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## Maternal Diabetes, Related Biomarkers and Genes, and Risk of Orofacial Clefts

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MATERNAL DIABETES, RELATED BIOMARKERS AND GENES, AND RISK OF  
OROFACIAL CLEFTS

by

Tiwaporn Maneerattanasuporn

A dissertation submitted in partial fulfillment  
of the requirement for the degree

of

DOCTOR OF PHILOSOPHY

in

Nutrition and Food Sciences

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Logan, Utah

2017

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## ABSTRACT

Maternal Diabetes, Related Biomarkers and Genes, and Risk of Orofacial Clefts

by

Tiwaporn Maneerattanasuporn, Doctor of Philosophy

Utah State University, 2017

Major Professor: Dr. Ronald G. Munger  
Department: Nutrition, Dietetics, and Food Sciences

Orofacial clefts (OFCs) are among the most common congenital birth defects and are characterized by incomplete development of the lip or the palate or both. The lip and palate develop separately at different times during the first trimester of pregnancy. The etiology of OFCs is multifactorial and includes a combination of genetic and environmental factors. This project aims to examine the role of maternal diabetes mellitus in orofacial clefts through studies of medical histories, biomarkers, and genes.

Firstly, the association between maternal pre-pregnancy weight and maternal diabetes mellitus and the risk of orofacial clefts (OFCs) in a population-based case-control study of birth certificate data in Utah was examined. The study found that maternal obesity increased the risk of OFCs with or without birth defects (non-isolated and isolated). Underweight mothers had a reduced risk of cleft lip only (CLO), and an increased risk of cleft palate only (CPO). Pre-existing diabetes and gestational diabetes mellitus (GDM) increased the risk of non-isolated OFCs. Mediation analysis indicated

that obesity had a direct effect of increasing the risk of OFCs without the mediating effect of known maternal diabetes.

Secondly, the association between maternal medical history and maternal biomarkers of metabolic syndrome and OFCs was examined using case-control interview and clinical examination data from the Utah population. This study was limited to isolated OFCs. Mothers having GDM in any pregnancy had an increased risk of OFCs. Mothers of children with cleft palate with or without cleft lip (CP/L), compared to controls, had higher mean levels of plasma glucose, insulin, triglycerides, waist circumference and systolic blood pressure, and lower HDL; these associations were not seen for mothers of children with CLO. Plasma IL-8 and leptin levels were associated with CP/L but not with CLO. Metabolic syndrome indices were associated with CP/L; these scores were not associated with CLO.

Finally, the association between genes related to GDM and the risk of OFCs was examined using data from a large scale genome-wide association study of European and Asian populations. Many genes previously known to be related to GDM were associated with OFCs through genetic effects alone and gene-environment interaction effects with periconceptional maternal multivitamin use, maternal smoking, and environmental tobacco smoke. These results support the hypothesis that GDM may be causally related to OFCs via multiple GDM susceptibility genes and interactions with environmental factors.

Individuals with OFCs face both physical and mental health problems, which require multi-specialty team care. OFC prevention and prediction are important to public health. This dissertation reported that maternal diabetes mellitus, maternal pre-pregnancy

weight and genes related to GDM had an association with the risk of OFCs. Mothers having an OFC child had an increased risk of developing metabolic abnormalities later in life. Potential risk factors that are reported in this dissertation may be useful for OFC prevention. This dissertation also reported potential biomarkers for predicting OFCs. Moreover, mothers having an OFC child require regular monitoring for maternal metabolic abnormalities later in life.

(442 Pages)

## PUBLIC ABSTRACT

Maternal Diabetes, Related Biomarkers and Genes, and Risk of Orofacial Clefts

Tiwaporn Maneerattanasuporn

Orofacial clefts (OFCs) are among the most common congenital birth defects and are characterized by incomplete development of the lip or the palate or both. The lip and palate develop separately at different times during the first trimester of pregnancy. The etiology of OFCs is multifactorial and includes a combination of genetic and environmental factors. This project aims to examine role of maternal diabetes mellitus in orofacial clefts through studies of medical histories, biomarkers, and genes.

In a study of Utah birth certificates, mothers with pre-existing diabetes and gestational diabetes mellitus (GDM) had an increased risk of OFCs, and obese mothers also had an increased risk. Mothers of children with OFCs were more likely than mothers of unaffected children to develop obesity, metabolic syndrome and gestational diabetes mellitus later in life. These result were more strongly related to cleft palate than cleft lip. Many genes related to GDM were associated with OFCs through genetic effects alone and gene-environment interaction effects with periconceptional maternal multivitamin use, maternal smoking, and environmental tobacco smoke. These results support the hypothesis that GDM may be causally related to OFCs via multiple GDM susceptibility genes and interactions with environmental factors.

Individuals with OFCs face both physical and mental health problem, which require multi-specialty team care. OFC prevention and prediction are important to public

health. This dissertation reported that maternal diabetes mellitus, maternal pre-pregnancy weight and genes related to GDM had an association with the risk of OFCs. Mothers having an OFC child had an increased risk of developing metabolic abnormalities later in life. Potential risk factors were reported in this dissertation that may be useful for OFC prevention. This dissertation also reported potential biomarkers for predicting OFCs. Moreover, mothers having an OFC child may require regular monitoring of metabolic abnormalities later in life.



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# CHAPTER 1

## INTRODUCTION

### 1.1 Background

Orofacial clefts (OFCs) are among the most common congenital birth defects and are characterized by incomplete development of the lip or the palate or both. The lip and palate develop separately at different times during the first trimester of pregnancy. Globally, approximately 1 in 700 newborns suffer from OFCs (1). The prevalence of OFCs is different among varying ethnic and racial groups; the highest rate was found in Asians and Native Americans (2 per 1000 births) (2). In the United States, Utah has a high rate of clefts (2.25 per 1000 births) (3).

Even though OFCs are not a major cause of death, they have an adverse effect on affected children and their families. Children with OFCs have difficulty in feeding, speaking, hearing, and socializing. The difficulties faced by children with OFCs include the need of multidisciplinary team clinical care (plastic surgery, speech therapy, audiology, otolaryngology, dentistry, orthodontics, and psychology) over their lifetime (4). Moreover, the parents of affected children have a greater risk of financial and mental health problems (5, 6).

The etiology of OFCs is multifactorial and includes a combination of genetic and environmental factors. The environmental factors that may increase the risk of OFCs include smoking, alcohol consumption, and certain medications during pregnancy and prior to pregnancy. Other environmental factors including maternal nutritional status, supplement intake, and eating behavior have also been associated with the incidence of OFCs (4). Moreover, some dietary patterns may have a protective effect on the rate of



OFCs such as the DASH (Dietary Approach to Stop Hypertension diet) and Mediterranean diet patterns (7, 8).

Insulin resistance and obesity are considered as risk factors of metabolic diseases and may be related to the risk of OFCs. Some studies have reported that mothers with high body mass index (BMI) have a higher rate of OFCs when compared with mothers with normal BMI (9). Moreover, an association between pre-gestational diabetes mellitus and OFCs has been reported, but has not been studied in detail (10).

The genetic factors of OFCs have been studied by many methods such as linkage studies and genome-wide association studies (GWAS). The gene-disease associations appear different among different ethnic groups (1, 11). The linkage study aims to identify genetic markers related to the disease in a family. These studies found have associations between non-syndromic orofacial clefts and genes related to growth factors, transcription factors, xenobiotic metabolism, immune response and one-carbon metabolism (12). GWAS have provided some similar and some different genetic markers associated with non-syndromic orofacial clefts but the data are limited. However, no study has reported the correlation between genes related to diabetes and metabolic syndrome and the risk of OFCs.

Due to the adverse effect of orofacial clefts on children and their family, studies of the complex etiology of OFCs are needed in order to reduce the incidence of OFCs. Moreover, studies of the health problems of mothers near the time of conception are needed to reduce the incidence of OFCs which occur in the first trimester of pregnancy.

## 1.2 Objectives

The overall objective is to examine role of maternal diabetes mellitus in orofacial clefts through studies of medical histories, biomarkers, and genes. The specific objectives and hypothesis are:

**Aim 1:** To determine the association between maternal diabetes mellitus and gestational diabetes mellitus and the risk of orofacial clefts using data from Utah birth certificates, Intermountain Healthcare (IHC) and University of Utah medical records, and the Utah Clefts 2 case-control study.

Hypothesis: Diabetes is more common among mothers of children with orofacial clefts compared to controls before, during, and after the index pregnancy.

**Aim 2:** To determine the association between the occurrence of maternal diabetes and maternal biomarkers of metabolic syndrome and isolated orofacial clefts (OFCs) using data from the Utah Cleft 2 case-control study.

Hypothesis: Mothers of children with orofacial clefts have a higher prevalence of maternal diabetes and abnormal biomarkers associated with metabolic syndrome compared to controls.

**Aim 3:** To determine the association between genes related to diabetes and obesity and risk of orofacial clefts

Hypothesis: Genes associated with diabetes mellitus and obesity are associated with the risk of orofacial clefts

### **1.3 Structure of the Dissertation**

This dissertation consists of six chapters. The first chapter introduces the recent problems related to OFCs and the way of the dissertation can contribute to solve these problems. The second chapter provided an overview of epidemiology, etiology, and risk factors of OFCs, diabetes mellitus, and metabolic syndrome. This chapter also presented biomarkers and genes related to diabetes and metabolic syndrome. The third chapter presented the finding of first aim, which is to determine the association between maternal diabetes mellitus and gestational diabetes mellitus and the risk of orofacial clefts. The fourth chapter presented the finding of second aim, which is to determine the association between maternal biomarkers of metabolic syndrome and orofacial. The fifth chapter showed the finding of third aim, which is to determine the association between genes related to diabetes and obesity and risk of orofacial clefts. The sixth chapter concludes the results from three aims, provides the public health significance, and gives suggestion for further research. The references for each chapter were listed at the end of each.

### **1.4 Study Design**

The study in this dissertation used the data from Utah Birth Defect Network (UBDN), the Utah Cleft 2 case-control study, and the International Genetic Epidemiology study of Oral Clefts.

The UBDN, operated by the Utah Department of Health (UDOH) is a statewide population-based surveillance system, identifies all prenatal or postnatal major structural birth defects in fetuses and neonates. The OFC classifications used in the data analyses

were based on the final UBDN diagnoses, which were reviewed by a medical geneticist. OFC cases were divided into cleft lip alone, cleft palate alone, cleft lip without cleft palate, and cleft lip with cleft palate and classified as isolated, syndromic, or multiple birth defect cases. The case mothers of a child with an OFC during 1995-2011 were linked to the Utah Population database (UPDB). The UPDB provides information for research on genetics, epidemiology, demography, and public health, which receives annual updates from birth and death certificates, hospitalization and ambulatory surgery records, and driver licenses. Controls were randomly selected from Utah birth certificates at a ratio of 4:1 to live-born cases matched by birth month and year. The anonymized identification numbers of cases and controls from UPDB were linked to the Utah Birth Certificate database. In addition, the UPDB provided information on OFC cases noted in fetal and neonatal death records. In total, 1,611 OFC live-born cases and 6,444 controls linked to the birth certificate records, in which 1,451 cases and 5,804 controls provided complete data, were used for analysis.

The Utah Cleft 2 study, a collaboration of Utah State University (USU), the University of Utah Health Sciences Center and the Utah Department of Health (UDOH), is a study of orofacial clefts in Utah. Cases and controls were selected from the participants in the Utah Cleft 1 case-control study (13) and the National Birth Defects Prevention Study (NBDPS) in Utah (14). In the Utah Cleft 1 study, case-mothers having a child with OFCs between January 1995 and June 2005 were recruited from UDOH, and control mothers were randomly selected, frequency matched by birth month and year, and gender of case child at ratio 1:1 by using Utah birth certificate files. The NBDPS in Utah,

also a state-wide population-based case-control study, recruited case mothers having a child with OFCs between 2005-2011 from UDOH database, and randomly selected control mothers from birth certificates. The OFC cases were limited to isolated OFCs; cases with multiple birth defects were excluded.

The International Genetic Epidemiology study of Oral Clefts, a part of the Gene-Environment Association Studies Initiative (GENEVA) of the National Institutes of Health (NIH). This study is a multi-center, international study of trios from Europe, the U.S., including Utah, China, Taiwan, Singapore, Korea, and the Philippines, which aims to investigate genes associated with oral clefts. Families were recruited from treatment centers or population-based registries. OFC cases were examined by either a clinical geneticist or an experienced clinician to minimize misclassification of the OFCs. All cases with cleft palate with or without cleft lip (CP) were analyzed together based on evidence that maternal obesity and diabetes have a specific effect on palate development. Trios having CL/P and CP (cleft palate with or without cleft lip) were analyzed in this study separately. For Asians, 892 CL/P and 910 CP trios, and for Europeans, 665 CL/P and 644 CP trios were analyzed in this dissertation.

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## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Orofacial Clefts

Orofacial clefts (OFCs) are craniofacial birth defects which can be divided into cleft lip only (CLO), cleft lip with cleft palate (CLP), cleft lip with or without cleft palate (CL/P), cleft palate only (CPO), and cleft palate with or without cleft lip (CP/L). The other terminology is used to describe OFCs: syndromic and non-syndromic (isolated). Isolated or non-syndromic OFCs refers to OFCs without other congenital malformations or anomalies. Syndromic OFCs means the OFCs with other known patterns of anomalies. OFCs with other deformations, which cannot classify to existing syndromes, are multiple birth defect OFCs. About 70% CL/P are isolated. Isolated OFCs are not typically a cause of mortality, but individuals with OFCs face difficulty with feeding, speaking, hearing, and socializing.

##### 2.1.1 Classification

Orofacial clefts have many classification systems depending on the purpose. The systems are divided into an anatomic system for surgeons and an embryology-based system for genetic counselling and research.

##### a) Anatomic system

The common form of CLP involves disruption of tissue planes above the lip extending into the nares and/or the hard and/or soft palate. For example, the Iowa system (1) classified OFCs into 5 groups

group 1: cleft of the lip only (either unilateral or bilateral cleft lip)

group 2: secondary palate cleft only

group 3: clefts of the lip, alveolus, and secondary palate (complete cleft lip and palate)

group 4: cleft of lip and alveolus (primary palate cleft and cleft lip)

group 5: miscellaneous

Additionally, Millard (2) suggested the ICPR system classifying OFCs into 3 groups

group 1: clefts of the primary palate (lip and/or alveolus)

group 2: clefts of the primary and secondary palate (lip and/or alveolus and palate)

group 3: clefts of the secondary palate (hard palate and/or soft palate)

#### **b) Syndromic clefts and multiple birth defects**

This system divides orofacial clefts into syndromic and non-syndromic clefts. Syndromic clefts are the orofacial clefts occurring with other birth defects related to known genetic syndromes such as Van der Woude syndrome, Treacher Collins Syndrome, and Apert Syndrome. Cases with multiple birth defect OFCs have OFCs with other congenital deformations, which cannot classify to existing syndromes. Non-syndromic clefts have no other structural or functional anomalies.

