study was conducted on 7,750 students from 31 primary schools in Hanoi (50 students/block, 250 students/school).

- Stage 2 - Case-control study: Case group included dyslipidemia children, control group included without dyslipidemia children. The objective of this stage is to analyze the association of some environmental factors and genetic factors with dyslipidemia in Hanoi primary school children. After exclusion of those with one of the exclusion criteria in section 2.3, 943 children were blood samples taken with the consent of the parents. Continue to exclude children whose blood samples for quality assurance DNA separation, the remaining 161 children with dyslipemia following NCEP classification standards (Section 2.4.1) were included in the case group, while 406 without dyslipidemia children were selected to the control group. The cases/controls ratio was approximately 1/3, satisfy the sample size for the Case-control study.

2.2. Subjects

The study subjects included 567 unrelated children in 31 primary school in Hanoi City. The ages of the subjects ranged from 6 to 10 years. All of them are Kinh-the major ethnic group in Vietnam. The subjects had no evidence of diseases related to atherosclerosis, CAD, diabetes and mental disorder. None of them were using lipid-lowering medication when the blood sample was taken.

2.4. Diagnostic criteria

2.4.1. Diagnostic criteria of dyslipidemia: Dyslipidemia was diagnosed according to the criteria of National Cholesterol Education Program (NCEP). Hypertriglyceridemia was defined as TG ≥ 100 mg/dL (with children age under 10

years) or TG ≥ 130 mg/dL (with children age uper 10 years). Hypercholesterolemia was defined as TC ≥ 200 mg/dL. Hyperbetalipoproteinemia was defined as LDL-C ≥ 130 mg/dL. Hypoalphalipoproteinemia was defined as HDL-C ≤ 35 mg/dL. The individuals with at least one of the four criteria above were defined as dyslipidemia.

2.4.3. Diagnostic criteria of nutritional status

Using the WHO (2007) standards to determine the nutritional status of children, using BMI Z-score by age and gender. Children with normal nutritional status can range from -2SD to 1SD, were obese with ≥ 2SD.

2.7. Research Methods

2.7.1. Collecting information

A questionnaire was provided along with consent forms and parents were asked to provide information regarding the child's general health and about family history of dyslipidemia.

2.7.3. Measurements

The body weight of participants was measured to the nearest 0.1 kg with standardized medical scales. Subjects were weighed without shoes and minimum of clothing. The height was measured to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Waist circumference (WC) was measured at the end of a normal expiration to the nearest 0.1 cm at the mid-point between the last floating rib and the top of the iliac crest. Hip circumference (HC) was measured at the level of the symphysis pubis and the greatest gluteal protuberance. Waist-to-hip ratio (WHR) was calculated as WC in cm divided by HC in cm.

2.7.7. Blood biochemical tests

Blood lipids and lipoproteins were measured on samples obtained after an overnight fast. Blood samples were collected in EDTA containing tubes. Serum TC and TG were determined with enzymatic method. Serum HDL-C and LDL-C were measured through a direct assay. All determinations were performed with

autoanalyzer (Type Architect C8000; Abbott Ltd., United States of America) in Medlatec Hospital of Hanoi City.

2.7.8. DNA preparation from whole blood method

Peripheral blood samples were obtained from each participants and genomic DNA was extracted from peripheral blood leukocytes, using Wizard® Genomic DNA Purification Kit (Promega Corporation, USA).

2.7.9. Genotyping method

Genotyping of 4 SNP was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method . Tất cả các mẫu nghiên cứu được xác định kiểu gen bằng phương pháp PCR -RFLP (Polymerase Chain Reaction - Fragment Length Polymorphism) included 4 steps (1) Amplification of SNP sequences; (2) electrophoresis the PCR products; (3) Restriction of the sequences with specific restriction enzyme for each SNP; (4) electrophoresis to determination of genotypes.

2.7.12. Verification of SNPs by sequencing

Samples with different genotypes detected by the RFLP method were also confirmed by direct sequencing in Axil Scientific Pte Ltd., Singapore.

