MINISTRY OF EDUCATION AND TRAINING
Hanoi National University of Education

NGUYEN THI TRUNG THU

PREVALENCE, SOME FACTORS OF ENVIRONMENT AND POLYMORPHISMS ASSOCIATED WITH PREDIABETES AMONG 40 - 64 YEAR OD PEOPLE IN HA NAM PROVINCE

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Advisors: **1. PhD. Tran Quang Binh**

2. Assoc.Prof. PhD. Nguyen Do Huy

Reviewer 1: **Assoc.Prof. PhD. Do Thi Hoa**

Hanoi Medical University

Reviewer 2: **Assoc.Prof. PhD. Nguyen Thi Hong Van**

VNU University of Science

Reviewer 3: **Assoc.Prof. PhD. Truong Tuyet Mai**

National Institute of Nutrition

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LIST OF ANNOUNCEMENT PAPERS RELATED TO THE THESIS

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1. **Nguyen Thi Trung Thu**, Pham Tran Phuong, Tran Quang Binh (2014), "The prevalence of prediabetes and related factors in men aged 40 - 64 years in rural area, Ha Nam province", *VNU Journal of Science: Science of Technology*, 30 (1S), pp. 202-209.
2. **Nguyen Thi Trung Thu**, Pham Tran Phuong, Tran Quang Binh (2014), "Association of SNP rs7903146 in *TCF7L2* gene with prediabetes among middle-aged women in rural area, Ha Nam province", *Journal of preventive medicine*, 24 (11), pp. 42-29.
3. Binh TQ, **Thu NTT**, Phuong PT, Nhung BT, Nhung TTH (2015), "*CDKN2A*-rs10811661 polymorphism, waist-hip ratio, systolic blood pressure, and dyslipidemia are the independent risk factor for prediabetes in a Vietnamese population", *BMC Genetics*, 16 (107). Doi:10.1186/s12863-015-0266-0.
4. **Nguyen Thi Trung Thu**, Pham Thu Trang, Pham Tran Phuong, Nguyen Do Huy, Tran Quang Binh (2015), "Relationship between *FTO* rs9939609 polymorphism and the risk of prediabetes in Ha Nam middle-aged men", *VNU Journal of Science: Science of Technology*, 31(4S), pp. 395-401.
5. **Nguyen Thi Trung Thu**, Pham Tran Phuong, Nguyen Do Huy, Tran Quang Binh (2015), 'The risk of prediabetes in middle-aged women in rural area, Ha Nam province", *VNU Journal of Science: Science of Technology*, 31(4S), pp. 388-394.
6. **Nguyen Thi Trung Thu,** Tran Quang Binh (2016), "Using allele specific PCR method to genotype the *KCNJ11* E23K polymorphism in Vietnamese population", Research and teaching Biology Conference in Viet Nam 2nd, pp. 753-761.
7. **Nguyen Thi Trung Thu**, Tran Quang Binh (2016), "Metabolic syndrome and risk factors in middle-aged people with prediabetes", *VNU Journal of Science: Science of Technology*, 32 (receipt posting paper).
8. **Nguyen Thi Trung Thu**, Pham Tran Phuong, Tran Quang Binh (2017), "Determine rs10811661 gene CDKN2A polymorphism in Vietnamese population using AS-PCR and RFLP-PCR methods, *HNUE Journal of Science: Natural Science*, 62 (receipt posting paper).

INTRODUCTION

1. Reasons

Prediabetes is the condition in which blood glucose is higher than the normal level, but still lower than the diabetes diagnosed level. According to the American Diabetes Association (ADA)'s standard in 2003, prediabetes was defined as fasting blood glucose (FPG) stays from 5.6 to 6.9 mmol/L and/or blood glucose 2 hours after oral glucose tolerance test (OGTT) stays from 7.8 to 11.0 mmol/L.

The prevalence of prediabetes is often 2 - 3 times higher than diabetes and different between populations, ethnic groups. The prevalence of prediabetes increases with age, especially after the age of 40. People living in urban areas have prevalence of prediabetes 2 times higher than those living in rural areas. The concern is that prediabetic status almost has no symptoms, so most people do not know they have prediabetes. Most of people (up to 70%) are with prediabetes, and if it is not detected early and timely managed on, it can develop into diabetes type 2 and other dangerous diseases throughout life. So medical system should screen to detect early prediabetic status based on risk factors and intervene timely to reduce the prevalence of prediabetes and limit the progression of diabetes and other serious illnesses.

Prediabetes is a multifactorial genetic disorder influenced by environmental factors and genes, and there have been many studies on prediabetes. However, the results of an association of environmental and genetic factors with prediabetes were inconsistent among ethnic groups. The reported factors were associated with prediabetes such as: habitat, education, obesity, hyperension, dyslipidemia, history of family diabetes, physical activity, characterized eating and genetic polymorphisms. Genome-wide studies (GWAs) have found many single nucleotide polymorphisms

(SNPs) in genes which have significant association with diabetes and prediabetes. Based on genes reported with strong association with diabetes type 2 and prediabetes from GWAs, especially in Asian populations, and with the understanding of gene function, this study seleted 4 gene polymorphisms: *FTO*-rs9939609, *TCF7L2*-rs7903146, *CDKN2A*-rs10811661 and *KCNJ11*-rs5219 to study the association with prediabetes.

In Vietnam, most studies focuse on the prevalence of prediabetes and analyze a number of risk factors from environment in big cities such as Hanoi and Ho Chi Minh - where unbanization is happenning strongly, only a few studies in rural area were proceeded. In addition, almost no study on gene polymorphisms and an combined association of environmental and genetic polymorphisms was performed.

Due to above reasons, we conducted the thesis entitled: "*Prevalence, some factors of environment and polymorphisms associated with prediabetes among 40 - 64 year old people in Ha Nam province*".