#### **2.1.2 Embryological development of OFCs**

OFCs results from non-closure of facial structures associated with lip and palate formation during the fourth through the twelfth week of pregnancy. The development of

face and jaws involves cell migration from the cranial neural crest, proliferation, differentiation and apoptosis (3).

The lip develops between the fourth and eighth week of pregnancy, which correlates with the formation of the frontonasal prominence, the paired maxillary processes, and the paired mandibular process. By the end of the fourth week of gestation, migrating neural crest cells of the first pharyngeal arch form the frontonasal prominence. The lower portion of the frontonasal prominence is divided into paired medial and lateral nasal processes. By the end of the sixth week of embryogenesis, the medial nasal processes merge with each other and with the bilateral maxillary processes to form the upper lip and the primary palate, giving rise to the premaxilla (central upper lip, maxillary alveolar arch and four teeth, and hard palate anterior to the incisive foramen). A cleft lip results from a failure to maintain an epithelial bridge due to lack of mesodermal penetration and proliferation from the maxillary and nasal processes.

The development of the secondary (soft) palate occurs after the primary palate (alveolar ridge and a triangular area of the anterior hard palate) during weeks 6-12. During the sixth week of development, the maxillary processes of paired palatal shelves initially rise vertically down the sides of the developing tongue, and grow to a horizontal position above the tongue and come into contact and fuse to form a midline epithelial seam during the seventh week. The palatal shelves also fuse in the midline with the primary palate anteriorly and with the nasal septum dorsally. These fusion processes are complete by the tenth week of pregnancy, separating the oral and nasal cavities, permitting simultaneous respiration and mastication. Clefts of the secondary palate are

due to lack of fusion of the palatal shelves. Normal development occurs sequentially; thus a cleft lip may or may not be associated with a cleft palate.

### **2.1.3 Epidemiology of OFCs**

Orofacial clefts (OFCs) remains a health issue in both developed and developing countries. OFCs occur in every 0.4 to 2 per 1000 births depending on geographic location, racial and ethnic groups, maternal age, environmental exposures, and socioeconomic status (4, 5). The global average prevalence of OFCs is 1.43 in 1000 live births (6, 7). The Centers for Disease Control and Prevention (CDC) reported the incidence during 2004-2006 of CL/P was 1.06 in 1000 live births and 0.64 in 1000 live births for CP (8). National Birth Defects Prevention Network reported the prevalence of OFCs during 2002-2006 of CL/P was 1.33 per 1000 live births, and 0.73 per 1000 live births for CP, which is higher than The US prevalence during 1999-2001 (1.05 and 0.64 per 1000 live births for CL/P and CP respectively) (9). International data from 57 registries for 1993-98 suggest a variation in prevalence at birth of cleft lip with or without cleft palate of 0.34-2.29 per 1000 births, and an even more pronounced variation for isolated cleft palate, with prevalence of 0.13-2.53 in 1000 births (10). Asians and Native Americas present the highest rate (2 in 1000 births), Europeans present at a rate around 1 in 1000, and Africans have the lowest rate (0.4 in 1000 births) (11). The National Center on Birth Defects and Developmental Disabilities updated the prevalence of CL/P and CP in the United States during 2006-2010 showing that Alaska (2.01 and 1.77 per 1000 births respectively), North Dakota (1.58 and 1.51 per 1000 births respectively), Oklahoma (0.80 and 1.33 per 1000 births respectively), Utah (1.37 and 0.

65 per 1000 births respectively), Washington (1.14 and 0.88 per 1000 births respectively), and Colorado (1.17 and 0.82 per 1000 births respectively) are the five states with the highest prevalence of CL/P and CP (1.14 and 1.10 per 1000 births respectively for overall prevalence in the United States) (12). Butali and Mossey reported that prevalence of OFCs differed among African populations (13), including; Uganda (0.75 per 1000 births in 1968), Kenya (1.65 per 1000 births during 1963-1964), South Africa (0.33 per 1000 births during 1983-1984), Nigeria (0.3 per 1000 births during 1976-1980), Tunisia (1.5 per 1000 births during 1983-1984), Zaire (0.46 per 1000 births during 1977-1979), Malawi (0.67 per 1000 births during 1998-1999), and Sudan (0.9 per 1000 births during 1997-2000). Recent studies also reported the different prevalence among regions of Africa, including; 0.313 per 1000 births for South Africa during 2002-2006 (14), 0.5 per 1000 births for Nigeria during 2006-2010 (15), 0.63 per 1000 births for Ghana in 2006 (16), 0.77 per 1000 births for Uganda during 2005-2010 (17). These studies showed that the prevalence of OFCs in South Africa, Nigeria and Uganda increased from the past.

The rate of CL/P differs by sex. The ratio of CL/P in males to females is 2:1; a ratio of 1:2 of males to females was found in populations of isolated cleft palate. In white populations, the male to female ratio for cleft lip with or without cleft palate is about 2:1 (18). In Japanese populations, CL/P shows a significant excess for males, but this excess is not seen for cleft lip alone (19). These findings are similar to studies done in China and Nigeria (15, 20). Moreover, among unilateral cleft lip cases, the left: right ratio is 2:1 (18).

#### 2.1.4 Risk factors of OFCs

The etiology of orofacial clefts is not fully understood. The updated evidence suggests that there are the multiple factors for this defect including both genetic and environmental factors.

##### a) Environmental factors

##### i) Maternal smoking

Maternal smoking has consistently been reported to increase the risk of both cleft lip with or without cleft palate and isolated cleft palate. An international population-based study, including Norway and the United States, reported that mothers with active smoking increased risk of all types of isolated clefts (odds ratio (OR): 1.38, 95% confidence interval (95% CI): 1.24-1.53 for all OFCs; OR: 1.42, 95% CI: 1.26-1.61 for CL/P, OR: 1.27, 95% CI: 1.08-1.51 for CP) (21). A case-cohort study from Denmark by Bille et al. (22) collecting first trimester maternal life style data found that maternal smoking increased the risk of orofacial clefts with statistical significance (OR: 1.50; 95% CI: 1.05-2.14). The meta-analysis study, analyzing 22 case-control studies, also reported that maternal tobacco smoking increased the risk of both CL/P and CP (relative risk (RR):1.34, 95% CI: 1.25-1.44 for CL/P and RR: 1.22, 95% CI: 1.10-1.35) (23). Another study from Brazil by Leite et al. (24), however, did not present a statistically significant association between maternal smoking during the first trimester of gestation and orofacial

clefts (OR: 1.13, 95% CI: 0.81-1.57). The inconsistent association between maternal smoking and OFCs from the Brazil study may result from the small sample size and quality of data (recall bias from retrospective study).

The effect of environmental tobacco smoke from passive exposure on the risk of OFCs still appears to be inconsistent. The study from Brazil (24) reported that maternal passive smoking during pregnancy associated with CL/P with statistical significance (OR: 1.39, 95% CI: 1.01-1.98), except for CP (OR: 1.67, 95% CI: 0.9-3.11). Case-control studies in different cities (Shenyang and Heilongjiang) in China stated that passive smoke exposure of mothers increased the risk of OFCs (Shenyang: OR: 2.05, 95% CI: 1.47-2.87 for OFCs and Heilongjiang: OR: 2.52, 95% CI: 1.90-3.33 for CL/P, OR: 1.87, 95% CI: 1.28-2.75 for CP) (20, 25). The slight association between maternal passive smoke and isolated OFCs and CL/P except CP was reported in the international population-based study (OR: 1.14, 95% CI: 1.03-1.25 for OFCs; OR: 1.14, 95% CI: 1.02-1.28 for CL/P, OR: 1.12, 95% CI: 0.95-1.31 for CP) (21). However, Honein et al. reported that environmental tobacco smoke exposure was not associated with CL/P and CP (26).

## ii) Alcohol consumption

Maternal alcohol consumption has been advanced as a risk factor, although the evidence is still inconsistent. The study in Brazil by Leite et

al. (24) reported maternal alcohol consumption during first trimester was associated with CL/P and CP with statistical significance (OR: 2.08, 95% CI: 1.27, 3.41 and OR: 2.89, 95% CI: 1.25-8.30, respectively). A recent study in Brazil by Bezerra et al. (27) also found that alcohol drinking during pregnancy increased risk of non-syndromic OFCs (OR: 3.64, 95% CI: 1.6-8.3). The Danish National Birth Cohort (22) presented a slight positive association between alcohol consumption during the first trimester of gestation without statistical significance (OR: 1.11; 95% CI: 0.79-1.55). A case-control study in Norway (28) which collected the data from 1996 to 2001 showed that mother consuming greater than or equal to 5 drinks per setting (binge drinking pattern: high dose of alcohol consumption in short periods) had increased the risk of CL/P (OR: 2.2, 95% CI: 1.1-4.2) and the risk of CP (OR: 2.6, 95% CI: 1.2-5.6). The Iowa case-control study reported that increased alcohol consumption increased risk of isolated CL/P except isolated CP (OR: 1.5, 95% CI: 0.9-2.4 for 1-3 drinks/month; OR: 3.5, 95% CI: 0.8-15.4 for 4-10 drinks/month; OR: 4.0, 95% CI: 1.1-15.1 for >10 drinks/month; p-trend 0.007) (29). Additionally, a meta-analysis evaluating data from 33 studies (23 case-control and 10 cohort studies) reported that maternal alcohol consumption during pregnancy was not associated with the occurrence of OFCs (30).

### iii) Coffee and caffeine-containing beverages consumption



A Norwegian case-control study by Johansen et al. (31) reported a statistically significant correlation between coffee consumption and the risk of CL/P (OR: 1.39, 95% CI: 1.01-2.39) for 1-2 cups per day and (OR: 1.59, 95% CI: 1.05-2.39) for 3 cups or more per day. There was no statistically significant association between coffee consumption and CP. Moreover, the study in Norway (31) presented a negative correlation between daily tea consumption of 3 or more cups and both CL/P and CP (OR: 0.55, 95% CI 0.32-0.95 and OR: 0.58, 95% CI: 0.31-1.07) when compared with no tea intake. However, the evidence for an association between coffee intake and orofacial clefts is still inconsistent. Kurppa et al. (32) reported that coffee intake of more than 4 cups a day was not associated with the risk of orofacial clefts (OR 1.0, 95% CI: 0.6-1.6). On the contrary, the cohort study in Denmark showed that daily coffee consumption of more than 5 cups reduced the risk of orofacial clefts by 37% (95% CI: 0.32-1.44) when compared with no coffee consumption, though the confidence intervals in this study are biased and included 1.0 (22).

#### **iv) Maternal age**

A meta-analysis (23 published case-control studies) showed that a maternal age of 40 years or more increased the risk of CLP by 56% compared to a maternal age between 20 and 29 years (33). A population-based case-control study in China supported the hypothesis of maternal

age being associated with OFCs (34). The study reported that mothers older than 35 years old experienced an increased risk of CL/P (OR: 2.12, 95% CI: 1.26-3.57).

**v) Medication**

Therapy with anticonvulsant drugs, notably diazepam, phenytoin, and phenobarbital in maternal epilepsy increased the risk of both CL/P (OR: 7.77, 95% CI: 2.02-26.0) and CP (OR: 3.61, 95% CI: 0.08-26.5) (35, 36). Use of valproic acid monotherapy in the first trimester of pregnancy increased the risk of cleft palate (OR: 5.2, 95% CI: 2.8-9.9) (37).

Maternal amoxicillin use in the third month of gestation increased the risk of both CL/P and CP with OR: 4.3, 95% CI: 1.4-13.0 and OR: 7.1, 95% CI: 1.4-36, respectively, and increased the risk of CL/P with OR 2.0, 95% CI: 1.4-4.1 for the first trimester use of amoxicillin (38).

The association between maternal corticosteroid use and risk of OFCs is still inconsistent. Maternal corticosteroid use had positive associations with OFCs (OR: 3.35, 95% CI: 1.97-5.69) (39). The data from MADRE project, an international women's human right organization, showed the association between corticosteroid use in the first trimester and the occurrence of CL/P (OR: 2.59, 95% CI: 1.18-5.67)(40). The case-control study in Norway presented the positive association of dermatologic corticosteroids with both CL/P (OR: 2.3, 95% CI: 0.71-7.7)

and CP (OR: 3.4, 95% CI: 0.87-13) (41), which is similar to the result from study in Denmark (42).

The population-based case-control study in China reported analgesics or antipyretics (aspirin, aminopyrine, and phenacetin) increased the risk of OFCs (OR: 7.85, 95% CI: 3.15-19.58) (20).

The association between OFCs and medication used for depression and anxiety has been reported in many studies. A case-control study found maternal benzodiazepine use increased the risk of CLP (43). Shiono et al. reported that maternal diazepam use during the first trimester had no significant association with orofacial clefts (RR: 1.22, 95% CI: 0.17-8.95) (44). The National Birth Defect Prevention Study found the association between OFCs and venlafaxine use one month before conception and during early pregnancy (OR:1.5, 95% CI: 0.5-4.3 for CL/P and OR:3.3, 95% CI: 1.1-8.8 for CP) (45).

#### **vi) Folate nutritional status**

Folate is a cofactor in the metabolism of one-carbon (the transfer and utilization of one-carbon-groups), the synthesis of DNA (donation of one-carbon units in the process of DNA -biosynthesis), and the remethylation of homocysteine to methionine (46). Maternal folate status has been suggested to influence the risk of OFCs (5). The case-control study in the Netherlands (47) found that the mothers of children with CL/P consumed dietary folate in quantities less than the mothers in the control

group. In addition, increasing quartiles of dietary folate intake was associated with decreased the risk of CL/P (quartile 1 is reference, quartile 2 (OR: 0.74; 95% CI: 0.40-1.37), quartile 3 (OR: 0.73; 95% CI: 0.39-1.35), quartile 4 (OR: 0.63; 95% CI: 0.32-1.23) quartile 5 (OR: 0.54; 95% CI: 0.27-1.05); p-trend 0.06). A recent case-control study in Brazil reported that low folate levels (< 7 ng/ml) increased risk of non-syndromic OFCs (OR: 2.18; 95% CI: 1.12-5.67) (27).

The data from a Utah case-control study reported that plasma folate and erythrocyte folate levels in mothers of children with isolated clefts (CL/P and CP) were lower than the controls group with statistical significance (48). A report in the Philippines by Munger et al. also presented a similar association, and noted that the folate association depended on the background of vitamin B6 level (49). However, Bille et al. studied the effect of IgG and IgM autoantibodies on folate receptor alpha (FRalpha) in pregnant women and found that the levels of folate receptor antibody were not associated with increased risk of oral clefts (50).