2.8. Statistical analysis

Qualitative variables were expressed as percentages. Quantitative variables were expressed as means ± SD if variables were normal distribution or median (interquartile range) if variables were not normal distribution. Chi-square test or one-way ANOVA or independent-sample t test or Kruskal-Wallis test or Mann-Whitney U test were used when appropriate. Allele frequency was determined for Hardy-Weinberg equilibrium (HWE) by Fisher's exact test. Five genetic models were tested (dominant, co-dominant, over-dominant, recessive, and additive model). Akaike’s Information Criterion and Bayesian Information Criterion were applied to estimate the best-fit model for each SNP. Linkage disequilibrium (LD) between pair of SNPs was calculated using the Lewontin’s standardized disequilibrium coefficient (D’). The procedure was performed in SNPstat

software (Solé et al., 2006). The association of genotypes and WHR with dyslipidemia was tested by binary logistic regression analysis, entering the best-fit model of inheritance for each SNP. Age, gender, residence, province and each of obesity-related traits (BMI, WC, HC, and WHR) were adjusted for the statistical analysis. The power of sample size was calculated by the Quanto software version 2.4.1. The statistical analyses were done with the statistical software package SPSS 16.0 (SPSS Inc., Chicago, Illinois).

2.9. Ethics research

The research protocol was approved by The Ethics Committee of the National Institute of Nutrition, Vietnam. Written consent to participate in the study was given by the parents of all subjects.

CHAPTER 3: RESULTS AND DISCUSSION

3.1. Subject characteristics

No differences in gender and age between the two study groups ($P > 0.05$). Meanwhile, the rate of obesity, BMI, WC, HC and WHR in the case group was significantly higher than that of the control group ($P < 0.05$). The serum TG, TC, LDL-C concentrations were higher; serum HDL-C concentrations of case group was lower than that of the control group, $P < 0.05$.

3.2. The association of the four SNP in *APOA5, APOE, APOC3* gene with dyslipidemia in primary school children in Hanoi

3.2.1. Genotypic and allelic frequencies

The genotypic distributions for rs429358 and rs7412 were in Hardy-Weinberg equilibrium in cases and controls ($P > 0.05$). There was no difference of minor allele frequency between the two groups. At rs429358, the minor allele (C allele) frequency was 7.51% in controls and 10,87% in cases. At rs7412, the minor allele (T allele) frequency was 9.73% in controls and 9.32% in cases.

At *APOA5*-rs662799, the minor allele (G allele) frequency in cases was significantly higher than that in controls (40,91% vs 32,13%) with $P = 0,01$. The genotypic distributions for this SNP was in Hardy-Weinberg equilibrium in controls.

At *APOC3*- rs2854116, the minor allele (G allele) frequency was 41.01% in controls and 46.58% in cases. There was no difference of minor allele frequency between the two groups. The genotypic distributions for this SNP was not in Hardy-Weinberg equilibrium in controls.

3.2.2. Serum lipid levels between genotypes

At *APOE* rs429358, the statistically significant differences among the three genotype groups were found in serum TC concentration for cases (the subjects with T/C genotype had higher TC concentration than that of subjects with T/T and C/C genotype). At *APOE* rs7412, there was no differences of serum lipid levels among the three genotype groups. At *APOA5* rs662799, the statistically significant differences among the three genotype groups were found in serum TG concentration for cases (the subjects with G/G genotype had higher TG concentration than that of

subjects with G/C and C/C genotype). At *APOC3* rs2854116, the statistically significant differences among the three genotype groups were found in serum HDL-C concentration for controls and in serum TC, HDL-C, LDL-C concentration, TC/HDL-C ratio for cases.

3.2.3. The association of the four SNP with dyslipidemia in primary school children in Hanoi

Genetic factors can affect to one or multiple different lipid parameters including TG, TC, HDL-C, LDL-C. Therefore, we conducted a association analysis of each SNP with dyslipidemia as well as its components with different assumption models. The division of the model based on the hypothesis assumes pathogenic allele (the minor allele) is dominant or recessive allele.

Through this analysis, we can choose the optimal model for association of each SNP to dyslipidemia as well as its components based on criteria (1) the lowest of AIC (Akaike Information Criterion) and BIC (Bayesian Information Criterion) value; (2) the smallest of 95%CI range; (3) the lowest of *P* values.

3.2.3.1. At APOE-rs429358 polymorphism

Analytical results after adjusted for age and gender showed that *APOE*-rs429358 polymorphism did not associate with dyslipidemia in all assumption models ($P > 0.05$).