2. Objectives

- **Objective 1**: To determime the prevalence of prediabetes among 40 - 64 year old people in Ha Nam province.
- **Objective 2:** To understand the association of environmental factors and prediabetes among 40 - 64 year old people in Ha Nam province.
- **Objective 3:** To determine the effects of polymorphisms in *FTO, TCF7L2, CDKN2A, KCNJ11* to prediabetes among 40 - 64 year old people in Ha Nam province.
- **Objective 4:** To analysis of combined effects of environmental factors and genetic polymorphisms among 40 - 64 year old people in Ha Nam province.

3. Application of the study

3.1. Sicentific application

Procedure for determining SNP genotypes can be widely used in laboratories.

The result of SNP genotype and allele frequencies can help estimate the distribution of genotypes and alleles in the population and is the basis for calculating the sample size for next studies.

The relationship of environmental factors and genetic polymorphisms and prediabetes is the basic for longitudinal studies and studies on related factors in direct and indirect impacts with prediabetes.

The rerults of study is the basic to perform further studies on the molecular effects of polymorphisms to prediabetes among Vietnamese people.

3.2. Practical application

The prevalence of prediabetes is the basic to determine the importance of prediabetic status and to provide early warnings of developing diabetes type 2 and other diseases in the future.

Results provide an association of environmental factors (such as diet, physical activity, economic-social characteristics, obesity, hypertension and dyslipidemia), some genetic polymorphisms and prediabetes. Thereby, this study helped to change lifestyles with increase efficiency treatment based on genetic interactions to reduce the risk of developing diabetes and other diseases.

The topic is the basis for making optimal model predicts of the risk of prediabetes based on the characteristics of environmental factors and genetic polymorphisms.

4. New findings of the thesis

- This is the first study in Vietnam on the association of genetic polymorphisms: *FTO*-rs9939609, *TCF7L2*-rs7903146, *CDKN2A*-rs10811661 and *KCNJ11*-rs5219 with prediabetes in 40 - 64 year old people.
- The topic has provided data on combined association of environmental factors and genetic polymorphisms with prediabetes among 40 - 64 year old people.

Chapter 1 - OVERVIEW

Chapter 2 - SUBJECTS AND METHODS

2.1. Study design

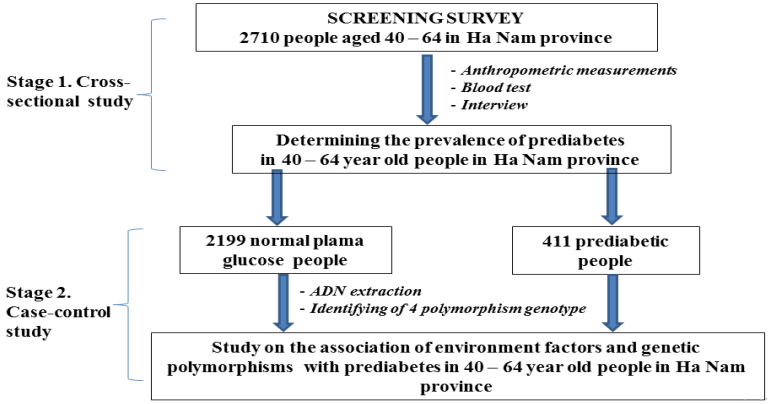

Figure 2.1. Design and implementation of study diagram

The study included two stages (Fig 2.1):

- Stage 1: Cross-sectional study. This was the initinal screening survey to determine the prevalence of prediabetes in 40 - 64 year old people in Ha Nam province.

- Stage 2: Case-control study. From subjects in cross-sectional study, the study selected all normal glucose and prediabetes people to determine the association of environment factors and genetic polymorphisms with prediabetes

2.2. Criteria for selecting study subjects

2.2.1. Criteria for selecting subjects in cross-sectional study

Selection criteria: 40 - 64 year old people living in 30 communes, wards and towns in Ha Nam province.

Exclusion criteria: people with acute disease, chronic such as tuberculosis, HIV/AIDS, drugs which made raise blood glucose,

pregnant, people with neurological problems, birth defects and who did not have the ability to answer questions.

2.2.2. Criteria for selecting subjects in case-control study

Subjects in the study were selected based on classification criteria of ADA in 2003 [88] and those criteria were used by the Healthy Ministry in 2014 [7] (Table 2.1)

Table 2.1. Blood glucose diagnostic criteria for case group and control group according ADA in 2003

Criteria	**Normal glucose**	**Prediabetes**		
		IFG	**IGT**	**IFG-IGT**
FPG (mmol/l)	< 5.6	5.6 - 6.9	< 5.6	5.6 - 6.9
2h Glucose (mmol/l)	< 7.8	< 7.8	7.8 - 11.0	7.8 - 11.0

2.3. The sample size of study

2.4. Time and place of study

The study was conducted in Ha Nam Province which consists of Phu Ly and 5 districts (Binh Luc, Duy Tien, Kim Bang, Ly Nhan, Thanh Liem) with 30 wards, towns and communes.

The study was conducted from 7/2011 to 10/2015.

2.5. Materials of study

2.6. Methods of data collection

2.6.1. Methods of collecting information about subjects

Interview according to the questionnaire to collect data on the characteristics of subjects (Appendix 3).

2.6.2. Anthropometric Measurement

2.6.3. Draw blood method

Subjects were taken 2 - 3 ml of blood from arm vein after fasting overnight. Then, subjects were drunk 75 grams glucose mixed with 250 ml water after 120 minutes, taken arm vein blood 2th.

2.6.4. Blood biochemical test

2.6.5. DNA extraction method

2.6.6. Genotyping using AS - PCR

100% of samples were determined by genotyping methods AS-PCR.

2.6.7. Genotyping using RFLP - PCR

Random 10% of the samples were checked using RFLP - PCR.

2.7. Study variables

2.8. Statistical analysis

Using softwares: Epidata 3.0. Excel 2010. SPSS 16.0 and R 2.14.0 to import and process data.

Using Kolmologov-Smirnov test, Student-T test, Man-Withney-U test, test $\chi 2$ test or Fisher Exact for analysing.

The association of environmental factors and genetics with prediabetes were analyzed using logistic regression. The genetic models used in the analysis of the association of polymorphisms with prediabetes include: dominant model, codominant model, over-dominant model, recessive model, additive model.