#### **vii) Vitamin B6 nutritional status**

Vitamin B6, pyridoxine, is a co-factor required for amino acid, glucose, and lipid metabolism including the metabolism of folate and homocysteine (51). Mouse studies reported the protective effect of vitamin B6 supplementation on incidence of corticosteroid-induced OFCs

(52-55) and dexamethasone-induced cleft palate (56). Davis et al. presented that mice with vitamin B6 deficiency resulted in birth defects including cleft palate (57). Another mouse study found that dietary vitamin B6 deprivation led to isolated cleft palate in 20% of the offspring (58). Moreover, animal studies reported that vitamin B6 prevented the induction of OFCs by vitamin A excess (59), cyclophosphamide (60), and  $\beta$ -aminopropionitrile (61).

The association between vitamin B6 nutritional status and OFCs in human study is limited. A case-control study in Netherland by Krapels et al. showed no association between dietary vitamin B6 consumption and risk of OFCs after adjustment for dietary folate intake (62). This study also reported increased dietary consumption of vitamin B6 significantly reduced risk of OFCs in periconceptional supplement group (quintile 1: 1.07-1.51 mg/day is reference, quintile 2: 1.52-1.61 mg /day (OR: 0.69; 95% CI: 0.24-1.99), quintile 3: 1.62-1.72 mg /day (OR: 0.49; 95% CI: 0.16-1.54), quintile 4: 1.72-1.84 mg /day (OR: 0.20; 95% CI: 0.07-0.61), quintile 5: 1.84-2.42 mg /day (OR: 0.22; 95% CI: 0.08-0.64), p-trend = 0.0006), but the association was not found in non-supplementary group . A human study in Philippines (49) reported the positive association between OFCs and maternal vitamin B6 activation coefficient (increased levels indicate poorer vitamin B6 status) in both sites (Negros Occidental: tertile 1 is reference, tertile 2 (OR: 4.01; 95% CI: 1.09-14.75), tertile 3 (OR:

6.54; 95% CI: 1.93-22.24), p-trend = 0.002 and Davao: tertile 1 is reference, tertile 2 (OR: 2.65; 95% CI: 1.16-6.09), tertile 3 (OR: 6.01; 95% CI: 2.53-14.30), p-trend < 0.001. A case-control study in Netherland (63) showed that mothers having pyridoxal-5'- phosphate (PLP) level  $\leq$  44 nmol/L increased risk of OFCs (OR: 2.9; 95% CI: 1.2-7.1), and the risk increased in mothers without periconceptional supplement (OR: 16.4; 95% CI: 1.8-152.2). However, the association between vitamin B6 status and OFCs was not found in a case-control study in Utah, the United States (48).

#### **viii) Vitamin B12 nutritional status**

Vitamin B12 (cobalamin) functions as a coenzyme in the synthesis of methionine by methylation of homocysteine to methionine and demethylation of N<sup>5</sup>-methyl-tetrahydrofolate to tetrahydrofolate. In addition, vitamin B12 is a coenzyme of methylmalonyl-CoA mutase. Vitamin B12 is involved in the metabolism of fatty acids and amino acids, and affecting DNA synthesis and regulation(46). Lu et al. conducting a mouse study reported that vitamin B12 prevented the dexamethasone-induced cleft palate (64). However, Zho et al. found no association between vitamin B12 and cleft palate induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and dexamethasone (65).

A case-control study in Netherland by Krapels et al. (62) found that increased dietary intake of vitamin B12 increased risk of OFCs with

marginally statistical significance (quintile 1: 2.20-4.13  $\mu\text{g}/\text{day}$  is reference, quintile 2: 4.14-4.92  $\mu\text{g}/\text{day}$  (OR: 0.88; 95% CI: 0.44-1.76), quintile 3: 4.92-5.53  $\mu\text{g}/\text{day}$  (OR: 1.30; 95% CI: 0.66-2.54), quintile 4: 5.53-6.24  $\mu\text{g}/\text{day}$  (OR: 1.18; 95% CI: 0.60-2.35), quintile 5: 6.27-42.92  $\mu\text{g}/\text{day}$  (OR: 1.79; 95% CI: 0.90-3.56),  $p$ -trend = 0.06). Another case-control study in Netherland by Van Aooil et al. (63) reported that that mothers having low serum vitamin B12 levels ( $\leq 185$  pmol/L) increased risk of OFCs (OR: 3.1; 95% CI: 1.3-7.4 for total group and OR: 4.4; 95% CI: 1.1-18.2 for none periconceptional supplement.

#### **ix) Zinc nutritional status**

Zinc plays role in the absorption of folate by polyglutamate hydrolase, a zinc-dependent enzyme, and is involved in the conversion of 5-methyltetrahydrofolate into tetrahydrofolate by the zinc- dependent methionine synthase enzyme. Maternal zinc deficiency can disrupt the normal function of trophoblasts, the production and secretion of hormones, establishment of the maternal-fetal barrier, and the mediation of metabolic exchanges across the maternal-fetal barrier (66). A Dutch study (67) used a food frequency questionnaire to assess nutrient intake and compare it between mothers of OFCs children and the control group mothers. This study reported that zinc intake in mothers in the control group was statistically significantly higher than mothers of the OFCs group.

A case-control study in Philippines (68) showed the correlation between poor maternal zinc nutritional status and increased risk of OFCs. The mean level of plasma zinc concentration in the control group was higher ( $10.1 \pm 1.6 \mu\text{mol/l}$ ) than in the group of mothers with CL/P children ( $9.6 \pm 1.2 \mu\text{mol/l}$ ) with statistical significance and the group of mothers with CP children ( $9.4 \pm 1.1 \mu\text{mol/l}$ ) without statistical significance. In addition, after adjusting for potential confounding factors, increasing quartile of plasma zinc was associated with a decreased the risk of both CL/P (quartile 1:  $\leq 8.9 \mu\text{mol/l}$  is reference, quartile 2:  $9.0-9.8 \mu\text{mol/l}$  (OR: 0.95; 95% CI: 0.38-2.35), quartile 3:  $9.9-10.9 \mu\text{mol/l}$  (OR: 0.81; 95% CI: 0.33-1.97), quartile 4:  $\geq 11.0 \mu\text{mol/l}$  (OR: 0.32; 95% CI: 0.11-0.92); p-trend 0.037) and CP (quartile 1:  $\leq 8.9 \mu\text{mol/l}$  is reference, quartile 2:  $9.0-9.8 \mu\text{mol/l}$  (OR: 0.65; 95% CI: 0.16-2.68), quartile 3:  $9.9-10.9 \mu\text{mol/l}$  (OR: 0.27; 95% CI: 0.05-1.45), quartile 4:  $\geq 11.0 \mu\text{mol/l}$  (OR: 0.07; 95% CI: 0.01-0.73); p-trend 0.007). The case-control study in Poland and the Netherlands presented a similar correlation between concentrations of zinc and risk of OFCs (69, 70). However, the study in Utah with a much higher level of zinc status did not present the association between maternal plasma zinc concentration and OFCs (71).

#### x) **Maternal supplement use**

Maternal use of multivitamin supplements in early pregnancy has been linked to decreased risk of OFCs; in a meta-analysis on overall 25%



reduction in birth prevalence of OFCs with multivitamin use was reported (72). Johnson and Little reported the negative association between maternal multivitamin use and both CL/P (OR: 0.75, 95% CI: 0.65-0.88) and CP (OR: 0.88, 95% CI: 0.76-1.01) (73).

Folic acid supplementation has been recommended to reduce the risk of OCFs although the evidence is still controversial. A case-control study in the Netherlands reported that higher folate supplementation lowered risk of CL/P (quartile 1: 152 µg/day is reference, quartile 2: 178 µg/day (OR: 0.74; 95% CI: 0.40-1.37), quartile 3: 196 µg/day (OR: 1.73; 95% CI: 0.39-1.35), quartile 4: 213 µg/day (OR: 0.63; 95% CI: 0.32-1.23), quartile 5: 242 µg/day (OR: 0.54; 95% CI: 0.27-1.05); p-trend 0.06) with marginal statistical significance (47). Data from the Hungarian Congenital Anomaly Registry showed that the high dose of folic acid (6 mg) in the first month of gestation reduced only the risk of CP (OR: 0.50, 95% CI: 0.68-0.96 for CP and OR: 0.89, 95% CI: 0.67-1.20 for CL/P) (74). Studies in China from different cities (Heilongjiang and Xuzhou) reported the protective effect of folic acid (Heilongjiang Province: OR: 0.43, 95% CI: 0.21-0.88 for CL/P and OR: 0.69, 95% CI: 0.28-1.69 for CP and Xuzhou city: OR: 0.23, 95% CI: 0.10-0.55 for OFCs) and multivitamin (Heilongjiang Province: OR: 0.04, 95% CI: 0.01-0.11 for CL/P and OR: 0.08, 95% CI: 0.02-0.25 for CP and Xuzhou city: OR: 0.16, 95% CI: 0.04-0.58 for OFCs) supplement during preconception period on OFCs (25,

75). These studies found a weaker association between high dose of folic acid containing multivitamin in the second month and risk of OFCs (OR: 0.82, 95% CI: 0.64-1.03 for CL/P and OR: 0.70, 95% CI: 0.48-1.02 for CP). The protective effect of folic acid containing multivitamin on risk of OFCs was supported by a case-control study in Norway (76) and the United States (77). However, later data from the National Birth Defects Prevention Study (NBDPS) found no association between maternal use of supplement containing folic acid and risk of CL/P (OR: 1.01; 95% CI: 0.82-1.24) and CP (OR: 1.02; 95% CI: 0.77-1.34) (78).

Moreover, a study in the United States presented that food fortification programs with folic acid decreased the prevalence of orofacial clefts (prevalence ratio 0.94, 95% CI: 0.92-0.96) (79). A retrospective population-based study in Canada reported food fortification program, cereal grain products fortified with folic acid, did not decrease the prevalence of orofacial clefts (prevalence ratio 1.06, 95% CI: 0.86-1.30) (80).

#### **xi) Maternal obesity**

A cohort study in Sweden presented a positive association between maternal obesity and the occurrence of OFCs. Maternal BMI greater than 29 increased the risk of OFCs 31 % after adjusting the potential variables (81). Blomberg and Kallen provided a similar correlation between maternal overweight (BMI 25-29.9) and obesity (BMI  $\geq$  30) and orofacial

clefts (adjusted OR: 1.15, 95% CI: 1.04-1.28 and adjusted OR: 1.26, 95% CI: 1.09-1.95; respectively) (82). The international consortium of case-control studies, Utah, Iowa, Norway, and the U.S. National Birth Defects Prevention Study, found that maternal obesity (pre-pregnancy BMI >35) increased the risk of isolated CLP and CP except CL (OR: 1.30, 95% CI: 1.05-1.60 for CLP, OR: 1.29, 95% CI: 1.02-1.64 for CP, and OR: 1.03, 95% CI: 0.78-1.37 for CL) when compared to mothers with normal weight (83). These studies found increased BMI increased risk of all cleft palate (p-trend = 0.004) but not isolated cleft lip. A study in Washington State also reported the same BMI result as the former studies (adjusted OR: 1.26, 95% CI: 1.03-1.55) (84). Moreover, the meta-analysis from 18 articles showed that maternal obesity increased risk of both cleft palate (OR: 1.23, 95% CI: 1.03-1.47) and cleft lip and palate (OR: 1.20, 95% CI: 1.03-1.40) (85).

### **xii) Diabetes mellitus (pre-gestational and gestational)**

Many studies have reported the association between maternal diabetes and the risk of orofacial cleft in offspring. Spilson et al. investigated the 1996 National Center for Health Statistics States Natality database and reported that diabetic mothers increased the risk of CL/P, (adjusted OR 1.35, 95% CI: 1.00-1.82; P<0.05) (86). Carinci et al. reported the positive association between familial diabetes and isolated cleft palate (P=0.0014) (87). Data from a large international consortium

from the U.S., Denmark, and Norway confirmed that maternal diabetes increased the incidence of OFCs (OR 1.33, 95% CI: 1.14-1.55) after adjusting for maternal age, education levels, multivitamin use, maternal BMI categories, and history of smoking (88). A few studies reported that maternal diabetes increased birth defects including orofacial clefts. The Atlanta Birth Defects Case-Control Study (89) evaluated the risk of malformations from diabetic pregnancy. The study showed that being an insulin-dependent diabetic mother increased the risk of cleft palate (RR: 23.7, 95% CI: 3.1-183.1). Correa et al. analyzed the data from the National Birth Defect Prevention Study (NBDPS) and reported an association between maternal diabetes mellitus and both isolated defects and multiple defects of CL/P and CP (90). Both pre-gestational (type 1 or 2) and gestational diabetes mellitus increased the risk of isolated CL/P (OR: 2.93, 95% CI: 1.45-5.87 and OR: 1.45, 95% CI: 1.03-2.04, respectively) and CP (OR: 1.80, 95% CI: 0.67-4.87 and OR: 1.54, 95% CI: 1.01-2.37, respectively).

Data from the a case-control study of Utah births during 1995-2005 (83) showed that GDM increased the risk of both isolated (OR: 2.63, 95% CI: 1.30-5.34) and non-isolated clefts (OR: 2.66, 95% CI: 1.02-6.97).

#### **b) Established Genetic factors**

Jugessur et al. reported that the majority of OFCs are isolated OFCs (around 70% of all CLP cases and around 50% of all CPO) (91). Syndromic and

multiple birth defects make up the minority of OFCs. Around 75% of syndromic orofacial clefts can be described by known genetic conditions including: Van der Woude syndrome, Bamforth–Lazarus syndrome, Kabuki syndrome, Pierre Robin syndrome, and Treacher Collins syndrome (7).

Genetic studies have reported genes related to syndromic OFCs and provided clear associations between cleft phenotypes and the mutations of genes. However, some published genes for isolated OFCs often remain with questions of genetic etiology and there is need for more research to confirm and explain these associations (91).

**i) IRF6 (Interferon regulatory factor 6)**

Nine interferon regulatory factors have been identified in humans. Interferon regulatory factor family relates to innate immunity and interferon signaling, while IRF6 is essential for normal epidermal differentiation and development. In both vitro and vivo studies found that IRF6 suppressed growth and promoted differentiation of keratinocytes, cell type in the epidermis, and mammary carcinoma cells (92).

IRF6 has been reported the association with both syndromic and isolated OFCs. IRF6 was found to be the cause of Van der Woude syndrome and Popliteal Pterygium syndrome (93). Zuccherro et al. (94) confirmed the association between IRF6 and non-syndromic CL/P in a large study of 10 populations with ancestry in Asia, Europe, and South America. Subsequent candidate gene studies confirmed the correlation of

IRF6 with non-syndromic CL/P in Belgian (95), Taiwanese (96), and Norwegian populations (97). The results from genome-wide linkage studies (98) and genome-wide association studies (99-101) also reported similar result with candidate gene studies.