Rs429358 polymorphism did not associated with hypertriglyceridemia and hypercholesterolemia but associated strongly with hypoalphalipoproteinemia and hyperbetalipoproteinemia. Table 3.8 showed that, rs429358 polymorphism related to hypoalphalipoproteinemia in co-dominant, dominant and overdominant models. In particular, overdominant model had smallest *P*, AIC and BIC values after adjustment. Therefore, this model was used for further analysis. Similarly, the rs429358 polymorphism also strongly related to hyperbetalipoproteinemia. The optimal model in predicting hyperbetalipoproteinemia was the dominant model.

Table 3.8. The association of *APOE*-rs429358 polymorphism with dyslipidemia in Hanoi primary school children

Models	OR (95% CI)	*P*	AIC	BIC
Hypoalphalipoproteinemia				
Co-dominant				
T/T	1			
T/C	3.0 (1.2 - 7.8)	**0.021**	190.5	212.2
C/C	-			
Dominant				
T/T	1	**0.029**	189.5	206.9
T/C+C/C	2.9 (1.1 - 7.3)			
Overdominant				
T/T+C/C	1	**0.026**	188.9	206.2
T/C	2.9 (1.1 - 7.3)			
Hyperbetalipoproteinemia				
Co-dominant				
T/T	1	**0.024**	135.9	157.6
T/C	3.7 (1.2 - 11.5)			
C/C	-			
Dominant				
T/T	1			
T/C-C/C	3.4 (1.1 - 10.6)	**0.033**	134.6	152.0
Overdominant				
T/T-C/C	1			
T/C	3.7 (1.2 - 11.7)	**0.022**	134.2	151.5

OR, P-values obtained by multivariate logistic regression after adjusted for age and gender. 95% CI (95% Confidence interval).

3.2.3.2. At APOE rs7412 polymorphism

Rs7412 polymorphism has no association to dyslipidemia and its components in Hanoi primary school children in all models. Different from our study, the NHANES

III (Third National Health and Nutrition Examination Survey) study in 6016 American from 17-60 years old, rs7412 polymorphism closely related to LDL-C concentration (β = -22.52, $P < 0.0001$) and TC concentration (β = -20.68, $P < 0.0001$) after adjustment for age and gender [43]. This difference may be due to differences in age and genetic features ethnic between the two studies.

3.2.3.3. At APOA5-rs662799 polymorphism

Table 3.11. The association of *APOA5*-rs662799 polymorphism with dyslipidemia in Hanoi primary school children

Models	**OR (95% CI)**	***P***	**AIC**	**BIC**
Dyslipidemia				
Co-dominant				
A/A	1			
A/G	1.7 (1.1 - 2.6)	**0.024**	645.8	667.3
G/G	1.7 (1.0 - 2.9)			
Dominant				
A/A	1			
A/G-G/G	1.7 (1.2 - 2.5)	**0.0062**	643.8	661.0
Additive G allele	1.4 (1.1 - 1.8)	**0.015**	645.4	662.6
Hypertriglyceridemia				
Dominant				
A/A	1	**0.026**	598.5	615.6
A/G-G/G	1.6 (1.1 - 2.4)			
Additive G allele	1.3 (1.0 - 1.7)	**0.034**	598.6	615.8

OR, P-values obtained by multivariate logistic regression after adjusted for age and gender. 95% CI (95% Confidence interval)

Table 3.11 showed that rs662799 polymorphism associated with dyslipidemia in the co-dominant, dominant and additive model after adjusting for age and gender. The dominant model had smallest AIC and BIC value so this model was used for further studies.

Further analysis the association of rs662799 polymorphism with lipid component disorder, the results indicated that this polymorphism did not related to hypercholesterolemia, hyperbetalipoproteinemia, hypoalphalipoproteinemia but increases hypertriglyceridemia risk with the dominant model (smallest AIC and BIC value).

3.2.3.4. At APOC3-rs2854116 polymorphism

Rs662799 polymorphism did not related to dyslipidemia, hypertriglyceridemia, hyperbetalipoproteinemia, hypoalphalipoproteinemia but increases risk hypercholesterolemia after adjusting for age and gender (Table 3.14). The additive model was the optimal model in predicting hypercholesterolemia.

Table 3.14. The association of *APOC3*-rs2854116 polymorphism with hypercholesterolemia in Hanoi primary school children

Models	**OR (95% CI)**	***P***	**AIC**	**BIC**
Co-dominant				
A/A	1			
A/G	1.7 (0.6 - 5.4)	**0.017**	209.6	231.3
G/G	4.4 (1.5 - 13.0)			
Recessive				
A/A-A/G	1	**0.006**	208.3	225.6
G/G	3.2 (1.4 - 7.2)			
Additive G allele	2.2 (1.0 - 3.7)	**0.005**	207.9	225.2

OR, P-values obtained by multivariate logistic regression after adjusted for age and gender. 95% CI (95% Confidence interval).