Models suitable for prediabetes were determined by backward continuous logistic regression with *P* from Hosmer - Lemeshow test. Determined ROC curve and AUC were estimated to caculate predictability of models. Determine the probability that the risk factors included in predictive prediabetes models was caculated Bayesian model average (BMA).

P values < 0.05 in two sides were considered statistical significance.

2.8. Study ethics

The study was approved by the Ethics Committee of the National Institute of Hygiene and Epidemiology, Vietnam.

Chapter 3 - RESULTS AND DISCUSSION

3.1. Charateristics of subjects

3.1.1. Charateristics of subjects in cross - sectional study

Table 3.1. Charateristics of subjects in cross - sectional study

Characteristics	**Female (n = 1761)**	**Male (n = 949)**	**Total (n = 2710)**	***P***
Rural n(%)	1663 (94.4)	897 (94.5)	2560 (94.5)	0.926[a]
Age (years)	50.5 (45.1 - 56.0)	52 (47.3 - 57.0)	51.3 (46.0 - 56.3)	**< 0.0001[b]**
Height (cm)	152 (149 - 156)	162 (158 - 165)	156 (150 - 160)	**< 0.0001[b]**
Weight (kg)	49.2 (44.8 - 53.8)	55.7 (50.2 - 60.8)	51.8 (46.3 - 56.6)	**< 0.0001[b]**
Body fat (%)	30.3 (27.0 - 33.2)	22.1 (18.1 - 25.5)	27.2 (23.3 - 31.7)	**< 0.0001[b]**
Waist (cm)	73.0 (68.1 - 78.3)	76.3 (70.3 - 82.5)	74.4 (68.5 - 80.0)	**< 0.0001[b]**
Hip (cm)	87.3 (84.1 - 91.2)	88.5 (85.0 - 92.4)	87.9 (84.0 - 91.6)	**< 0.0001[b]**
Waist/hip ratio	0.83 (0.79 - 0.87)	0.86 (0.82 - 0.90)	0.85 (0.80 - 0.88)	**< 0.0001[b]**

Table 3.2. Charateristics of subjects in case - control study

Charateristics	**Control group (n = 2199)**	**Prediabetic group (n = 411)**	***P***
Rural n (%)	2106 (95.8%)	363 (88.3%)	**< 0.0001[a]**
Female n (%)	1447 (65.8)	259 (63.0)	0.276[a]
Age (years)	51 (46 - 56)	53 (47 - 58)	**< 0.0001[b]**
BMI (kg/m^2)	21.1 (19.3 - 22.9)	21.5 (19.6 - 23.4)	**0.012[b]**
Body fat percentage	27.5 (23.0 - 31.5)	28.2 (23.7 - 31.9)	0.119[b]
Waist/hip ratio	0.84 (0.80 - 0.87)	0.85 (0.81 - 0.90)	**< 0.0001[b]**
Systolic blood pressure (mmHg)	110 (100 - 128)	120 (110 - 138)	**< 0.0001[b]**
Diastolic blood pressure (mmHg)	70 (65 - 80)	80 (70 - 85)	**< 0.0001[b]**
Total cholesterol (mmol/l)	4.2 (3.9 - 4.9)	4.6 (4.1 - 5)	**< 0.0001[b]**
HDL-C (mmol/l)	1.23 (0.99 - 1.60)	1.19 (0.97 - 1.60)	**< 0.0001[b]**
LDL-C (mmol/l)	2.79 (2.31 - 3.31)	3.10 (2.64 - 3.70)	0.103[b]
Triglyceride (mmol/l)	1.34 (1.0 - 2.10)	1.8 (1.12 - 2.55)	**< 0.0001[b]**
FPG (mmol/l)	4.5 (4.0 - 4.9)	5.9 (5.5 - 6.1)	**< 0.0001[b]**
2h Glucose (mmol/l)	5.1 (4.6 - 5.9)	6.8 (5.6 - 8.4)	**< 0.0001[b]**

3.2. Prevalence of prediabetes among 40 - 64 year old people in Ha Nam province

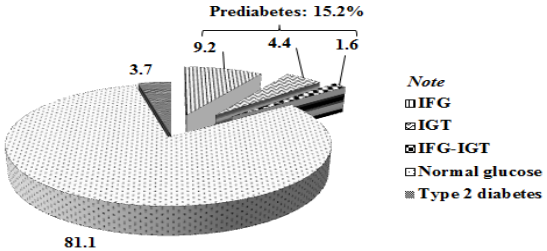

Figure 3.1. Prevalence of prediabetes among 40 - 64 year old people in Ha Nam province (%)

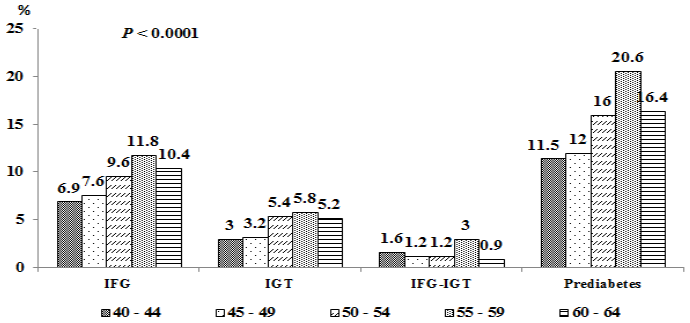

Figure 3.2. Prevalence of prediabetes among 40 - 64 year old people in Ha Nam province according age groups

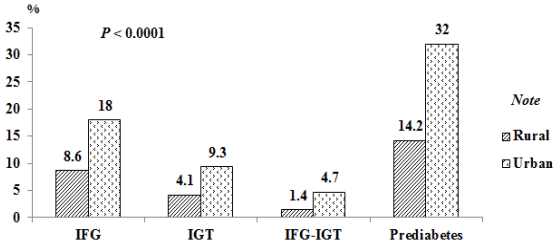

Figure 3.3. Prevalence of prediabetes among 40 - 64 year old people in Ha Nam province according region group