Mouse models have also demonstrated the role of IRF6 showing that *Irf6* mutant mice presented a hyper-proliferative epidermis causing failure to undergo terminal differentiation. The hyper-proliferative epidermis causes multiple epithelial adhesions which can occlude the oral cavity and lead to a cleft palate (102, 103).

**ii) MAFB (v-maf musculoaponeurotic fibrosarcoma oncogene homolog B)**

MAFB gene encodes a basic leucine zipper transcription factor which regulates lineage-specific hematopoiesis. MAFB is expressed in the palate shelves and the medial edge epithelia during palate formation (101). Moreover, MAFB regulates the functions of establishing, differentiating, and developing the function of cells, tissues and organs, including pancreatic alpha and beta islets (104), which may be related to diabetes.

Genome-wide association studies in Chinese Han (105), Hispanic (106), European and Asian (101) populations reported an association between MAFB and non-syndromic orofacial clefts. However, no association was found in a Brazilian population (107).

**iii) ABCA4 (ATP-binding cassette, sub-family A (ABC1), member 4)**

ABCA4 gene is a member of ATP-binding cassette (ABC) family.

The ABCA4 gene plays an obvious role in retina photoreceptor cells.

ABCA4 accelerates the clearance of all-*trans*-retinal to all-*trans*-retinol by translocating all-*trans*-retinal from the luminal to the cytoplasmic side of the disk membrane. ABCA4 mutation had strong association with vision disease such as Stargardt disease, cone-rod dystrophy, and autosomal recessive retinitis pigmentosa (108). The function of ABCA4 in other organs is unknown.

The genome-wide association study in the GENEVA Cleft Consortium provided the statistical evidence of linkage and association between ABCA4 and non-syndromic CL/P (101). This evidence was supported with other genome-wide association studies in other populations such as Hispanic (106) and Brazilian (107) populations. However, the genome-wide association study in a Chinese Han population was not associated with the risk of non-syndromic orofacial clefts (105).

**iv) 8q24**

8q24 is a 640-kb region on chromosome 8 with no well-annotated genes in this interval. 8q24 has been associated with non-syndromic CL/P and many types of cancer such as breast (109, 110), prostate (111-113), bladder (114), colon (115-117), lung (118), ovarian (119), pancreatic

(120), and brain (121) cancer. The 8q24 gene was strongly associated with non-syndromic CL/P in GWAS (99, 100).

**v) FOXE1 (Forkhead box E1)**

FOXE1, a member of the forkhead family, is a thyroid-specific transcription factor. FOXE1 is essential for the development and differentiation of thyroid, and the maintenance of thyroid differentiated state in adults (122). FOXE1 expression was observed in the rostral epithelium of the oral pharynx, the caudal epithelium of the nasal and maxillary processes, and epithelium involved in the fusion between the medial nasal and maxillary processes, which represents the role of FOXE1 in lip development (123). FOXE1 mutation is also associated with thyroid agenesis, cleft palate, and choanal atresia (124). Moreno et al. reported that FOXE1 had a significant association with non-syndromic orofacial clefts (123). This association was confirmed by the subsequent candidate gene study in European (125, 126), Thai (127), Arab (128) populations. The GWAS in the Hispanic population also reported the association (106).

**vi) VAX1 (Ventral anterior homeobox 1)**

VAX1 is a transcription factor containing a DNA-binding homeobox domain in developing anterior ventral forebrain (129). Mouse studies found that VAX1 related to forebrain development and neuronal differentiation (129), and VAX1 mutations affected the development of basal forebrain and the formation of visual system (130).



With GWAS, Mangold et al. and the GENEVA Cleft Consortium reported the association between VAX1 and risk of non-syndromic CL/P (101, 131). Figueiredo et al. reported VAX1 was associated with the risk of orofacial clefts in the Southeast Asian population, but not in the African population (132). An animal study found that the mouse knockout for *Vax1* developed cleft palate and VAX1 was expressed in developing craniofacial structures (130)

#### **vii) Other genes**

The associations between the risk of orofacial clefts and the gene related to folate metabolism (MTHFR: Methylenetetrahydrofolate gene, MTHFD: Methylenetetrahydrofolate dehydrogenase gene, MTR: 5-Methyltetrahydrofolate-homocysteine methyltransferase, MTRR: Methionine synthase reductase, RFC1: Reduced folate carrier 1, FOLR: Folate receptor, BHMT: Betaine-homocysteine methyltransferase, and CBS: Cystathionine beta-synthase) is still controversial (133). In addition, no GWAS has reported the association between OFCs and diabetes-related genes. Published genes associated with OFCs have various function, only MAFB gene has function on pancreatic alpha and beta cells, which may related to diabetes.

#### **c) Gene-environment interaction**

Many studies have suggested that the development of CL/P and CP results from interaction of genetic and maternal environmental exposures.

Indeed, interaction between maternal exposures and specific allelic variants may have more significant relevance for the occurrence of CL/P and CP than studies in maternal exposures or genes alone.

Maternal smoking has been associated with the increased risk of CL/P due to interference of genes in the metabolic pathways related to CLP. GSTT1 (glutathione S-transferase theta), NOS3 (nitric oxide synthase 3), MSX1 (Msh homeobox homolog1), TGFA (transforming growth factor alpha) and TGFB3 (transforming growth factor beta 3) genes influence the risk of orofacial clefts in infants of mothers who smoked during the peri-conceptual period (134-138). Beaty et al. (139) reported GRID2 (glutamate receptor, ionotropic, delta 2) and ELAVL2 (ELAV like neuron-specific RNA binding protein 2) provided the strong evidence for a gene-smoking interaction among European maternal smoker but those did not show significant evidence of genotypic effects alone. Infants with allelic variants at the MSX1 (Msh homeobox homolog1) site had significantly elevated risk of CL/P with maternal alcohol consumption ( $\geq 4$  drinks/month) and CP with maternal smoking ( $\geq 10$  cigarettes/day) (140). Offspring polymorphism of BMP4T538C (bone morphogenetic protein 4) was associated with an increased risk of non-syndromic CL/P for maternal passive smoking, and a decreased the risk of CL/P for maternal multivitamin use (141). Jia et al. (142) stated that IRF6 (Interferon regulatory factor 6) provided a similar result to BMP4T538C. The presence of ADH1C (alcohol dehydrogenase 1C) is associated with risk of orofacial clefts that mothers consume alcohol (143).

Moreover, CBS and MTHFD2L in Asian population and DHFR, MMAA, MTR, and TCN2 in European population show association between orofacial clefts and maternal multivitamin use (144).

## 2.2 Diabetes Mellitus

### 2.2.1 Overview

Diabetes Mellitus is a disorder of the regulation of blood glucose. Hyperglycemia is a characteristic of diabetes mellitus resulting from a defect in insulin secretion or insulin action or both. Diabetes mellitus can be divided into four groups as follows (145):

#### a) Type 1 diabetes mellitus

Type 1 diabetes mellitus, accounts for around 5% of all diabetes cases, and results from a cellular-mediated autoimmune destruction of  $\beta$ -cells of the pancreas. Patients with type 1 diabetes mellitus are typically diagnosed as children or young adults.

#### b) Type 2 diabetes mellitus

Type 2 diabetes mellitus is the most common type of diabetes (90-95%). This type of diabetes results from insulin resistance or insulin deficiency or both, which is caused by genetic and lifestyle factors. The most significant factors leading to type 2 diabetes mellitus are overweight, abdominal obesity, and physical inactivity. Many patients with type 2 diabetes mellitus are undiagnosed for many years because of no significant signs and symptoms during the early stages of the disease.

### c) Gestational diabetes mellitus (GDM)

GDM is defined as glucose intolerance that begins or is first recognized during pregnancy. The cause of GDM is not clear, but it has been suggested that it is from the effect of placental and adipose tissue hormones. Placental hormones such as human placental lactogen, progesterone, cortisol, placental growth hormone and prolactin decreases phosphorylation of insulin receptor substrate 1, which leads to insulin resistance. Decreased adiponectin and increased tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and leptin during pregnancy are also associated with insulin resistance during pregnancy.

### d) Other specific types

In addition to the causes of type 1 and type 2 diabetes mellitus and gestational diabetes mellitus, there are many causes that can lead to diabetes mellitus.

- i) Genetic defects of  $\beta$ -cells function result in impaired insulin secretion because  $\beta$ -cells lose the function to convert pro-insulin to insulin. (e.g. maturity-onset diabetes of the young (MODY), mitochondrial disorders, et al.).
- ii) Genetic defects in insulin action result from the mutation of insulin receptor, which leads to hyperinsulinemia and hyperglycemia. (e.g. Leprechaunism, congenital lipotrophic disorders). Moreover, other genetic syndromes associated with diabetes include chromosomal abnormalities such as Prader-Willi syndrome, Wolfram's syndrome, Turner syndrome, etc.).

- iii) Diseases of the exocrine pancreas such as pancreatitis, trauma, pancreatectomy, neoplasia, fibrocalculous pancreatopathy, pancreatic carcinoma, et al., result in damaged  $\beta$ -cells. Decreased  $\beta$ -cells in the pancreas lead to reduced insulin secretion.
- iv) Endocrinopathies related to an excess amount of hormones antagonizing insulin action such as growth hormone, cortisol, glucagon, epinephrine, etc. (e.g. acromegaly, Cushing's syndrome, thyrotoxicosis).
- v) Drug- or chemical-induced diabetes occurs when drugs and chemicals impair insulin action or damage  $\beta$ -cells. (e.g. glucocorticoids, thiazides, phenytoin, antiretroviral therapy).
- vi) Infections, especially viral infections, destroy  $\beta$ -cells. (e.g. congenital rubella, Cytomegalovirus, HIV/AIDS, coxsackievirus B, mumps, et al.).

Uncommon forms of immune-mediated diabetes are the autoimmune disorder interfering with the insulin receptor, such as anti-insulin receptor antibodies.

### 2.2.2 Criteria for diagnosis of type 2 diabetes mellitus

The American Diabetes Association updated the criteria for the diagnosis of type 2 diabetes mellitus in 2010 (145). Persons having HbA1c  $\geq 6.5\%$  or Fasting Plasma Glucose (FPG)  $\geq 126$  mg/dl (7.0 mmol/l) or 2-hr plasma glucose  $\geq 200$  mg/dl (11.1 mmol/l) during an oral glucose tolerance test (OGTT) or random plasma glucose  $\geq 200$  mg/dl with hyperglycemic symptoms is diagnosed as having diabetes mellitus. In 2006 the World Health Organization (WHO) and the International Diabetes Federation (IDF)(146) recommended Fasting Plasma Glucose (FPG)  $\geq 126$  mg/dl (7.0 mmol/l) or 2-

hr plasma glucose  $\geq 200$  mg/dl (11.1 mmol/l) as the diagnostic criteria for diabetes. Moreover, HbA1c  $\geq 6.5\%$  was recommended in 2011 by WHO for diagnosing diabetes mellitus (147). WHO/IDF suggested Fasting Plasma Glucose (FPG)  $\geq 126$  mg/dl (7.0 mmol/l) and 2-hr plasma glucose  $\geq 140$  and 200 mg/dl (7.8-11.1 mmol/l) as the diagnostic criteria for Impaired Glucose Tolerance (IGT), and Fasting Plasma Glucose (FPG)  $> 110$ -125 mg/dl (6.1-6.9 mmol/l) for Impaired Fasting Glucose (IFG) (146).

### 2.2.3 Criteria for diagnosis of GDM

The American Diabetes Association (148) proposed one-step and two-step approaches for diagnosing GDM. The one-step approach is based on a 75 gram OGTT at 24-28 weeks of gestation with fasting. The diagnosis of GDM is made when the plasma glucose level  $\geq 92$  mg/dl (5.1 mmol/L) when fasting, or  $\geq 180$  mg/dl (10.0 mmol/L) at 1 hour after loading, or  $\geq 153$  mg/dl (8.5 mmol/L) at 2 hours after loading. In addition, the two-step approach is based on 50 gram at 24-28 weeks of gestation with non-fasting. If the plasma glucose level at 1 hour after glucose loading is equal to or over 140 mg/dl (7.8 mmol/L), 100 gram OGTT is required. The diagnosis of GDM is made if at least two of the four measured plasma glucose levels meet the criteria. The GDM criteria for 100 gram OGTT is  $\geq 95$  mg/dl (5.3 mmol/L) at fasting,  $\geq 180$  mg/dl (10.0 mmol/L) at 1 hour after loading,  $\geq 155$  mg/dl (8.6 mmol/L) at 2 hours after loading, and  $\geq 140$  mg/dl (7.8 mmol/L) at 3 hours after loading. By the 2006 WHO criteria for diabetes (149), the diagnosis of GDM is made if at least one criteria is met  $\geq 126$  mg/dl (7.0 mmol/L) at fasting, and  $\geq 140$  mg/dl (7.75 mmol/L) at 2 hours after 75 gram OGTT loading.

### 2.2.3 Epidemiology of diabetes mellitus

The International Diabetes Federation (IDF) reported that the global prevalence of diabetes was 366 million in 2011 and estimated that the prevalence will be 552 million in 2030 (150). Additionally, the IDF estimated the global prevalence of diabetes in 2035 will be 600 million, which will be a 57% increase over the projection of 382 million for 2013 (151). Studies from China, Japan, and Sweden, supported the projected increase in the prevalence of diabetes (152-154). Men show a higher prevalence of diabetes than women in some but not all studies (198 million for men and 184 million for women) (155). The Chinese study by Wang C also reported that the prevalence of diabetes was higher in males (42.75%) compared to females (26.9%) in 2012 (153). However, a study done in Southern Iraq reported the prevalence of diabetes in women to be slightly higher than for men (52.6% for women and 47.3% for men) (156).

The National Diabetes Statistics Report (157) released in June 2014 stated that in 2012, 29.1 million or 9.3% of the American population had diabetes, which is a 12.8% increase over 2010. Moreover, it is estimated that 8.1 million (27.8%) people have undiagnosed diabetes. In the American population, Indians/Alaskan Natives have the highest rate of diabetes (15.9%) followed by non-Hispanic blacks (13.2%) and Hispanics (12.8%). The IDF (151) reported that the western pacific region has the highest number of people with diabetes, but the diabetic population in Africa, the Middle East and North America, and South-East Asia will increase 100%, 96%, and 71% respectively by 2035. The Hispanic Community Health Study/Study of Latinos (HCHS/SOL) reported that the prevalence of diabetes was 10.2% in South Americans and 13.4% in Cubans, increasing

to 17.7% in Central Americans, 18.0% in Dominicans and Puerto Ricans, and 18.3% in Mexicans. The prevalence of diabetes is negatively associated with educational levels and household incomes (158).