3.2.4. The combined analysis of the association of gene with dyslipidemia in 6-11 years old children in Hanoi

Each SNP had different relevance to different phenotypes. Especially, the combination of rs429358 and rs7412 form different apoE polymorphism. Besides, these SNPs has strong linkage disequilibrium (D' = 0.99, *P* = 0.0001). The combination of these two SNPs on the same chromosome will create different haplotype. Haplotype is understood as a set of specific nucleotide sequences in a

group of closely linked genes on chromosome likely inherited together during segregation, and therefore likely to be conserved throughout many generation.

Therefore, we present (1) the association of combine rs429358 and rs7412 as genotype and haplotype; (2) association of combine four SNP with dyslipidemia and its components.

3.2.4.1. The association of combine rs429358 and rs7412

No difference in genotypic, allelic, haplotypic frequency between cases and controls. Genotype ε3/ε3, ε3 allele and haplotype T-C were the most common in the population with the frequency in the control group was 69.0%, 82.8% and 82.8%, respectively.

Table 3.15. The association of *APOE* polymorphism with dyslipidemia in Hanoi primary school children

	OR (95% CI)	***P***
Genotype		
Hypoalphalipoproteinemia		
ε3/ε3	1	
ε4 (ε3/ε4, ε4/ε4)	3.1 (1.2 - 8.3)	**0.023**
Hyperbetalipoproteinemia		
ε3/ε3	1	
ε4 (ε3/ε4, ε4/ε4)	3.1 (1.0 - 10.9)	**0.048**
Haplotype		
Hypoalphalipoproteinemia		
T-C	1	
C-C	8.2 (1.9 - 36.2)	**0.006**

OR, P-values obtained by multivariate logistic regression after adjusted for age and gender. 95% CI (95% Confidence interval).

Table 3.15 showed that ε4 carrier also had higher hypoalphalipoproteinemia and hyperbetalipoproteinemia risk than ε3/ε3 carrier. Our study results consistent with numerous other studies have been published. Especially, children without C-C

haplotype have higher hypoalphalipoproteinemia risk than those with T-C haplotype (OR = 8.2, *P* = 0.006).

3.2.4.1. The association of combine four SNPs

Genetic risk score is an effective tool in building risk measurements to the disease. Therefore, we analyzed association of genetic risk score from four SNPs with dyslipidemia.