3.3. The association of enviromental factors with prediabetes among 40 - 64 year old people in Ha Nam province

3.3.1. Socio - economic status

Table 3.3. The association of socio - economic status with prediabetes among 40 - 64 year old people in Ha Nam province

Characteristics		**Control group (n = 2199)**	**Prediabetic group (n = 411)**	**OR**	***P***
Age				1.19	**< 0.0001**[a]
Region	Rural	2106 (95.8%)	363 (88.3%)	1	
	Urban	93 (4.2%)	48 (11.7%)	2.76	**< 0.0001**
History of family diabetes	No	2016 (96.6%)	377 (94.5%)	1	
	Yes	71 (3.4)	22 (5.5%)	1.77	**0.023**
Marriage	Yes (alive)	2014 (91.6%)	366 (89.1%)	1	
	No	34 (1.5%)	14 (3.4%)	2.28	**0.012**
	Others	151 (6.9%)	31 (7.5%)	1.06	0.79

Table 3.4. Some socio - economic status which had an association with types of prediabetes among 40 - 64 year old people in Ha Nam province

Charateristics		**Control group**	**Prediabetic group**	**OR**	***P***
IFG		**n = 2199**	**n = 248**		
Age				1.18	**0.001**[a]
Region	Rural	2106 (95.8%)	221 (89.1%)	1	
	Urban	93 (4.2%)	27 (10.9%)	2.55	**< 0.0001**
IGT		**n = 2199**	**n = 120**		
Age				1.21	**0.008**
Region	Rural	2106 (95.8%)	106 (88.3%)	1	
	Urban	93 (4.2%)	14 (11.7%)	2.70	**0.001**
History of family diabetes	No	2016 (96.6%)	108 (93.1%)	1	
	Yes	71 (3.4%)	8 (6.9%)	2.27	**0.035**
Income level	Level 1	566 (25.7%)	26 (21.7%)	1	
	Level 2	569 (25.9%)	43 (35.8%)	1.66	**0.049**
	Level 3	530 (24.1%)	20 (16.7%)	0.83	0.573
	Level 4	534 (24.3%)	31 (25.8%)	1.21	0.485

Charateristics		Control group	Prediabetic group	OR	*P*
IFG-IGT		**n = 2199**	**n = 43**		
Region	Rural	2106 (95.8%)	36 (83.7%)	1	
	Urban	93 (4.2%)	7 (16.3%)	4.24	**0.001**
Marriage	Yes (alive)	2014 (91.6%)	36 (83.7%)	1	
	No	34 (1.5%)	4 (9.3%)	6.1	**0.022**
	Others	151 (6.9%)	3 (7.0%)	0.98	0.981
Education	Elementary	220 (10%)	9 (20.9%)	1	
	Junior school	1368 (62.2%)	18 (41.9%)	0.34	**0.012**
	High school	286 (13.0%)	4 (9.3%)	0.39	0.134
	Colleges	325 (14.8%)	12 (27.9%)	0.99	0.996

3.3.2. Dietary behaviors

Table 3.5. The association of dietary behaviors with prediabetes và types of prediabetes among 40 - 64 year old people in Ha Nam province

Dietary behaviors		Control group	Prediabetic group	OR	*P*
Prediabetes		**n = 2199**	**n = 411**		
Alcohol consumption	No	1433 (65.2%)	241 (58.6%)	1	
	1-4 glasses/month	263 (12.9%)	58 (14.1%)	1.40	**0.045**
	> 4 glasses/month	503 (22.9%)	112 (27.3%)	1.58	**0.017**
IFG		**n = 2199**	**n = 248**		
Alcohol consumption	No	143 (65.2%)	139 (56.0%)	1	
	1-4 glasses/month	263 (12.0%)	34 (13.7%)	1.50	0.053
	> 4 glasses/month	503 (22.9%)	75 (30.2%)	2.21	**0.01**

3.3.3. Physical activities

The results did not observe any association of physical activities (watching television time, sleep time, walking, sport) with prediabetes and types of prediabetes among 40 - 64 year old people in Ha Nam province ($P > 0.05$).

3.3.4. Obesity, systolic blood pressure and dyslipidemia

Table 3.8. The associaton of obesity, systolic blood pressureand dyslipidemia with prediabetes among 40 - 64 year old people in Ha Nam province

Indices		**Control group (n = 2199)**	**Prediabetic group (n = 411)**	**OR**	***P***
WHR				1.27	**< 0.0001**
Body fat percentage				1.15	**< 0.0001**
Systolic blood pressure				1.15	**< 0.0001**
HDL-C	Normal	1091 (54.7%)	187 (49.9%)	1	
	Low	903 (45.3%)	188 (50.1%)	1.26	**0.048**
LDL-C	Normal	1506 (76.8%)	219 (60.2%)	1	
	High	454 (23.2%)	145 (39.8%)	2.12	**< 0.0001**
Total cholesterol	Normal	1677 (84.1%)	305 (81.3%)	1	
	High	317 (15.9%)	70 (18.7%)	1.17	0.275
Triglyceride	Normal	1249 (62.7%)	168 (44.8%)	1	
	High	742 (37.3%)	207 (55.2%)	2.0	**< 0.0001**

Table 3.8. Obesity, Systolic blood pressureand dyslipidemia associated with types of prediabetes among 40 - 64 year old people in Ha Nam province

Indices		**Control group**	**Prediabetic group**	**OR**	***P***
IFG		**n = 2199**	**n = 248**		
WHR				1.19	**0.018**
Systolic blood pressure				1.15	**< 0.0001**
Triglyceride	Normal	1249 (62.1%)	107 (49.5%)	1	
	High	742 (37.3%)	109 (50.5%)	1.6 4	**0.003**
IGT		**n = 2199**	**n = 120**		
WHR				1.51	**< 0.0001**
Body fat percentage				2.09	**< 0.0001**
Systolic blood pressure				1.17	**< 0.0001**
Nutritional status	Normal	1637 (75.6%)	78 (65.5%)		
	Overweight	527 (24.4%)	41 (34.5%)	1.60	**0.02**