The percentage of women suffering from GDM is close to type 2 diabetes mellitus prevalence. The IDF (151) reported the global prevalence of hyperglycemia in pregnancy is 16.9%. The International Association of Diabetes and Pregnancy Study Groups (IADPSG), an international consensus group with representatives from multiple obstetrical and diabetes organizations, including the American Diabetes Association (ADA) also reported more than 200,000 GDM cases each year, or around 7% of all pregnancies (ranging from 1 to 14%, depending on the population and the diagnostic criteria) were diagnosed with GDM (159). The South-East Asian regions have the highest prevalence (25%) while the lowest prevalence is found in North America and the Caribbean (10.4%). In addition, the prevalence of GDM in Haryana, India, was 13.9%, which is higher than the prevalence in the United States (8.5%) during 2009-2010 (160). The GDM prevalence in Australia in 2000-2009 was 5.1% for the indigenous population and 4.5% for the non-indigenous, an increase from 1999 (154). The results of these studies show that the majority of cases of hyperglycemia during pregnancy occurred in low- and middle-income countries.

#### **2.2.4 Risk factors of diabetes mellitus**

##### **a) Age**

A Turkish study reported on the positive correlation between maternal age and the incidence of type 2 diabetes. The incidence of individuals over age 50



developing type 2 diabetes is higher than it is for individuals between the ages of 20-49 years old (OR: 4.53, 95% CI: 1.98-10.33, P 0.0003) (161). The Tromsø study reported that increased age increased the risk of diabetes (OR: 1.3, 95% CI: 1.1-1.5) (162).

A positive correlation between age and risk of GDM has been reported, similar to the correlation seen in type 2 diabetes. IDF reported that the age of the mother is associated with the prevalence of hyperglycemia in pregnancy; mothers over 45 years old having the highest prevalence (47.7%) (151). The Nurses' Health Study II presented similar results (163). Increased maternal age increased the risk of GDM (age  $\geq$  40 years vs 25-29 years, RR: 2.24, 95% CI 1.26-3.98, p-trend  $<0.01$ ). A Chinese study by Yang H reported that, when compared to mothers less than 30 years old, mothers age 30 years or older had an increased risk of GDM (OR: 2.18, 95% CI: 1.88-2.52) (164). This is similar to the results of a study done in Iran (OR: 3, 95% CI: 1.8-5) (165).

#### **b) Family history**

A study in Japan examined the incident risk of type 2 diabetes over a 7 year period. The study showed that participants with a family history of diabetes had an increased risk of diabetes when compared with participants without a family history of diabetes (HR 1.82; 95% CI: 1.36-2.43) (166). Moreover, participants with only the mother having diabetes showed a higher rate of type 2 diabetes than those with a family history of diabetes (HR: 2.6; 95% CI: 1.71-3.97). The MONICA (Monitoring of Trends and Determinants in Cardiovascular

Disease) Augsburg cohort study reported a significant association between family history of diabetes and the risk of diabetes (167).

A family history of diabetes has also been reported as a risk factor for GDM (168). Pregnant women with a history of diabetes in their family have a higher risk of GDM when compared to women without a family history of diabetes (RR: 1.68, 95% CI: 1.39-2.04) (163). Moreover, data from the National Health and Nutrition Examination Survey III, provided similar results (169). Women with maternal, paternal, or sibling histories of diabetes had an increased risk of developing GDM (OR: 3.0, 95% CI: 1.2- 7.3; OR: 3.3, 95% CI: 1.1-10.2; OR: 7.1, 95% CI: 1.6 -30.9, respectively). A history diabetes in family increased risk of type 2 diabetes and GDM.

### c) **Body weight**

Many studies have reported the positive correlation between body weight and risk of type 2 diabetes. The Nurses' Health Study reported that both overweight (BMI 25-30 kg/m<sup>2</sup>) and obese (BMI ≥ 30 kg/m<sup>2</sup>) individuals showed a statistically significant increase in the risk of type 2 diabetes (170). The Finnmark study followed subjects for 12 years and found that increased BMI increased risk of type 2 diabetes in both genders (BMI ≤ 27 as a reference, BMI ≥ 35 kg/m<sup>2</sup> RR: 27.89; 95% CI: 12.27-63.42 for men; RR: 11.07, 95% CI: 4.63-26.46 in women) (171). Studies in Lebanon and Qatar also found that mother having BMI ≥ 30 kg/m<sup>2</sup> increased the risk of type 2 diabetes (OR: 2.29, 95% CI: 1.74-3.02, and OR: 1.5, 95% CI: 1.2-1.9, respectively) (172, 173).

Pre-pregnancy bodyweight has been shown to have a strong association with the risk of GDM. A meta-analysis study (174) pooling the data from 70 studies, also revealed that when compared with women of normal weight (20-24.9kg/m<sup>2</sup>), the odds ratio for overweight (25-29.9≤ 30 kg/m<sup>2</sup>), moderate obesity (30-34.9kg/m<sup>2</sup>), morbid obesity (≥ 35 kg/m<sup>2</sup>) were 1.97 (95% CI 1.77- 2.19), 3.01 (95% CI 2.34-3.87) and 5.55 (95% CI 4.27- 7.21) respectively. Moreover, the study also presented a protective effect of being underweight (≤ 20 kg/m<sup>2</sup>) on the incidence of GDM (OR: 0.75, 95% CI: 0.69-0.82). A retrospective study in Poland (175) reported that increased BMI significantly increased the risk of GDM (BMI > 35 kg/m<sup>2</sup> vs BMI 18.5-20.9 kg/m<sup>2</sup>, OR 9.01, 95% CI 3.47-23.3, p-trend 0.027). Many studies have been confirmed that high pre-pregnancy BMI increases the risk of GDM (164, 165, 168, 176-179). Nevertheless, a study in Thailand (180) reported the obese women did not have an increased risk of GDM when compared with normal weight women (RR: 0.9, 95% CI: 0.6-1.4). Individuals with overweight or obesity have a tendency to increase risk of type 2 diabetes and GDM.

#### **d) Hypertension**

The 8 year follow up of the San Antonio Heart Study, reported that participants who converted to non-independent diabetes mellitus had higher systolic blood pressure than subjects without diabetes in both men and women, the results were statistically significant (181). The Augsburg study showed a similar correlation between high blood pressure and the risk of diabetes. The

study stated that diabetic subjects had both higher systolic and diastolic blood pressure than non-diabetic subject (167). A study in Norway showed that subjects developing diabetes had higher systolic blood pressure than non-diabetics (162). A study from Qatar reported the positive association of type 2 diabetes with systolic blood pressure (SBP) (OR = 1.5, 95% CI = 1.2 – 2.0 for SBP 120–139 mmHg, OR = 2.2, 95% CI = 1.6-3.1 for SBP 140–159 mmHg, and OR = 3.2, 95% CI = 2.0 – 5.3 for SBP > 160 mmHg) (172). In addition, the study in Western Asia, Lebanon, also suggested that hypertension is a predictor of type 2 diabetes (OR: 1.75, CI: 1.54-2.00, p-value <  $2 \times 10^{-16}$ ) (173). The association between high blood pressure and GDM has not been reported. Hypertension is strongly associated with diabetes. Both hypertension and type 2 diabetes are related to metabolic syndrome, and have common risk factors (obesity, diet, and inactivity).

#### e) **Smoking**

Many cohort studies have reported the positive association between smoking and the risk of type 2 diabetes and GDM. The studies showed that tobacco smoking was correlated with the impairment of glucose metabolism, insulin sensitivity, and insulin secretion (182, 183). Willi et al. (184) analyzed 25 cohort studies and reported that current smoking increases the risk of type 2 diabetes (OR: 1.44, 95% CI: 1.31-1.58). Moreover, heavy smokers ( $\geq 20$  cigarettes/day) showed the highest incidence of diabetes (OR: 1.61, 95% CI: 1.43-1.80) when compared to lighter smoking (<20 cigarettes/day) (OR: 1.29, 95% CI: 1.13-1.48) and former smokers (RR: 1.23, 95% CI: 1.14-1.33). The high

incidence of type 2 diabetes in heavy smokers was confirmed by studies in Japan (OR: 1.37, 95% CI: 1.05-1.80)(185), Finland (OR: 1.57, 95% CI: 1.34-1.84 in men and OR: 1.87, 95% CI: 1.36-2.59 in women) (186), and China (OR: 1.25, 95% CI: 1.00-1.56) (187). In addition, Kim et al. reported that early onset of smoking increased the risk of type 2 diabetes in both Korean and US populations (188).

However, some studies showed that the risk of type 2 diabetes increased after 3-5 years of smoking cessation (189-191). As a consequence, smoking cessation was associated with increased body weight, which is a risk factor of type 2 diabetes. However, increased duration of smoking cessation significantly decreased the risk of type 2 diabetes (191). A meta-analysis analyzing 4 cohort studies reported that passive smoking increased the risk of type 2 diabetes (OR: 1.28, 95% CI: 1.14-1.44)(192).

A prospective study in the United States (163) reported that women who smoked cigarettes had an increased risk of GDM (RR: 1.43, 95% CI: 1.14-1.80). A prospective cohort study in Massachusetts (193) showed that women who smoked prior to pregnancy had an increased risk of GDM when compared with non-smokers, but the results did not show statistical significance.

#### **f) Gestational diabetes mellitus (GDM) and subsequent risk of DM**

Women with previous GDM show increased risk of type 2 diabetes 17-63% in 5-16 years after diagnosis of GDM (194). A study in northwestern Ontario (195) reported that 70% of the women diagnosed with GDM developed type 2

diabetes, and the average duration from diagnosis of GDM to type 2 diabetes was three years. Sivaraman et al. (196) showed that women diagnosed with GDM had a significantly increased risk of type 2 diabetes, finding 6.9% at five years (95% CI: 3.8%-9.9%) and 21.1% at ten years (95% CI: 14.1%-27.5%) after diagnosis of GDM. Lui et al. (197) showed that increased postpartum body weight (>7 kgs) increased the risk of diabetes by 86% and pre-diabetes, impaired glucose tolerance or impaired fasting glucose, by 32%, whereas decreased postpartum body weight reduced the risk of pre-diabetes by 45%.

#### **g) Dietary intake**

The association between food intake and incidence of diabetes has been showed to have both positive and negative effects. The Nurses' Health Study (198) reported the inverse association between dietary fiber intake and risk of type 2 diabetes (RR=0.72, 95% CI, 0.58-0.90, P trend=.001), this was confirmed by the Health Professionals Follow-up Study (199), the Iowa Women's Health Study (200), and the Black Women's Health Study (201). The protective effect of fiber intake against GDM was reported in the Nurses' Health Study II, showing that the risk of GDM was 0.67 (RR: 0.67, 95% CI: 0.51-0.9) when comparing the highest quintile with the lowest quintile of total daily fiber intake (202). Moreover, each 10 g/day increase in total fiber intake decreased the risk of GDM 26% (RR: 0.74, 95% CI: 0.51-0.91). A study focusing on the association between fruit intake and the risk of GDM reported that whole fruit consumption reduced the GDM risk without statistical significance (203).

The association between total carbohydrate intake and the risk of type 2 diabetes is controversial (198, 199, 204-206). The Nurses' Health Study (207) reported a positive correlation between glycemic index and the risk of type 2 diabetes (p-trend 0.001), and no association between glycemic load and the risk of type 2 diabetes. However, the Health Professionals Follow-up Study (199), the Japan Public Health Center-based Prospective Study (208), and a study in China (204) reported that both glycemic index and glycemic load increased the risk of type 2 diabetes. A cohort study in a US population, The Health Professionals Follow-up Study and the Nurses' Health Study I and II, stated that the high white rice intake ( $\geq 5$  serving per week) increased the risk of type 2 diabetes (OR: 1.17, 95% CI: 1.02-1.36), while high brown rice intake ( $\geq 2$  serving per week) reduced the risk of type 2 diabetes (OR: 0.89, 95% CI: 0.81-0.97) (209). This result demonstrated the adverse effect of high glycemic index foods and the benefit of fiber on the incidence of type 2 diabetes. The correlation between carbohydrate intake and GDM was reported on a study by Bao W. and colleagues. The study found that a low carbohydrate dietary pattern, with high protein and fat from animals, has a negative association with the risk of GDM (p-trend 0.03) (210).

Additionally, the association between dietary fat intake (total, saturated, polyunsaturated, and monounsaturated fat) and occurrence of diabetes is inconsistent. The 14 year follow up of the Nurses' Health Study (211) reported the protective effect of polyunsaturated fat on type 2 diabetes; on the contrary, the Health Professionals Follow-up Study (199) reported no correlation between

polyunsaturated fat intake and the risk of type 2 diabetes. Bowers K. and colleagues (212) reported that increased total fat intake, especially animal fat increased the risk of GDM (p-trend 0.05). Analyzing the association between fatty acid intake and GDM risk revealed that MUFA and cholesterol significantly increased the risk of GDM (p-trend 0.04 for both MUFA and cholesterol). The Alpha Case-Control Study (213) also showed an association between consumption of cholesterol and the risk of GDM (Q4 ( $\geq 294$  mg/day) vs Q1 ( $< 151$  mg/day) OR: 2.35, 95% CI: 1.35, 4.09, p-trend 0.021).

High fiber and low carbohydrate intake reduce the risk of both type 2 diabetes and GDM. The association dietary fat intake and risk of diabetes is controversial.

#### **h) Dietary pattern**

The Multi-Ethnic Study of Atherosclerosis, MESA, (214) reported that a dietary pattern characterized by high intake of tomatoes, beans, refined grains, high-fat dairy, and red meat was associated with increased the risk of type 2 diabetes (OR: 1.18, 95% CI: 1.06-1.32, p-trend 0.004). This study showed that the pattern characterized by high intake of whole grains, fruit, nuts/seeds, green leafy vegetables, and low-fat dairy decreased the risk of type 2 diabetes (OR: 0.85, 95% CI: 0.76-0.95, p-trend 0.005). The insulin Resistance Atherosclerosis Study followed non-diabetics for five years and reported that food intake pattern (high intake of dried beans, low-fiber bread and cereal, fried potatoes, tomato



vegetables, red meat, eggs, and cheese and including low intake of wine) was associated with the risk of the type 2 diabetes (215).

A cohort study in the US showed an association between the western dietary pattern or a prudent dietary pattern and the risk of type 2 diabetes. The study showed that the western dietary pattern increased the incidence of type 2 diabetes (OR: 1.59, 95% CI: 1.32-1.93), while the prudent dietary pattern decreased the occurrence of type 2 diabetes (OR: 0.84, 95% CI: 0.70-1.00) (216). The effect of prudent dietary pattern on the risk of type 2 diabetes was confirmed by a study in Finland (217). The MESA study (218) reported that the Mediterranean dietary pattern (high consumption of monounsaturated fatty acids, fruits, vegetables, and whole grains as well as low fat dairy products intake) reduced insulin levels, but had no significant association with incidence of diabetes.