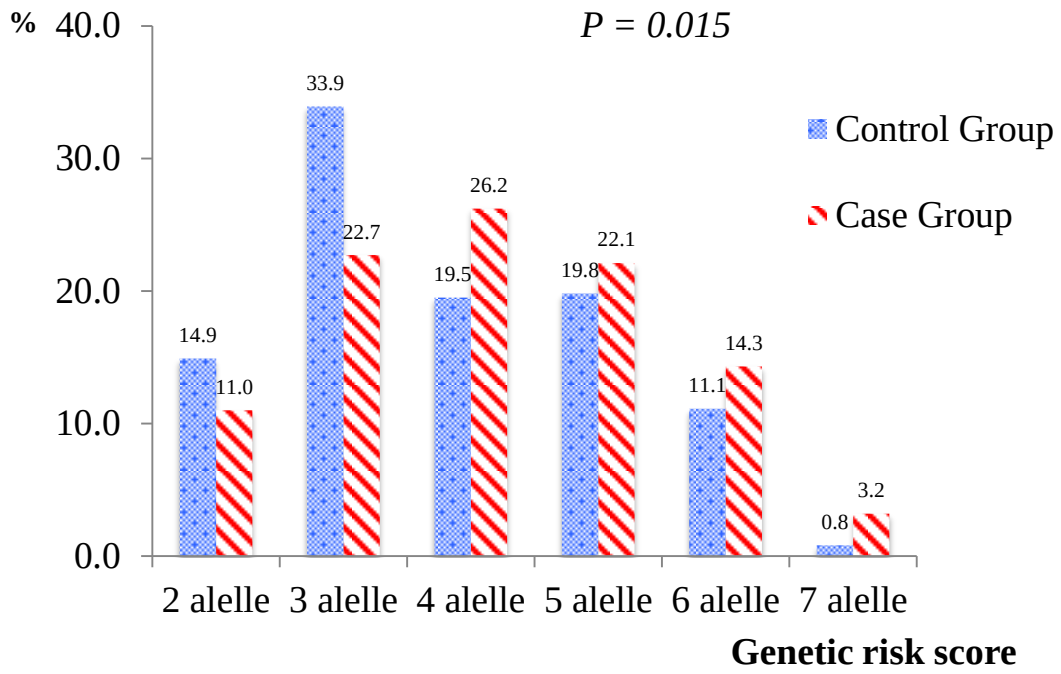

Figure 3.1. The distribution of genetic risk score between cases and controls

There was differences in the distribution of genetic risk score between cases and controls (Figure 3.1). The percentage of children with 2 and 3 risk alleles in the control group was higher than that of case group, while the percentage of children with more than 4 risk alleles in cases was higher than that in the control group (*P* = 0.015).

Genetic risk score associated to dyslipidemia, hypertriglyceridemia and hypercholesterolemia in primary school children after adjusting for age and gender (Table 3.19).

Table 3.19. The association of genetic risk score with dyslipidemia in Hanoi primary school children

Genetic risk score	OR (95% CI)	*P*
Dyslipidemia		
2-3	1	
4-5	1.8 (1.2 - 2.8)	**0.005**
6-7	2.1 (1.2 - 3.7)	**0.010**
Each alelle	1.5 (1.2 - 2.0)	**0.002**
Hypertriglyceridemia		
2-3	1	
4-5	1.7 (1.1 - 2.7)	**0.018**
6-7	2.2 (1.2 - 3.9)	**0.010**
Each alelle	1.2 (1.0 - 1.4)	**0.014**
Hypercholesterolemia		
2-3	1	
4-5	5.1 (1.7 - 15.9)	**0.005**
6-7	5.1 (1.3 - 19.7)	**0.020**
Each alelle	1.6 (1.2 - 2.3)	**0.004**

OR, P-values obtained by multivariate logistic regression after adjusted for age and gender. 95% CI (95% Confidence interval).

3.3. The association of some environmental factors with dyslipidemia in 6-11 years old children in Hanoi

3.3.1. Socio-economic status

The analyzed results of association of some socio-economic factors with dyslipidemia showed that children living in families with food costs more than 2 million vnđ/person/month had 3.2 times higher hypercholesterolemia risk compared with children living in families with food costs less than 1 million vnđ/person/month after adjusting for age and gender (P = 0.041).

There was no relationship between residence, number of persons living in a household and dyslipidemia, hypoalphalipoproteinemia and hyperbetalipoproteinemia.

3.3.2. Neonatal characteristics

Neonatal characteristics include babies delivery types (normal delivery, cesarean delivery), the birth weight, weaning time, time began to take a punch did not associate with dyslipidemia ($P > 0,05$). There is a negative correlation between the characteristics of difficulty to take a punch with dyslipidemia and hypertriglyceridemia. Drinking formula milk during the first four months tend to increase the risk of hypoalphalipoproteinemia. Caesarean delivery increases the risk of hyperbetalipoproteinemia (Table 3.21).

Table 3.21. The association of Neonatal characteristics with dyslipidemia in Hanoi primary school children

Characteristics	OR (95%CI)	*P*
Dyslipidemia		
Difficulty to take a punch	1.6 (1.1 - 2.3)	**0.025**
Hypertriglyceridemia		
Difficulty to take a punch	1.6 (1.0 - 2.4)	**0.037**
Hypoalphalipoproteinemia		
Drinking formula milk during the first 6 months	2.5 (1.0 - 6.6)	0.059
Hyperbetalipoproteinemia		
Caesarean delivery (vs normal delivery)	3.4 (1.3 - 4.9)	**0.049**

OR, P-values obtained by multivariate logistic regression after adjusted for age, gender and residence. 95% CI (95% Confidence interval).

3.3.3. Dietary behaviors

Gluttonous increased dyslipidemia and hyperglyceridemia risk in children. Children have submeal had lower dyslipidemia and hyperglyceridemia risk. Liking eating eggs increases the dyslipidemia, hyperglyceridemia and hyperbetalipoproteinemia risk. Eating slowly reduce the hypercholesterolemia and

hyperbetalipoproteinemia risk. Eating fast increases the dyslipidemia and hypoalphalipoproteinemia risk. Liking fatty food increase the hypoalphalipoproteinemia risk. Eating before bed increases the hypercholesterolemia risk. Liking sweeteen food increased the hyperglyceridemia (Table 3.22).