Indices		Control group	Prediabetic group	OR	*P*
HDL-C	Normal	1091 (54.7%)	44 (37.9%)	1	
	Low	903 (45.5%)	72 (62.1%)	2.08	**0.001**
LDL-C	Normal	1506 (76.8%)	53 (48.6%)	1	
	High	454 (23.2%)	56 (51.4%)	3.13	**< 0.0001**
Triglyceride	Normal	1249 (62.7%)	45 (38.8%)	1	
	High	742 (37.3%)	71 (61.2%)	2.63	**< 0.0001**
IFG-IGT		**n = 2199**	**n = 43**		
HDL-C	Normal	1091 (54.7%)	13 (30.2%)	1	
	Low	903 (45.3%)	30 (69.8%)	2.21	**0.03**
LDL-C	Normal	1249 (62.7%)	15 (36.6%)	1	
	High	742 (37.3%)	26 (63.4%)	5.0	**< 0.0001**
Triglyceride	Normal	1249 (62.7%)	16 (37.2%)	1	
	High	742 (37.3%)	27 (62.8%)	2.70	**0.004**

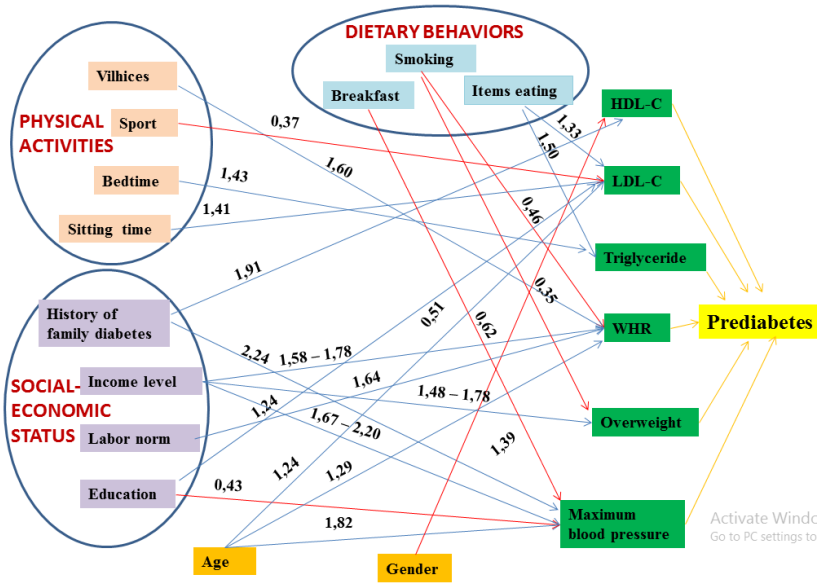

Figure 3.5. The association of environmental factors with prediabetes indirectly through obesity, Systolic blood pressureand blood lipid

: Increased risk, : Reduced risk, : Related directly with prediabetes.

The index in Figure was odd ratio (OR).

3.4. The association of genes with prediabetes among 40 - 64 year old people in Ha Nam province

3.4.1. Prevalance of genotypes and alleles of 4 polymorphisms in cases and controls

Table 3.10. *Prevalances of genotypes and alleles of 4 polymorphisms in cases and controls*

SNP		Prediabetic group (n = 411)	Control group (n = 2199)	*P*
FTO-rs9939609				
Genotypes	AA	13 (3.2%)	85 (3.8%)	
	AT	115 (28.2%)	643 (29.4%)	0.68
	TT	280 (68.6%)	1462 (66.8%)	
Alleles	A	141 (17%)	813 (19%)	
	T	675 (83%)	3567 (81%)	0.39
TCF7L2-rs7903146				
Genotypes	CC	387 (94.9%)	2101 (96%)	0.55
	CT	20 (4.9%)	85 (3.9%)	
	TT	1 (0.2%)	3 (0.1%)	
Alleles	C	794 (97%)	4287 (98%)	0.27
	T	22 (3%)	91 (2%)	
***CDKN2A-rs*10811661**				
Genotypes	CC	64 (15.9%)	420 (19.5%)	
	CT	185 (4.9%)	1014 (47.1%)	0.09
	TT	154 (38.2%)	719 (33.4%)	
Alleles	C	313 (39%)	1854 (43%)	**0.03**
	T	493 (61%)	2452 (57%)	
***KCNJ11-rs*5219**				
Genotypes	CC	186 (45.8%)	957 (44.2%)	0.84
	CT	176 (43.4%)	960 (44.4%)	
	TT	44 (10.8%)	246 (11.4%)	
Alleles	C	548 (67%)	2874 (66%)	0.56
	T	264 (33%)	1452 (34%)	

3.4.2. Characteristics of blood glucose of genotypes

The result did not receive any differences of glycemic charateristics of genotypes.

3.4.3. The association of each polymorphism with prediabetes

3.4.3.1. FTO-rs9939609

Table 3.13. The association of *FTO*-rs9939609 with prediabetes among 40 - 64 year old people in Ha Nam province

Models		**Control group**	**Prediabetic group**	**Univariate analysis**		
				OR	***P***	**AIC**
Codominant	TT	1462 (66.8%)	280 (68.6%)	1		2248.8
	AT	643 (29.4%)	115 (28.2%)	0.93	0.544	
	AA	85 (3.9%)	13 (3.2%)	0.79	0.435	
Dominant	TT	1462 (68.6%)	280 (68.6%)	1		
	AT-AA	728 (33.2%)	128 (31.4%)	0.91	0.43	2247.1
Recessive	TT-AT	2105 (96.1%)	395 (96.8%)	1		
	AA	85 (3.9%)	13 (3.2%)	0.8	0.46	2247.2
Over-dominant	AA-TT	1547 (70.6%)	293 (71.8%)	1		
	AT	643 (29.4%)	115 (28.2%)	0.94	0.61	2247.5
Additive with each allele A				0.91	0.36	2246.9

3.4.3.2. TCF7L2-rs7903146

Table 3.14. The association of *TCF7L2*-rs7903146 with prediabetes among 40 - 64 year old people in Ha Nam province