The effect of dietary pattern on the risk of GDM has been shown to be similar to the effect seen on type 2 diabetes risk (protective effects: Prudent diet and Mediterranean diet and negative effect: Western Diet). The Nurses' Health Study II (219) reported a protective effect of the prudent pattern (high intake of fruit, green leafy vegetables, poultry and fish) on the GDM risk (p-trend 0.018). In addition, the western dietary pattern, which is characterized by high intakes of red meat, processed meat, sugary desserts, high-fat foods, and refined grains, is positively associated with the risk of GDM (P-trend 0.0011). Tobias DK. and colleagues (220) examined the association between GDM risk and dietary

patterns, namely the alternate Mediterranean (aMED), Dietary Approaches to Stop Hypertension (DASH), and alternate Healthy Eating Index (aHEI). When comparing the highest with the lowest quartile of dietary pattern score, aMED, DASH, and aHEI significantly decreased the risk of GDM (RR: 0.76, 95% CI: 0.60-0.95, P-trend = 0.004 for aMED; RR: 0.66, 95% CI: 0.53-0.82, P-trend = 0.0005 for DASH; RR: 0.54, 95% CI: 0.43-0.68, P-trend < 0.0001 for aHEI). The cohort study in China (221) reported the protective effect of a vegetable dietary pattern on the risk of GDM (T3 vs T1: RR: 0.79, 95% CI: 0.64-0.97, p-trend 0.036). Moreover, sweets and seafood dietary patterns increased the risk of GDM (T3 vs T1: RR: 1.23, 95% CI: 1.02-1.49, p-trend 0.01).

Dietary patterns characterized by high fiber intake (whole grain, fruit, and vegetable), and low animal fat such as Prudent diet, DASH diet, and Mediterranean diet reduces risk of type 2 diabetes and GDM. The Western dietary pattern (low intake of fiber (fruit and vegetable) and high intake of red meat, high-fat foods, processed meat and sugar) increased the risk of diabetes.

#### **i) Physical inactivity**

Several studies have reported the protective effect of physical activity and the adverse effect of sedentary behaviors on the incidence of type 2 diabetes. The Nurses' Health Study (222) showed that sedentary behaviors increased the risk of type 2 diabetes. The studies showed that increased duration of sitting while watching television increased the risk of diabetes (0-1 hr/week is reference, OR: 1.22; 95% CI: 1.06-1.42 for 2-5 hrs/week), OR: 1.42; 95% CI: 1.24-1.63 for 6-20

hrs/week, OR: 1.65; 95% CI: 1.41-1.93 for 21-40 hrs/week, OR: 1.94; 95% CI: 1.51-2.49 for >40 hrs/week; p-trend <0.001), whereas increased standing or walking around at home decreased the risk (0-1 hr/week is reference, OR: 0.99; 95% CI: 0.79-1.24 for 2-5 hrs/week), OR: 0.87; 95% CI: 0.70-1.08 for 6-20 hrs/week, OR: 0.78; 95% CI: 0.63-0.97 for 21-40 hrs/week, OR: 0.77; 95% CI: 0.61-0.97 for >40 hrs/week; p-trend <0.001). A survey in Lebanon (173) showed that physical inactivity significantly increased the risk of type 2 diabetes. In addition, physical activity significantly decreased the risk of type 2 diabetes (OR: 0.55, 95% CI: 0.37-0.81 for moderate activity, OR: 0.46, 95% CI: 0.24-0.88 for heavy activity). The study in urban Shanghai, China (187) also showed a significant inverse association between the incidence of type 2 diabetes and metabolic equivalent values (METs), which is used to estimate intensity of physical activity. Physical activity reduces the incidence of type 2 diabetes by decreasing insulin resistance and improving insulin sensitivity (223).

However, the effect of physical activity on GDM development is controversial. The Nurses' Health Study II (224) reported that the physical activity score was inversely associated with the risk of GDM (p-trend 0.01). The study showed that brisk or very brisk walking 4 hours a week decreased the risk of GDM when compared with casual walking of the same duration (RR: 0.66, 95% CI: 0.46-0.95). However, the Australian Longitudinal Study found no association between physical activity and GDM risk (225). A case-control study determining the relation between recreational physical activity and risk of GDM (226) showed

an effect similar to brisk walking. For distances of  $\leq 2$  miles/day, brisk walking significantly reduced the GDM risk when compared with casual walking, but there was no significant protective effect in distances over 2 miles a day for either intensity. This study also reported that women participating in recreational physical activity during the year before pregnancy and during the first 20 weeks of pregnancy reduced the risk of GDM (OR: 0.45, 95% CI: 0.28-0.74 and OR: 0.52, 95% CI: 0.33-0.80 respectively). Physical activity can prevent diabetes both type 2 diabetes and GDM while physical inactivity increases the risk of diabetes.

## 2.3 Metabolic Syndrome

### 2.3.1 Overview

Metabolic syndrome is a major public health issue because it increases the risk of cardiovascular diseases (CVD), type 2 diabetes mellitus, stroke, etc. Metabolic syndrome is the grouping of visceral obesity, insulin resistance, hyperglycemia, dyslipidemia (hypertriglyceridemia and hypo-HDL cholesterolemia), and hypertension. The definitions of metabolic syndrome have been proposed by the World Health Organization (WHO), the National Cholesterol Education Program, Adult Treatment Panel III (NCEP/ATP III), the International Diabetes Federation (IDF), the American Heart Association/National Heart, Lung and Blood Institute (AHA-NHLBI), the American Association of Clinical Endocrinology (AACE), and the European Group for the Study of Insulin Resistance (EGIR) (Table 3.1). AACE proposed the definition of metabolic syndrome by using insulin resistance syndrome. The definition given by AACE can be applied to the

diagnosis of metabolic syndrome; relying on clinical judgment in non-diabetic patients.

Genetic, hormonal, and lifestyle factors lead to the development of the syndrome.

Risk of metabolic syndrome among obese persons can be split into two groups: metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUHO) (227). Metabolically healthy obesity (MHO) means obese people without metabolic abnormalities and have normal blood pressure, normal glucose tolerance, and normal lipid profiles. MHO results from the interaction between genetic, environmental, dietary, and behavioral factors associated with the accumulation and distribution of fat and insulin resistance (228). Subcutaneous adipose tissue of MHO individuals has the propensity to accumulate peripheral fat rather than visceral fat depots in MUHO which leads to insulin resistance (227). A study of weight-discordant monozygotic twins (229) reported that the obese co-twins in the  $\geq 2\%$  liver fat group presented significantly higher adipocyte volume, AUC insulin and glucose, LDL-cholesterol, leptin, and CRP and lower HDL-cholesterol than the non-obese co-twins. In the group with different liver fat  $<2\%$ , obese co-twin had higher adipocyte volume and leptin than lean co-twins. This study stated that the MHO group (those with a low percentage of liver fat), had less inflammation, while the excess liver fat group presented with insulin resistance, dyslipidemia, and inflammation. This study of discordance among monozygotic twins indicates a role for non-genetic factors influencing the risk of metabolic syndrome.

Metabolically unhealthy obesity is characterized by abdominal visceral fat disposition, visceral and ectopic fat accumulation, and insulin resistance (228). Visceral fat is intra-abdominal fat and a marker of ectopic fat which means the fat accumulating in

and around the organs in abdominal cavity including the heart (230). Abdominal obesity has an effect on metabolic processes by the intra-abdominal visceral fat which has higher lipolysis rate than any other depots. Abdominal adipose tissue elevates free fatty acids (FFA), cytokines (tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6)), adipokines, and angiotensin II. Excess FFA circulation induces insulin resistance, which results from reduced hepatic insulin clearance, decreased skeletal muscle insulin sensitivity, increased hepatic cholesterol production with elevated triglycerides and very low density lipoprotein (VLDL), and altered endothelial function. Elevated levels of cytokines also impair insulin sensitivity. A similar result is seen with decreased adiponectin level. Adiponectin has a protective effect by regulating lipid and glucose metabolism, controlling energy metabolism, and increasing insulin sensitivity (231, 232). Obesity increases not only adipose tissue but also the systemic renin angiotensin system, which increases angiotensin II circulation. Angiotensin II causes decreased insulin sensitivity and vasoconstriction (233).

Table 2.1 Definition of metabolic syndrome

Criteria	WHO <sup>1</sup> (234)	NCEP/ATP III <sup>2</sup> (235)	IDF <sup>3</sup> (236)
	Abnormal glycemia plus 2 or more other criteria	3 or more criteria	Abdominal obesity plus 2 or more other criteria
Abdominal obesity	BMI > 30 kg/m <sup>2</sup> and/or Waist to hip ratio > 0.9 (men) > 0.85 (women)	Waist circumference > 102 cm (men) > 88 cm (women)	Waist circumference for US population > 94 cm (men) > 80 cm (women)
Glucose	Insulin resistance or impaired glucose regulation or diabetes	Fasting plasma glucose > 110 mg/dL or previous diabetes	Fasting plasma glucose > 100 mg/dL or previous diabetes
HDL-C	< 35 mg/dL (men) < 39 mg/dL(women)	< 40 mg/dL (men) < 50 mg/dL(women)	< 40 mg/dL (men) < 50 mg/dL(women)
Hypertension	≥ 140/90 mmHg	≥ 130/85 mmHg	≥ 130/85 mmHg
Triglyceride	≥ 150 mg/dL	≥ 150 mg/dL	≥ 150 mg/dL
Other	microalbuminuria (urinary albumin excretion rate ≥ 20 µg/min or albumin:creatinine ratio ≥ 30 mg/g)		

Table 2.1 Definition of Metabolic Syndrome (Cont.)

Criteria	AHA-NHLBI <sup>4</sup> (237)	AACE <sup>5</sup> (238)	EGIR <sup>6</sup> (239)
		3 or more criteria	No specific number of criteria for diagnosis; based on clinical judgment (only for non-diabetic subjects)
Abdominal obesity	Waist circumference > 102 cm (men) > 88 cm (women)	BMI $\geq$ 25 kg/m <sup>2</sup>	Waist circumference > 94 cm (men) > 80 cm (women)
Glucose	Fasting plasma glucose > 110 mg/dL or medication treatment for controlling glucose	Fasting plasma glucose 110-125 mg/dL or 2 hr post glucose challenge 140-200 mg/dL	Insulin resistance or impaired glucose regulation (but not diabetes)
HDL-C	< 40 mg/dL (men) < 50 mg/dL(women)	< 40 mg/dL (men) < 50 mg/dL(women)	< 39 mg/dL
Hypertension	$\geq$ 130/85 mmHg	$\geq$ 130/85 mmHg	$\geq$ 140/90 mmHg
Triglyceride	$\geq$ 150 mg/dL	$\geq$ 150 mg/dL	$\geq$ 150 mg/dL
Other		Other features of insulin resistance	

<sup>1</sup>World Health Organization

<sup>2</sup> National Cholesterol Education Program, Adult Treatment Panel III

<sup>3</sup>International Diabetes Federation

<sup>4</sup>American Heart Association/National Heart, Lung and Blood Institute

<sup>5</sup> American Association of Clinical Endocrinology, American College of Endocrinology

<sup>6</sup>European Group for the Study of Insulin Resistance



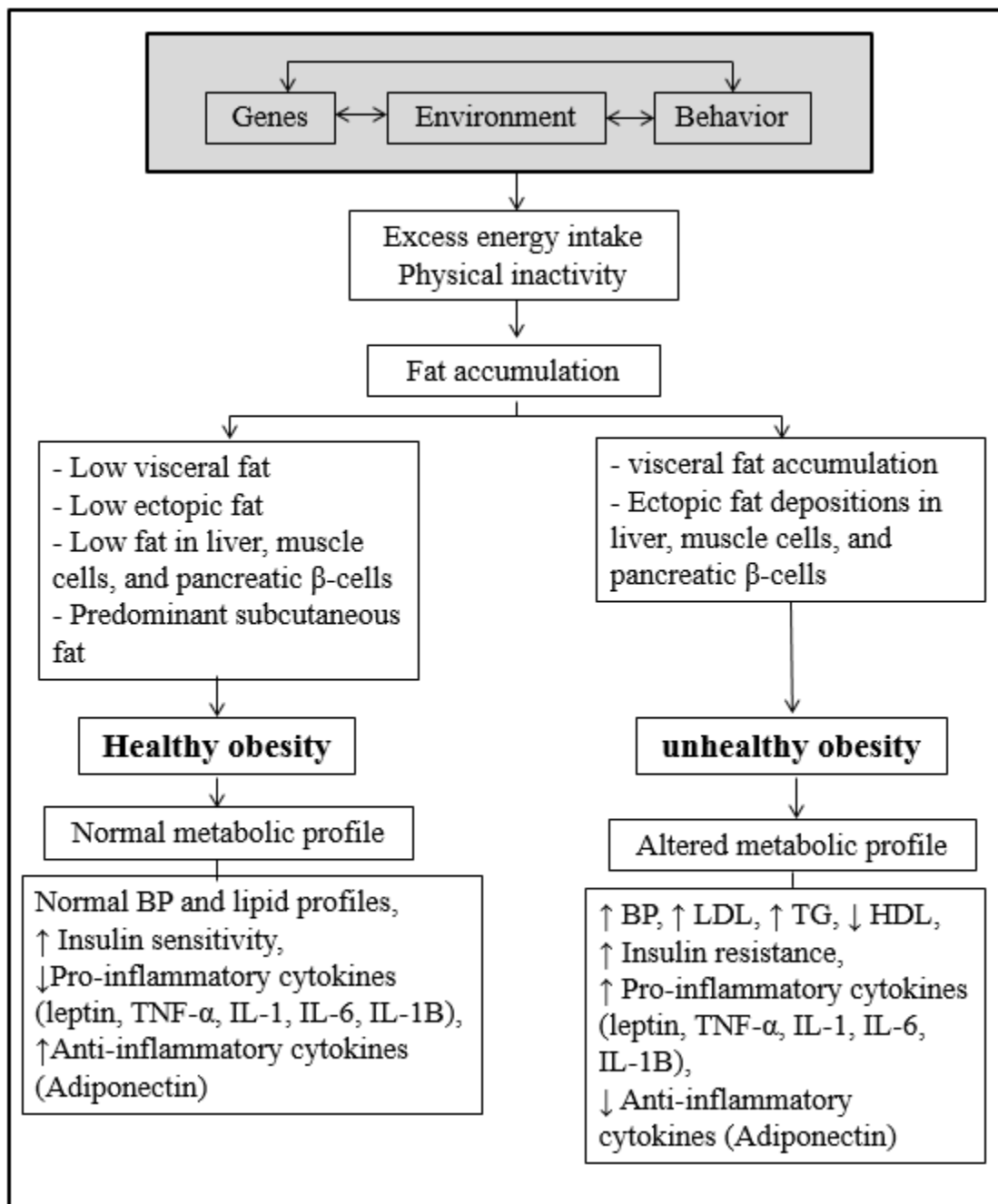


Figure 2.1 The difference between metabolically healthy and unhealthy obesity (227, 228)