Table 3.22. The association of dietary behaviors with dyslipidemia in Hanoi primary school children

Dietary behaviors	OR (95%CI)	*P*
Dyslipidemia		
Gluttonous	2.3 (1.5-3.4)	**<0.0001**
Eating fast	1.6 (1.1-2.5)	**0.023**
Gorge	1.9 (1.2-3.1)	**0.007**
Having submeal	1.7 (1.1-2.5)	**0.011**
Liking eating eggs	2.0 (1.2-3.3)	**0.006**
Hypertriglyceridemia		
Gluttonous	2.8 (1.8-4.3)	**<0.0001**
Having submeal	2.0 (1.3-3.1)	**0.001**
Liking eating eggs	2.1 (1.2-3.5)	**0.006**
Liking sweeteen food	1.5 (1.1-2.3)	**0.027**
Hypercholesterolemia		
Eating before bed	2.4 (1.1-5.3)	**0.037**
Eating slowly	0.4 (0.1-0.9)	**0.032**
Liking eating fruits and vegetables	0.5 (0.4-1.0)	0.058
Hypoalphalipoproteinemia		
Eating fast	5.2 (1.8-14.4)	**0.004**
Liking fatty food	3.3 (1.0-10.1)	**0.041**
Hyperbetalipoproteinemia		
Eating slowly	0.1 (0.1-1.0)	0.061
Liking eating eggs	5.2 (1.7-15.5)	**0.003**

OR, P-values obtained by multivariate logistic regression after adjusted for age, gender and residence. 95% CI (95% Confidence interval).

3.3.4. Physical activity

Result analysis adjusted for age, gender and residence showed that sleep duration less than 8 hours/day with dyslipidemia in children 6-11 years old in Hanoi (OR = 2.1 ; *P* = 0.019) (Table 3.21). Night sleep duration has important implications for health, especially in children. At ages 6-11, sleep time best evening between 9 pm and 6-7 am (ie 9-11 hours/day). Short sleep duration may lead to dark disorders such as cortisol secretion certain hormone, GH, leptin, ghrelin, the hormone related to RLLM.

Table 3.21. The association of sleep duration with dyslipidemia in Hanoi primary school children

Đặc điểm	OR (95%CI)	*P*
Sleep duration		
≥ 8 hours/day	1	
< 8 hours/day	2.1 (1.1-3.2)	**0.019**

OR, P-values obtained by multivariate logistic regression after adjusted for age, gender and residence. 95% CI (95% Confidence interval).

There was no association between several characteristics of physical activity including exercise, the way move to the school, the total time watching television and playing computer games, nap time with the dyslipidemia and its components in Hanoi primary school children. The level of physical activity is usually evaluated by the period of a week or a month through the toolkit IPAQ (International Physical Activity Questionnaires). However, for primary school children, the application of the questions are difficult. Therefore, in this study, physical activity levels have only been assessed through a number of simple and easy variables collected as time watching television and playing video games, playing sports or not, how to school and at bed time. These variables may not accurately reflect the level of physical activity of children thus have not found an association of these variables with elementary school children dyslipidemia in Hanoi. This is one of the limitations of the study. This can be explained that the students in our study learned in schools with the curriculum prescribed by the Ministry of Education and Training with a similar

timetable. Therefore, there is no difference in time to nap, time watching television and playing video games during the day, taking exercise activity between groups of students.

3.3.5. Obesity

Table 3.24. The association of obesity with dyslipidemia in Hanoi primary school children