Models		**Control group**	**Prediabetic group**	**Univariate analysis**		
				OR	***P***	**AIC**
Codominant	CC	2101 (96.0%)	387 (94.9%)	1		2248.2
	CT	85 (3.9%)	20 (4.9%)	1.25	0.386	
	TT	3 (0.1%)	1 (0.2%)	1.96	0.564	
Dominant	CC	2101 (96.0%)	387 (95.8%)	1		
	CT - TT	88 (4.0%)	21 (5.1%)	1.27	0.35	2246.4
Recessive	CC - CT	2186 (99.9%)	407 (99.8%)	1		
	TT	3 (0.1%)	1 (0.2%)	1.94	0.59	2247.0
Over-dominant	CC - TT	2104 (96.1%)	388 (95.1%)	1		
	CT	85 (3.9%)	20 (4.9%)	1.25	0.40	2246.5
Additive with each allele T				1.27	0.32	2246.3

3.4.3.3. CDKN2A-rs10811661

Table 3.15. The association of *CDKN2A*-rs10811661 with prediabetes among 40 - 64 year old people in Ha Nam province

Models		**Control group**	**Prediabetic group**	**Univariate analysis**		
				OR	***P***	**AIC**
Codominant	CC	420 (19.5%)	64 (15.9%)	1		2213.8
	CT	1014 (47.1%)	185 (45.9%)	1.18	0.294	
	TT	719 (33.4%)	154 (38.2%)	1.40	**0.039**	
Dominant	CC	420 (19.5%)	64 (15.9%)	1		
	CT - TT	1733 (80.5%)	339 (84.1%)	1.27	0.107	2212.9
Recessive	CC - CT	1434 (66.%)	249 (61.8%)	1		
	TT	719 (33.4%)	154 (38.2%)	1.24	0.058	2213.8
Over-dominant	CC - TT	1139 (52.9%)	218 (54.1%)	1		
	CT	1014 (47.1%)	185 (45.9%)	0.94	0.592	2216.2
Additive with each T allele				1.18	**0.032**	2211.8

Table 3.16. The association of *CDKN2A*-rs10811661 with IFG among 40 - 64 year old people in Ha Nam province

Models		**Control group**	**Prediabetic group**	**Univariate analysis**		
				OR	***P***	**AIC**
Codominant	CC	420 (19.5%)	38 (15.8%)	1		2114.2
	CT	1014 (47.1%)	108 (44.8%)	1.16	0.449	
	TT	719 (33.4%)	95 (39.4%)	1.45	0.064	
Dominant	CC	420 (19.5%)	38 (15.8%)	1		
	CT - TT	1733 (80.5%)	203 (84.2%)	1.28	0.179	2115.3
Recessive	CC - CT	1434 (66.6%)	146 (60.6%)	1		
	TT	719 (33.4%)	95 (39.4%)	1.31	0.057	2114.8
Over-dominant	CC - TT	1139 (52.9%)	133 (55.2%)	1		
	CT	1014 (47.1%)	108 (44.8%)	0.90	0.454	2114.9
Additive with each T allele				1.22	**0.045**	2113.8

3.4.3.4. KCNJ11 rs5219

Table 3.16. The association of *KCNJ11*- rs5219 with prediabetes among 40 - 64 year old people in Ha Nam province

Models		**Control group**	**Prediabetic group**	**Univariate analysis**		
				OR	***P***	**AIC**
Codominant	CC	246 (11.4%)	44 (10.8%)	1		2233.3
	CT	960 (44.4%)	176 (43.3%)	0.93	0.852	
	TT	957 (44.2%)	186 (45.8%)	0.90	0.553	
Dominant	CC	246 (11.4%)	44 (10.8%)	1		
	CT - TT	1917 (88.6%)	362 (89.2%)	0.92	0.680	2231.3
Recessive	CC - CT	1206 (55.8%)	220 (54.2%)	1		
	TT	957 (44.2%)	186 (45.8%)	0.93	0.456	2231.7
Over-dominant	CC - TT	1203 55.6%)	230 (56.7%)	1		
	CT	960 (44.4%)	176 (43.3%)	0.95	0.630	2231.6
Additive with each T allele				0.90	0.454	2231.3

3.5. The combined association of enviromental factors and genetic polymorphisms with prediabetes among 40 - 64 year old people in Ha Nam province

3.5.1. The combined association of environmental factors with prediabetes

Table 3.18. Models to study the association of environmental factors among 40 - 64 year old people in Ha Nam province

Models	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Number of factors	**13**	**12**	**11**	**10**	**9**	**8**	**7**	**6**
Age	X	X	X	X	X			
Gender	X	X	X	X	X	X		
Region	X	X	X	X	X	X	X	X
Marital status	X	X	X	X	X	X	X	X
History of family diabetes	X	X	X	X				
Extra meals	X	X						

Models	1	2	3	4	5	6	7	8
Alcohol consumption	X	X	X	X	X	X	X	
Waist/hip ratio	X	X	X	X	X	X	X	X
Fat percentage	X							
Systolic blood pressure	X	X	X	X	X	X	X	X
HDL-C	X	X	X					
LDL-C	X	X	X	X	X	X	X	X
Triglyceride	X	X	X	X	X	X	X	X
$P_{\text{H-L test}}$	0.693	0.707	0.986	0.971	0.823	0.917	0.603	0.782
AUC	0.691	0.690	0.689	0.688	0.684	0.682	0.680	0.678

Environmental factors helped predict risk of prediabetes among 40 - 64 year old people in Ha Nam 67.8% - 69.1%. Respectively, environmental factors helped predick risk of IFG 63.9% - 64.8%, IGT 76.1% - 76.4% and combined IFG-IGT 80.4% - 80.9% (Appendix 5 – Fig 1, Fig 2, Fig 4, Fig 5, Fig 7, Fig 8). To choose a suitable model to predict prediabetes and types of prediabetes from environmental factors, BMA analyzes were conducted to determine probability of factors involved in models. Results were presented in Figure 3.8, Appendix 5 - Figure 3, Figure 6, Figure 9.