BP, blood pressure; LDL, low density lipoprotein; TG, triglyceride; HDL, high density lipoprotein; TNF- $\alpha$ , tumor necrosis factor alpha; IL-1, interleukin 1; IL-1B, interleukin 1 beta; IL-6, interleukin 6

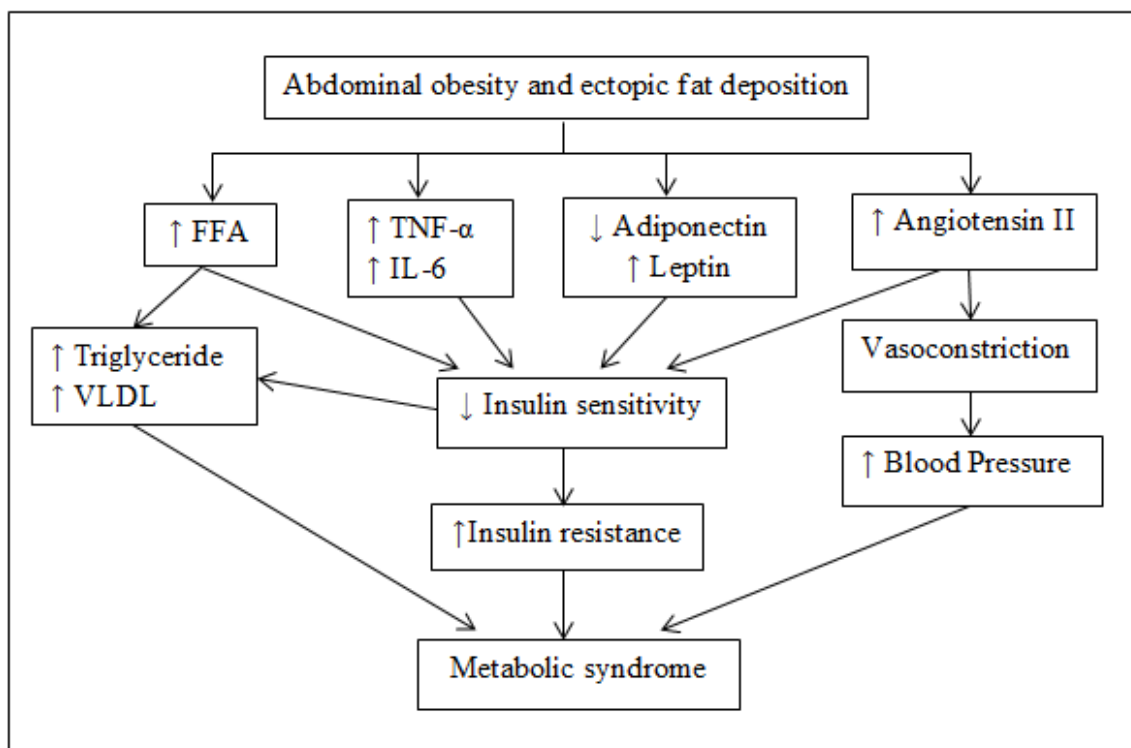


Figure 2.2 Mechanism of metabolic unhealthy obesity

FFA, free fatty acid; VLDL, very low density lipoprotein; TNF- $\alpha$ , tumor necrosis factor alpha; IL-6, interleukin 6

### 2.3.2 Epidemiology of metabolic syndrome

Global prevalence of metabolic syndrome is around 10% to 84%, which depends on region, sex, age, and the definition of metabolic syndrome used (232). The prevalence of metabolic syndrome in the United States was 27% during 1999-2000, and increased to 34.2% during 1999-2006 (240), 36.1% during 2007-2008, 34.2% during 2009-2010, and 34.7% during 2011-2012 (241). The MARE consortium collaborating among 10 countries in Europe (Lithuania, Greece, Spain, Germany, Netherlands, United Kingdom, Italy, Portugal, Belgium, and Sweden) and the United States reported that overall prevalence of metabolic syndrome is 24.3% (24.6% in women and 23.9% in men) (242).

This study also found that Lithuania had the highest prevalence of metabolic syndrome (around 63%) and Italy presented the lowest prevalence (around 7%). Misra and Khurana reported the prevalence of metabolic syndrome in developing countries (33.5% in South Africa, 16.3% in Morocco, 21% for Oman, 33.4% in Turkey, 31.2% in Venezuela, 25.4 in Brazil, and 33.7% in Iran.) (243). The China Health and Nutrition Survey in 2009 presented that metabolic syndrome prevalence is 21.3% (95%CI: 20.3-22.2) for NCEP ATP III criteria and 18.2% (95%CI: 17.3-19.1) for IDF criteria after adjustment for age (244). The prevalence of metabolism of rural women in Bangladesh and India is also as high as the prevalence in other developing and developed countries (36.4% in India and 31.25% in Bangladesh) (245, 246).

Higher prevalence of metabolic syndrome in women than in men has been reported in the United States, Germany, France, Greece, Finland, and Sweden (241, 242, 247), but not in Northeast of China, Spain, Netherlands, Portugal (242, 247, 248). Data from the National Health and Nutrition Examination Survey (NHANES) (2003-2012) in the United States reported that the highest prevalence of metabolic syndrome was 38.6% in Hispanic population, and 37.4% in Non-Hispanic Whites, 35.5% in Blacks, and 23.4 in other races (241).

### **2.3.3 Risk factors of metabolic syndrome**

#### **a) Age**

The NHANES (2003-2012) reported that prevalence of metabolic syndrome increased when ages increased (18.3% among ages 20-39 years, and 46.7 among ages 60 years or over (241). The prevalence of metabolic syndrome is

considered to increase with age. The LATIN America METabolic Syndrome (LATINMETS) multicenter study in Brazil reported that increased age groups increases risk of metabolic syndrome (20-29 years: OR:1.3, 95% CI: 0.5-3.2; 30-39 years: OR:5.6, 95% CI: 0.8-11.9; and  $\geq 40$  years: OR:26.3, 95% CI: 4.5-48.1) (249). The Chinese survey in 2009 (244) reported that risk of metabolic syndrome increased three time in age  $\geq 60$  years (OR: 36.7, 95% CI: 34.7-38.7) when compare with age 18-39 years (OR: 12.4, 95% CI: 10.9-13.9-38.7).

#### **b) Body weight**

A national Survey in the United States (1988-1994) (250) reported that increased BMI increased the incidence of metabolic syndrome in both men and women (BMI 18.5-24.9 is a reference, for men: BMI 25-29.9 kg/m<sup>2</sup> (OR: 5.2, 95% CI: 3.9-6.9), BMI 30-34.9 kg/m<sup>2</sup> (OR: 25.2, 95% CI: 19.3-32.9), BMI  $\geq 35$  kg/m<sup>2</sup> (OR: 67.7, 95% CI: 40.5-113.3); for women: BMI 25-29.9 kg/m<sup>2</sup> (OR: 5.4, 95% CI: 3.7-7.9), BMI 30-34.9 kg/m<sup>2</sup> (OR: 14.0, 95% CI: 9.1-21.4), BMI  $\geq 35$  kg/m<sup>2</sup> (OR: 34.5, 95% CI: 22.6-52.7)). Xi et al. found that overweight and obesity significantly increased risk of metabolic syndrome (BMI < 25 kg/m<sup>2</sup> is reference; BMI 25-27.49 kg/m<sup>2</sup>: OR: 4.32, 95% CI: 3.77-4.95; BMI  $\geq 27.5$  kg/m<sup>2</sup>: OR: 11.24, 95% CI: 9.53-13.26) (244).

#### **c) Smoking**

Smoking is associated with abdominal obesity and insulin resistance (251). Studies in Norway and Korean found that smoking more than 20 cigarettes per day increased risk of metabolic syndrome (HR: 1.25, 95% CI: 1.02-1.53, and

OR: 1.79, 95% CI: 1.10-2.91, respectively) when compared with non-smoker (252, 253). A population based cross-sectional study in China found that increased smoking increased risk of metabolic syndrome (never is reference; OR: 0.79, 95% CI: 0.51-1.25 for  $\leq 7.5$  packs/year; OR: 1.12, 95% CI: 0.69-1.79 for  $\leq 20$  packs/year, and OR: 1.81, 95% CI: 1.15-2.84 for  $> 20$  packs/year; p-trend 0.045) (251). A study in Iran also reported the protective effect of smoking on metabolic syndrome. (254)A study in China reported that high incidence of metabolic syndrome in heavy smoker ( $\geq 11$  cigarettes/day) (OR: 1.33, 95% CI: 1.04-1.71) when compare to lighter smokers ( $\leq 10$  cigarettes/day) (244). However, the study in Japan and Portugal that smoking had no association with metabolic syndrome (255, 256).

#### **d) Alcohol consumption**

Light or moderate alcohol consumption can reduce risk of coronary heart disease and stroke, but excessive consumption is toxic to many organs (257). The association between alcohol consumption and risk of metabolic syndrome is inconsistent. A meta-analysis study, analyzing 14 observational studies, found that both men having alcohol consumption 0.1-39.9 g/day and women having alcohol consumption 0.1-19.9 g/day had a lower prevalence of metabolic syndrome when compared with non-drinkers (OR: 0.84, 95% CI: 0.75-0.94 for men and OR: 0.75, 95% CI: 0.64-0.89 for women) (258). The Third National Health and Nutrition Examination Survey in the United States also reported the protective effect of alcohol consumption on the incidence of metabolic syndrome

(< 1 drink/month is reference, OR: 0.65, 95% CI: 0.54-0.79 for 1-19 drinks/month: OR: 0.34, 95% CI: 0.26-0.47 for  $\geq 20$  drinks/month, p-trend = 0.0001) (259). A study in China (251) also reported the protective effect of alcohol consumption (0 g/day is reference, OR: 0.81, 95% CI: 0.63-1.05 for  $\leq 5.7$  g/day: OR: 0.72, 95% CI: 0.56-0.94 for  $\leq 17.7$  g/day; OR: 0.73, 95% CI: 0.56-0.95 for  $> 17.7$  g/day; p-trend  $< 0.0001$ ). However, a survey in China demonstrated that increased alcohol intake increased metabolic syndrome risk (<1 time/month is reference; 1-3 times/month (OR: 1.82, 95% CI: 1.21-2.75; 1-2 times/week (OR: 2.03, 95% CI: 1.35-3.05); 3.4 times/week (OR: 2.07, 95% CI: 1.31-3.27); 1 times/day (OR: 2.16, 95% CI: 1.45-3.22)) (244). A cross-sectional study in Portugal reported no association between alcohol consumption and metabolic syndrome (255).

#### e) Dietary intake

The NHANES (1988-1994) found that high carbohydrate intake increased risk of metabolic syndrome in men (OR: 1.7, 95% CI: 1.2-2.5 in men and OR: 1.1, 95% CI: 0.8-1.4 in women) (250). A cross-sectional study in Finland found that increased fish consumption reduced risk of metabolic syndrome (tertile 1 is reference, tertile 2 (OR: 0.52, 95% CI: 0.32-0.83), tertile 3 (OR: 0.63, 95% CI: 0.4-1.0), p-trend 0.04 (260). This study also reported the protective effect of the consumption of berries, legumes and nuts on the incidence of metabolic syndrome. Many studies also reported the inverse association between fish consumption and risk of metabolic syndrome (261-263). A study in France

presented that increased consumption of cereal grains, and dairy products decreased risk of insulin resistance (quintile 1 is reference, for cereal grains: quintile 2 (OR: 1.24, 95% CI: 0.76-2.00), quintile 3 (OR: 0.79, 95% CI: 0.47-1.33), quintile 4 (OR: 0.55, 95% CI: 0.30-0.98), quintile 5 (OR: 0.76, 95% CI: 0.39-1.48), p-trend 0.05; for dairy products: quintile 2 (OR: 0.76, 95% CI: 0.46-1.23), quintile 3 (OR: 0.64, 95% CI: 0.39-1.07), quintile 4 (OR: 0.49, 95% CI: 0.28-0.83), quintile 5 (OR: 0.64, 95% CI: 0.37-1.09), p-trend 0.03) (262). This study also found that meat consumption was positively associated with insulin resistance (quintile 1 is reference, quintile 2 (OR: 0.96, 95% CI: 0.56-1.64), quintile 3 (OR: 1.10, 95% CI: 0.64-1.88), quintile 4 (OR: 1.70, 95% CI: 0.99-2.90), quintile 5 (OR: 2.29, 95% CI: 1.30-4.02), p-trend 0.0007). The protective effect of dairy consumption on insulin resistance was also reported in the Coronary Artery Risk Development in Young Adults (CARDIA) study (264).

#### **f) Physical activity**

Increased physical activity has a protective effect on metabolic syndrome because increased physical activity improves metabolic parameters by promoting weight reduction (251). A population based cross-sectional study in China, The Nantong Metabolic Syndrome Study (NMSS), reported that occupational physical activity reduced the incidence of metabolic syndrome (no or sedentary work is reference; light intensity (OR: 0.77, 95% CI: 0.55-0.88, moderate intensity (OR: 0.55, 95% CI: 0.57-0.90), and vigorous intensity (OR: 0.76, 95% CI: 0.63-0.91), p-trend 0.005) (251). The Tromsø Study (252) reported that increased intensity of

physical activity significantly decreased risk of metabolic syndrome, which is similar to the result from NMSS. However, A study in Portugal reported no association between physical activity and metabolic syndrome (255). A study in Taiwan found that watching television  $\geq 21$  hours/week increased the incidence of metabolic syndrome when compared with watching television  $\leq 5$  hours/week (OR: 3.69, 95% CI: 1.05-12.95) (265). The Third National Health and Nutrition Examination Survey in the United States (1988-1994) found that physical inactivity increase risk of metabolic syndrome (OR: 1.4, 95% CI: 1.0-2.0 for men and OR: 1.2, 95% CI: 1.0-1.4 for women) (250).

#### **2.3.4 Metabolic syndrome and risk of diabetes mellitus**

Pathogenic studies reported that obesity leads to insulin resistance, the impairment of insulin sensitivity in sites of glucose disposal, which can develop into type 2 diabetes mellitus and GDM. Enlarged fat cells lead to increased free fatty acid level, which result in insulin resistance and impaired insulin secretion. Enlarged fat cells cause fat overflow to muscles, the liver, and the pancreas, which also affects insulin resistance and impairs insulin secretion (266). In a study focusing on the correlation between type of obesity and risk of diabetes, the presence of visceral fat, or the accumulation of fat in the central abdominal area, was associated with diabetes (267). Moreover, other studies presented waist circumference, correlating it with the level of abdominal visceral fat, as a predictor of non-insulin independent diabetes mellitus (268-270).