	OR (95%CI)	***P***
Dyslipidemia		
Obesity (vs normal weight)	4.0 (2.6-6.1)	**<0.0001**
BMI (each kg/m^2)	1.2 (1.1-1.2)	**<0.0001**
Waist circumference (each cm)	1.1 (1.0-1.1)	**<0.0001**
Hip circumference (each cm)	1.1 (1.0-1.1)	**<0.0001**
Waist to hip ratio > 0,9 (vs ≤ 0,9)	1.9 (1.2-2.8)	**0.004**
Hypertriglyceridemia		
Obesity (vs normal weight)	5.1 (3.2-8.1)	**<0.0001**
BMI (each kg/m^2)	1.2 (1.1-1.3)	**<0.0001**
Waist circumference (each cm)	1.1 (1.0-1.1)	**<0.0001**
Hip circumference (each cm)	1.1 (1.0-1.1)	**<0.0001**
Waist to hip ratio > 0,9 (vs ≤ 0,9)	2.6 (1.6-4.0)	**<0.0001**
Hypoalphalipoproteinemia		
Having overweight-obesity parents (vs having normal weight parents)	4.2 (1.3-14.2)	**0.020**
Obesity (vs normal weight)	5.7 (2.0-16.4)	**0.001**
BMI (each kg/m^2)	1.2 (1.0-1.3)	**0.001**
Waist circumference (each cm)	1.1 (1.0-1.1)	**0.002**
Hip circumference (each cm)	1.1 (1.0-1.1)	**0.007**
Waist to hip ratio > 0,9 (vs ≤ 0,9)	3.3 (1.1-9.3)	**0.022**

OR, P-values obtained by multivariate logistic regression after adjusted for age, gender and residence. 95% CI (95% Confidence interval).

Obesity and related-obesity traits related strongly to dyslipidemia, hyperglyceridemia and hypoalphalipoproteinemia in Hanoi primary school children (Table 3.24).

In this study we did not find the association between obesity and hypercholesterolemia and hyperbetalipoproteinemia while one study shows that obese children have high levels of LDL-C more 9 times compared with normal weight children.

The results of this study similar to other studies such as the study of Finn et al (2015), Korsten-Reck et al (2008), Sanchez et al (2006) and Yoshinaga et al (2005).

3.3.6. The combined analysis of the association of some environmental factors with dyslipidemia in 6-11 years old children in Hanoi

The traits related to obesity and nutritional status of children strongly correlated with each other. Therefore, the two indexes most easily calculated and no correlation to each other were selected for inclusion in the next analysis is BMI and WHR (r = 0.5, $P < 0.001$). In the characteristic eating habits, characteristics bulimia, eating speed and extent of eating together correlated (r = 0.6, $P < 0.001$). Therefore, in the 3 traits, characteristics "gluttonous" was selected for inclusion in the multivariate analysis.

Combining the results of analysis of continuous backward multivariate logistic regression method and BMA analysis, optimization model from environmental factors with dyslipidemia and it’s components have been identified as six:

Optimal model to determine the relationship between some environmental factors in children with dyslipidemia in primary Hanoi factors including BMI and characteristics "liking eating eggs".

Optimal model to determine the relationship between some environmental characteristics with hypertriglyceridemia including BMI, "liking eating eggs" and "having submeal".

Optimal model to determine the relationship between some environmental characteristics with hypercholesterolemia includes characteristics "eating before bed".

Optimal model to determine the relationship between some environmental characteristics with hypoalphalipoproteinemia including features "liking fatty food" and "drinking formula milk during the first 6 months".

Optimal model to determine the relationship between some environmental characteristics with LDL-C blood disorder includes characteristics "caesarean delivery", "eating slowly" and "liking eating eggs".

3.4. The combined analysis of the association of some environmental factors and gene with dyslipidemia in 6-11 years old children in Hanoi

To determine the effects of genes simultaneously and some environmental factor as well as disorder RLCHLM indices of lipid components, analysis and association of genetic factors (based on the best model selected from the analysis adjusted for age and gender and integrated analysis model) and environmental factors (based on analytical models optimal synthesis) were conducted.

The results of analysis of the combined effects of genes and some environmental factors to dyslipidemia and it’s components showed that *APOA5*-rs662799 polymorphism associated independently with dyslipidemia and hypertriglyceridemia; *APOE*-rs429358 polymorphism associated independent with hypoalphalipoproteinemia; *APOC3*-rs2854116 polymorphism associated independent with hypercholesterolemia after adjustment under certain environmental factors. E4 allele also independently associated with hypoalphalipoproteinemia. Increased genetic risk score associated independently with dyslipidemia, hypertriglyceridemia and hypercholesterolemia in primary school children in Hanoi.

Can be summed the best predictive models of dyslipidemia, hypertriglyceridemia, hypercholesterolemia, hypoalphalipoproteinemia, hyperbetalipoproteinemia based on BMA analysis results combined with the logistic regression analysis results of in Figure 3.11.