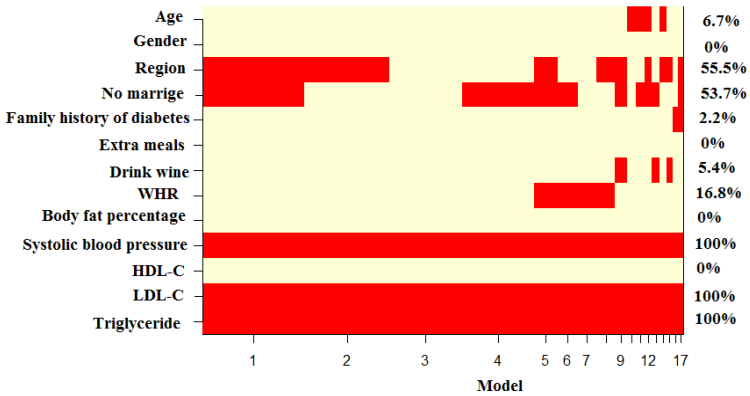

Figure 3.7. Probability of some environmental factors in models predicting prediabetes among 40 - 64 year old people in Ha Nam province

Types	Indices	Prediabetes	IFG	IGT	IFG-IGT
Models		**6**	**2**	**5**	**4**
Urban	OR Probability	2.19 55.5%	2.56 97.2%	2.56 10.5%	
Not married	OR Probability	2.49 53.7%			6.15 85.3%
Junior school	OR Probability				0.36 16.6%
Alcohol consumption	OR Probability		1.42 - 1.87 6.9%		
WHR	OR Probability	1.21 16.8%	1,15 10,2%	1.43 46.7%	
Systolic blood pressure	OR Probability	1.14 100%	1.16 100%	1.14 60.5%	
Low HDL-C	OR Probability			2.14 75%	2.32 3.8%
High LDL-C	OR Probability	1.79 100%		2.41 100%	4.23 100%
High triglyceride	OR Probability	1.64 100%	1.46 100%	1.46 100%	2.65 6.9%
AUC		67.8%	64.8%	76.1%	80.4%

3.5.2. The combined association of polymorphisms with prediabetes

3.5.2.1. The association of numbers of risk alleles with prediabetes

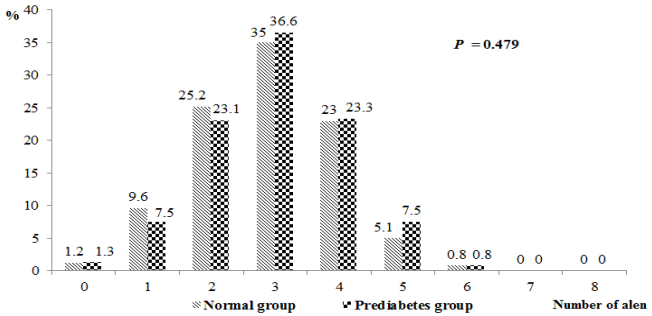

Figure 3.10. Distribution of the risk alleles in case group and control group

Table 3.10 The association of numbers of risk alleles with prediabetes among 40 - 64 year old people in Ha Nam province

Number of risk alleles	**Control group (n = 2199)**	**Prediabetic group (n = 411)**	**Association**	
			OR	***P***
0 - 1	576 (27.0%)	99 (24.8%)	1	
2 - 4	1502 (70.5%)	291 (72.9%)	1.11	0.414
5 - 6	52 (2.4%)	9 (2.3%)	1.27	0.932
Add one risk allele			1.03	0.553

3.5.2.2. The combined association of 4 polymorphisms with prediabetes

Models	**1**	**2**	**3**	**4**
Number of risk factors	**4**	**3**	**2**	**1**
FTO-rs9939609	X			
TCF7L2-rs7903146	X	X	X	
CDKN2A- rs10811661	X	X	X	X
KCNJ11-rs5219	X	X		
$P_{\text{H-L test}}$	0,.60	0.118	0.265	0.833
AUC	0.545	0.544	0.537	0.533

Figure 3.11. Predictive models to study the effects of genetic polymorphisms among 40 - 64 year old people in Ha Nam province

Model 4 was the suitable model for studying the association of gene with prediabetes.

3.5.3. The combined association of environmental factors and genetic polymorphisms with prediabetes among 40 - 64 year old people in Ha Nam province

Model	**1**	**2**	**3**	**4**	**5**	**6**
Numbers of risk factor	**14**	**13**	**12**	**11**	**10**	**9**
Age	X	X	X	X	X	
Gender	X	X	X	X		
Region	X	X	X	X	X	X
Marrige status	X	X	X	X	X	X

Model	**1**	**2**	**3**	**4**	**5**	**6**
History of family prediabetes	X	X	X			
Waist/hip ratio	X	X	X	X	X	X
Body fat percentage	X					
Systolic blood pressure	X	X	X	X	X	X
HDL-C	X	X	X	X	X	X
LDL-C	X	X	X	X	X	X
Triglyceride	X	X	X	X	X	X
Extra meals	X	X				
Alcohol consumption	X	X	X	X	X	X
CDKN2A-rs10811661	X	X	X	X	X	X
$P_{\text{H-L test}}$	0.023	0.287	0.156	0.043	0.140	0.623
AUC	0.703	0.701	0.700	0.697	0.696	0.692

Figure 3.15. Predictive models to study the combined effects of environmental factors and genetic polymorphisms with prediabetes among 40 - 64 year old people in Ha Nam province

Models	**1**	**2**	**3**
Number of risk factors	**8**	**7**	**6**
Age	X	X	X
Gender	X	X	X
Region	X	X	X
Alcohol consumption	X	X	
WHR	X		
Systolic blood pressure	X	X	X
Triglyceride	X	X	X
CDKN2A-rs10811661	X	X	X
$P_{\text{H-L test}}$	0.045	0.083	0.788
AUC	0.676	0.675	0.672

Figure 3.17. Predictive models to study the combined effects of environmental factors and genetic polymorphisms with IFG among 40 - 64 year old people in Ha Nam province

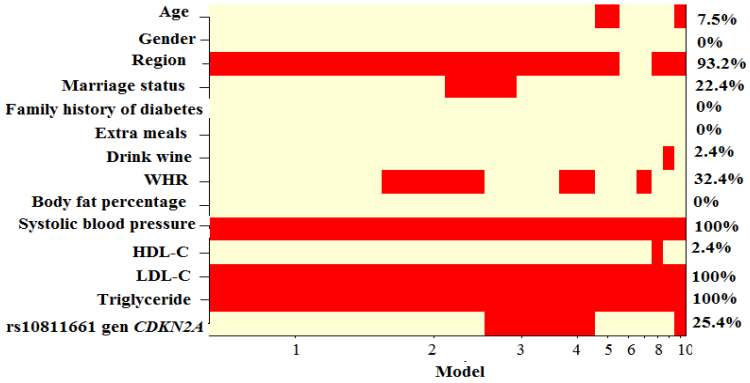

Figure 3.18. The probability of environmental factors and genetic polymorphisms among 40 - 64 year old people in Ha Nam province

Risk factor	**Index**	**Prediabetes**	**IFG**
Model		**6**	**2**
Urban	OR Probability	2.37 93.2%	2.50 97.2%
No married	OR Probability	2.06 22.4%	
Alcohol consumption	OR Probability	1.45 - 1.67 2.4%	1.49 - 2.0 7.2%
Waist/hip ratio	OR Probability	1.22 32.4%	
Systolic blood pressure	OR Probability	1.13 100%	1.14 100%
Low HDL-C	OR Probability	1.32 2.4%	
High LDL-C	OR Probability	1.70 100%	
High triglyceride	OR Probability	1.77 100%	1.51 63.0%
T allele CDKN2A-rs10811661	OR Probability	1.21 25.4%	1.27 13.9%
AUC		69.2%	67.2%

Figure 3.19. The effects of environmental factors and genetic polymorphisms with prediabetes and IFG

Thus, there were differences in the predictive model including environmental factors and/or genetic polymorphisms. Models involved environmental factors could predict prediabetes 67.8% and IFG 64.8%. And models which consisted of *CDKN2A*-rs10811661 could predict prediabetes and IFG 53,3% and 53,9%, respectively. When adding *CDKN2A*-rs10811661 polymorphism on enviromental models, the precdictabe prediabetes and IFG increased negligibly (1.4% and 2.4%).

One of the most of study strengths was that this was the first study on Vietnam human populations on the impact of genetic polymorphisms: *FTO*-rs9939609, *TCF7L2*-rs7903146, *CDKN2A*-rs10811661, *KCNJ11*-rs5219 and on combined effects of environmental factors and genetic polymorphisms with prediabetes among 40 - 64 year old pepole. Second, the study was conducted on large homogeneous populations in Red River Delta, Vietnam. Finally, the method used venous blood to determine FPG and glucose 2 hours after oral glucose tolerance test had high accuracy.

CONCLUSIONS AND RECOMMENDATIONS

1. Conclusions

- **Conclusion 1. Prevalences of prediabetes among 40 - 64 year old people in Ha Nam province**

Prevalence of prediabetes among 40 - 64 year old people in Ha Nam province was at high level (15.2%), with mainly impaired fasting blood glucose (9.2%). The prevalence of prediabetes increased from 40 - 44 year-old group (11.5%) and reached the highest in the group of 55 - 59 year-old (20.6%), then decreased in the group of 60 - 64 year-old group (16.4%). Prediabetes prevalence in urban areas (32%) was 2 times higher than that in rural areas (14.2%).

- **Conclusion 2. The association of environmental factors with prediabetes among 40 - 64 year old people in Ha Nam Province**

Environmental factors increased the risk of prediabetes included: urban (OR = 2.19 - 2.56), single (OR = 2.49 – 6.15), history of family diabetes (OR = 1.77 - 2.31), income level 2 (OR = 1.66), alcohol consumption (1.42 - 1.87), waist/hip ratio (OR = 1.21 - 1.43), overweight (OR = 1.28 – 1.60), increased systolic blood pressure (OR = 1.14 - 1.16), low HDL-C (OR = 2.14 - 2.32), high LDL-C (OR = 1.79), high triglyceride (OR = 1.46 - 2.65). While education level reduced the risk of combined impaired fasting glucose and impaired glucose tolerance (OR = 0.34).

- **Conclusion 3. The association of gene polymorphisms with prediabetes among 40 - 64 year old people in Ha Nam Province**

Among 4 genetic polymorphisms: *FTO*-rs9939609, *TCF7L2*-rs7903146, *CDKN2A*-rs10811661 and *KCNJ11*-rs5219, the research only found the significant association of rs10811661 polymorphism with prediabetes in codominant model (OR = 1.40) and additive with

each allele A model (OR = 1.18); with impaired fasting glucose in additive with each allele A model (OR = 1.22).

- **Conclusion 4. The combined association of environmental factors and genetic polymorphisms with prediabetes among 40 - 64 year old people in Ha nam Province**

Model which could predict prediabetes included: region, marriage, alcohol assumption, waist/hip ratio, systolic blood pressure, HDL-C, LDL-C, triglyceride, and *CDKN2A*-rs10811661.

Model which could predict imparied fasting glucose included: region, drinking wine, systolic blood pressure, triglyceride and *CDKN2A*-rs10811661.

2. Recommendations

- Population should be encouraged to enhance physical activities, to limit drinking alcohol, to take proper nutrition to prevent obesity, dyslipidemia and hypertension to reduce the risk of prediabetes and diseases to improve the quality of life of Vietnamese people.
- *CDKN2A*-rs10811661 should be used in the predictive prediabetic model to help reduce the potential progression of diabetes type 2 and its complications.
- It is necessary to study the association of diet, physical activities and increase the number of polymorphisms to improve the ability to predict prediabetes.
- It is important to study in different subjects to analyze the role of environmental factors and genetic polymorphisms in prediabetes among Vietnamese people.