Risk factors		Dys	High TG	High TC	Low HDL-C	High LDL-C
BMI	Probability β	100% 1.14	100% 1.16			
APOA5-rs662799	Probability β	92.4% 1.67	53.7% 1.85			
Liking eggs	Probability β	54.3% 1.34	67.1% 0.85			95.5% 1.58
Eating before bed	Probability β			50.5% 1.09		
APOC3-rs2854116	Probability β			17.5% 1.87		
APOE-rs429358	Probability β				37.0% 1.23	
Caesarean delivery	Probability β					38.2% 1.26
Total of factors		**7**	3	2	1	1

Figure 3.11. Summary of the risk factors in the best predictive models to dyslipidemia in children under logistic regression and BMA analysis

This study was conducted in children 6-11 years old, is the age of the blood lipid values are relatively stable at the same time limiting factors from environmental interference such as using stimulants especially the hormonal changes of puberty. Effect of four SNPs (*APOE*-rs429358, *APOE*-rs7412, *APOA5*-rs662799 and *APOC3*-rs2854116), of a number of environmental factors as well as the influence of genetic and environmental factors to dyslipidemia and it's components were analyzed. In addition to analyzing the relationship of each SNP, the second combined analysis SNP rs429358 and rs7412 on gene *APOE* form ε isoforms and haplotype; combined analysis of four SNPs in the form genetic risk score with dyslipidemia and it's components in Hanoi primary school children has also been conducted. In particular, the relationship between haplotype with dyslipidemia little analysis of published studies.

However, within the scope of the thesis, this research is still some limitations. The sample size of the study is still small and has not yet identified the diet and level of physical activity of the subjects studied. Besides, the SNP analyzed was small. So, in the future should continue to make extensive studies on a larger sample size, the

object of all ages and geographies more. Especially, research should be done vertically to more accurately assess the degree of influence of genetic and environmental factors to dyslipidemia and disorders of lipid index of blood components.

CONCLUSIONS AND RECOMMENDATIONS

1. Conclusions

From the results of this study can be concluded as follows:

The first conclusion: Polymorphisms of different genes have different association to the dyslipidemia and its components:

- *APOE* rs429358 polymorphism increased hypoalphalipoproteinemia risk (overdominant model, OR = 2.9; *P* = 0.026) and hyperbetalipoproteinemia risk (overdominant model, OR = 3.3; *P* = 0.039) in primary school children in Hanoi.

- *APOA5* rs662799 polymorphism increased dyslipidemia risk (dominant model, OR = 1.7, *P* = 0.006) and hypertriglyceridemia (dominant model, OR = 1.5, *P* = 0.041) in primary school children in Hanoi.

- *APOC3* rs2854116 polymorphism increased hypercholesterolemia risk (additive model, OR = 3.0, *P* = 0.029).

- ε4 allele increased hypoalphalipoproteinemia risk (OR = 2.1; *P* = 0.005).

- C-C haplotype from rs429358 và rs7412 had higher hypercholesterolemia risk than C-T and T-T haplotype (OR = 8.2; *P* = 0.006).

- 4 - 5 and 6 - 7 genetic risk score carriers had higher dyslipidemia, hypertriglyceridemia and hypercholesterolemia risk.

The second conclusion: Environmental factors in predicting the optimal model from analysis and logistic regression BMA in Hanoi elementary school children:

- Optimal model to determine the relationship between some environmental factors in children with dyslipidemia in primary Hanoi factors including BMI and characteristics "liking eating eggs".

- Optimal model to determine the relationship between some environmental characteristics with hypertriglyceridemia including BMI, " liking eating eggs" and "having submeal".

- Optimal model to determine the relationship between some environmental characteristics with the blood disorder includes characteristics TC "eating before bed".

- Optimal model to determine the relationship between some environmental characteristics with blood disorders including HDL-C features "liking fatty food" and "drinking formula milk during the first 6 months".

- Optimal model to determine the relationship between some environmental characteristics with LDL-C blood disorder includes characteristics "caesarean delivery", "eating slowly" and " liking eating eggs".

The third conclusion: The combined analysis of the association of some environmental factors and gene with dyslipidemia in 6-11 years old children in Hanoi.

2. Recommendations

- Continuing to expand research on multiple SNP to build predictive models based on early dyslipidemia DNA sequences from the neonatal period which nutrition counseling and physical activity early to subjects at risk improves muscle health, fitness.

- Continuing to expand research on many subjects in the age, different geographical areas to determine the extent of the influence of the risk factors to dyslipidemia Vietnamese people.

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