Metabolic syndrome is a cluster of cardio-metabolic risk factors which increase the risk of type 2 diabetes mellitus and cardiovascular disease. Many studies have



reported that metabolic syndrome increased the risk of type 2 diabetes mellitus, including the West of Scotland Coronary Prevention Study (RR: 7.26, 95% CI: 2.25-23.4 ) (271), the Beaver Dam Study (RR: 9.37, 95% CI: 2.22-39.59) (272), the San Antonio Heart Study (OR: 3.30, 95% CI: 2.27-4.80) (273), the Framingham Offspring Study (RR: 6.29, 95% CI: 4.47-10.81) (274), Australian Diabetes, Obesity, and Lifestyle (AusDiab) study (RR:7.8, 95% CI: 5.5–11.0) (275), and the Insulin Resistance Atherosclerosis Study (OR: 4.14, 95% CI: 2.79-6.16) (276). Insulin resistance and dyslipidemia in metabolic syndrome are important factors in type 2 diabetes mellitus development. Insulin resistance over time will develop to type 2 diabetes mellitus because of the inability of pancreatic beta cells to produce sufficient insulin to compensate for insulin resistance (232). Moreover, hypertriglyceridemia and hypo HDL-C as a risk factor for pancreatic beta cell dysfunction contribute to type 2 diabetes by reducing insulin secretion (277).

Most studies have reported the development of metabolic syndrome after gestational diabetes mellitus (GDM). Studies reported the risk factors of GDM, including: high fasting plasma glucose, insulin resistance, high systolic blood pressure, high triglyceride level, low HDL-C level at the first trimester of pregnancy, and overweight or obesity pre-gestation (174, 278-280). These factors are used for the diagnosis of metabolic syndrome. Women with a history of GDM also have increased risk of metabolic syndrome. A meta-analysis study (281), analyzing 17 studies, showed that the risk of metabolic syndrome increased in women with a history of GDM (OR: 3.96, 95% CI: 2.98-5.26). In addition, the subgroup analysis by ethnicity showed that Caucasian women have higher odds of developing metabolic syndrome after GDM than

Asian women (OR: 4.54, 95% CI: 3.78 -5.46 in Caucasian; and OR: 1.28, 95% CI: 0.64 to 2.56 in Asian). The subgroup analysis by BMI showed that women with prior GDM had an increased risk of metabolic syndrome (OR: 5.39, CI: 4.47- 6.50). This result may be explained by a study which reported that women with a history of diabetic pregnancy had higher CRP levels and lower adiponectin levels compared to controls (282). Moreover, higher level of CRP and IL-6 were present in women with metabolic syndrome and a history of GDM compared with the control group and women with a history of GDM, but who did not have metabolic syndrome (283, 284). Therefore, inflammation and adipose tissue relating to insulin sensitivity may be the link between GDM and metabolic syndrome.

Metabolic syndrome is not only a consequence of, but a risk factor for gestational diabetes mellitus. A study in Greece by Chatzi et al. (285) found that women with metabolic syndrome in early pregnancy increased the risk of GDM (RR: 3.17, 95% CI: 1.06-9.50).

### **2.3.5 Biochemical markers related to diabetes mellitus and metabolic syndrome**

Biochemical markers are hormones, enzymes, antibodies, or other substances in urine, blood, tissue, or other body fluids. These biomarkers have been used to detect abnormality or disease. Fasting plasma glucose (FBG), oral glucose tolerance test (OGTT), glycated hemoglobin (HbA<sub>1c</sub>), and random plasma glucose are the common biochemical markers for screening and diagnosing diabetes (145). The association between first trimester biomarker and the risk of developing GDM has been studied in lipid profiles, inflammatory biomarkers, and ferritin levels.

### a) Glucose and Insulin

Plasma glucose is a parameter for diagnosing diabetes by the American Diabetes Association, WHO, and IDF (145, 146). The homeostatic model assessment (HOMA) was proposed to assess insulin resistance (HOMA-IR) and  $\beta$ -cells function (HOMA-B) (286). HOMA model has been used to predict type 2 diabetes development. QUICKI (quantitative insulin sensitivity check index) was proposed later by Katz et al. in order to assess insulin sensitivity. Chen et al. determining the predictive accuracy of QUICKI concluded that QUICKI is an accurate index for assessing insulin sensitivity (287).

$$\text{HOMA-IR} = [\text{Fasting plasma glucose (mmol/L)} \times \text{Fasting plasma insulin (mU/L)}] / 22.5$$

$$\text{HOMA-B} = [20 \times \text{Fasting plasma insulin (mU/L)}] / [\text{Fasting plasma glucose (mmol/L)} - 3.5] \%$$

$$\text{QUICKI} = 1 / [\log(\text{fasting plasma insulin } (\mu\text{U/ml})) + \log(\text{fasting plasma glucose (mg/dl)})]$$

The Women's Health Initiative Observation Study (288), a multiethnic cohort of women in the United States, found that diabetic cases had higher HOMA-IR and lower HOMA-B than controls. This study reported the strong association between diabetes and HOMA-IR and HOMA-B in all ethnic groups (HOMA-IR; RR: 3.05, 95% CI: 2.63-3.53 for overall; RR: 3.79, 95% CI: 2.94-4.88 for Whites; RR: 2.59, 95% CI: 2.03-3.30 for Blacks; RR: 2.66, 95% CI: 1.80-3.91 for Hispanics; RR: 4.18, 95% CI: 2.18-8.04 for Asians; HOMA-B; RR:

0.52, 95% CI: 0.46-0.58 for overall; RR: 0.50, 95% CI: 0.42-0.59 for Whites; RR: 0.48, 95% CI: 0.39-0.60 for Blacks; RR: 0.49, 95% CI: 0.35-0.68 for Hispanics; RR: 0.59, 95% CI: 0.37-0.93 for Asians). The association between type 2 diabetes and HOMA-IR and HOMA-B was reported in many population including Mexican-American (289), non-Hispanic White (289), African-American (290), Mexican (291), Japanese (292), and Chinese (293). Yokoyama et al. found that type 2 diabetic patients had higher QUICKI than the healthy participants (294). A five year follow-up study in Finland (295) found that increased QUICKI increased risk of type 2 diabetes in obesity (tertile 1 is reference; tertile 2, OR: 5.29, 95% CI: 1.39-20.24; tertile 3, OR: 7.77, 95% CI: 1.63-37.04; p-trend 0.002).

Many studies found that pregnant women with GDM have higher HOMA-IR and lower QUICKI and HOMA-B than pregnant women with normal glucose tolerant (296-299). Ozcimen et al. suggested the cut-point of HOMA-IR in the first trimester for predicting GDM is 2.60 (300). However, a prospective cohort study in Turkey reported no association between HOMA-IR, HOMA-B, and QUICKI and risk of GDM (301). Therefore, HOMA and QUICKI have inconsistent correlations with the prevalence of type 2 diabetes and GDM.

A study in India conducted by Bhatnagar et al. reported that metabolic syndrome group had higher both fasting and post-prandial plasma glucose and serum insulin including HOMA-IR than the control group (302).

## b) Lipid profiles

Both high triglyceride and low high-density lipoprotein levels are associated with a pre-diabetic state, or insulin resistance syndrome (303). The San Antonio Heart Study with an 8 year follow up, reported that participants who converted to non-insulin dependent diabetes mellitus had higher triglyceride levels and lower HDL levels than subjects without diabetes. These results were statistically significant for both men and women (181). A longitudinal study in Norway also reported that both high triglyceride and low HDL had a significant association on the risk of diabetes (162). The Finnmark study with a 12 year follow up, presented a significant inverse correlation between HDL levels and the risk of diabetes mellitus only in women at not in men (HDL <1 mmol/l is reference, HDL 1.0-1.49 mmol/l :RR = 0.6), HDL  $\geq$  1.5 mmol/L: RR = 0.17), P-trend <0.0001)(171). In addition, a positive correlation between total cholesterol and risk of diabetes was reported in the study from Qatar (total cholesterol  $\geq$  240 mg/dl OR: 1.4, 95% CI: 1.0-1.9) (172).

The triglyceride and HDL levels have an association with the development of GDM. Pregnant women developing GDM have lower HDL levels and higher triglyceride levels during the first trimester than control pregnancies when adjusted for maternal age, BMI, gestational age at sampling, smoking, ethnicity, parity, conception status, and previous GDM (279). Guanghui Li and colleagues (304) studied the association between lipid profiles during the first trimester and risk of developing GDM. The study reported on two groups, a lean group (BMI <

24 kg/m<sup>2</sup>) and an obese group (BMI  $\geq$  24 kg/m<sup>2</sup>). After adjustment for age, parity, gravidity, family history of diabetes, and level of education, the risk of developing GDM had a positive association with triglyceride, LDL, and LDL/HDL ratio in both groups. In addition, the negative correlation between HDL level and the risk of GDM was found only in lean women. The results of this study were supported by a meta-analysis study which found that higher triglyceride and LDL levels and lower HDL levels during first trimester were associated with development of GDM (305).

#### c) **Fatty acids**

Fatty acids have been used as biomarkers for dietary fat intake, obesity, insulin resistance and diabetes. The Melbourne Collaborative Cohort Study (MCCS), four year follow up, presented a positive association between the incidence of type 2 diabetes and some plasma fatty acid proportions such as total saturated fatty acid levels (OR: 3.76, 95% CI: 2.43- 5.81), stearic acid levels (OR: 4.14, 95% CI: 2.65- 6.49), palmitoleic acid levels (OR: 5.61, 95% CI: 3.65-8.60), dihomo- $\gamma$ -linolenic acid levels (OR: 9.65, 95% CI: 5.48-16.97), and inverse association with linoleic acid levels (OR: 0.22, 95% CI: 0.14- 0.36), and *trans* fatty acid levels (OR: 0.20, 95% CI: 0.12- 0.32) when compared highest quintile with lowest quintile (306).

#### d) **Inflammatory markers: cytokines and adipokines**

Inflammatory markers and diabetes can be described with adipose tissue functions. Obesity causes adipose tissue to promote inflammatory response

(increased leptin, resistin, visfatin, chemerin, tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), plasminogen activator inhibitor 1, monocyte chemoattractant protein-1, and retinol binding protein-4, and decreased adiponectin and interleukin-10 (IL-10)) (307).

Inflammatory markers have been proposed as predictors for the incidence of type 2 diabetes. A meta-analysis study conducted by Wang et al. found that interleukin-6 (IL-6) and C-reactive protein (CRP) had positive associations with the risk of type 2 diabetes (RR: 1.31, 95% CI: 1.17-1.46 and RR: 1.26, 95% CI: 1.16-1.37, respectively) (308). The population-based MONItoring of trends and determinants in CARDiovascular disease (MONICA)/Cooperative Research in the Region of Augsburg (KORA) (309) studies found that increased interleukin-18 (IL-18) raised the risk of type 2 diabetes. The MONICA/KORA studies, however, did not observe an association between type 2 diabetes and IL-6 and CRP. A year later, the MONICA/KORA studies published result on the novel inflammatory markers associated with type 2 diabetes, namely monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8) and interferon-gamma-inducible protein-10 (IP-10) (310). A cohort study in the United Kingdom found that Interleukin-1 receptor antagonist (IL-1Ra) was positively associated with the risk of type 2 diabetes (OR: 1.48, 95% CI: 1.21-1.80) (311).

Adipokines have been reported to have an association with the incidence of type 2 diabetes. The data from the Third National Health and Nutrition Examination Survey (NHANES III) (312) showed that increases in leptin levels

significantly increased the incidence of diabetes mellitus in both genders (Q4 vs Q1; OR: 4.36, 95% CI: 2.15-8.85 for men, OR: 2.76, 95% CI: 1.32-5.77 for women, and OR: 3.79, 95% CI: 2.05-7.00 for overall) after adjustment for diabetes risk factors (age, sex, race/ethnicity, education, smoking, alcohol intake, hypertension, serum cholesterol and C-reactive protein). However, plasma leptin level had no association with diabetes mellitus after adjustment for diabetes risk factors and body mass index (Q4 vs Q1; OR: 1.07, 95% CI: 0.59–1.94 for men, OR: 0.86, 95% CI: 0.49–1.51 for women, and OR: 0.98, 95% CI: 0.56–1.72 for overall). A meta-analysis study (313) analyzing 24 articles presented that an increase in leptin levels of 1-log ng/ mL significantly increased the risk of type 2 diabetes only in men (RR: 1.37, 95% CI: 1.13-1.66 in men, and RR: 0.96, 95% CI: 0.90-1.03 in women) after adjustment for type 2 diabetes risk factors and body mass index. The British Regional Heart Study (314) , a prospective study of cardiovascular disease in men aged 60-79 years, reported that type 2 diabetes mellitus had a negative correlation with adiponectin (RR: 0.33, 95% CI: 0.19-0.56) and a positive correlation with leptin (RR: 4.98, 95% CI: 2.75-9.04) after adjusting for type 2 diabetes risk factors. The 10 year follow-up study in Aboriginal Canadian population (315) showed that type 2 diabetes mellitus had a positive association with leptin (OR: 1.50, 95% CI: 1.02-2.21) and a negative association with adiponectin (OR: 0.63, 95% CI: 0.48-0.83) and adiponectin-to-leptin ratio (OR: 0.54, 95% CI: 0.37-0.77), after adjustment for age, sex, triglycerides, HDL cholesterol, hypertension, and impaired glucose tolerance.



However, only adiponectin significantly correlated with the risk of type 2 diabetes (OR: 0.68, 95% CI: 0.51-0.90) after additional adjustment with waist circumference and body mass index.

Prospective studies have found that GDM is related to decreased adiponectin and anti-inflammatory cytokines (IL-4 and IL-10) and increased leptin and pro-inflammatory cytokines (IL-6 and TNF- $\alpha$ ) (316). Pregnancies with GDM have higher IL-6 concentrations during the first trimester than pregnancies without GDM (317). Adiponectin levels during the first trimester have a negative correlation with the risk of developing GDM (OR: 1.13, 95% CI: 1.03-1.24 per 1  $\mu$ g/ml decrease) after adjustment for age and waist circumference (318). When compared to the highest quantile, pregnant women with first trimester adiponectin concentrations ranging in the lowest quantile were 10.2 times (95% CI: 1.13-78.7) more likely to develop GDM (319). Other studies confirmed that pregnant women developing GDM have lower adiponectin level and higher leptin and CRP levels than women in the control group (279, 320-323). Increased first trimester CRP concentration increased the risk of developing GDM after adjusting for age, race/ethnicity, parity, blood pressure, smoking, and gestational age (T3:T1, OR: 3.6, 95% CI: 1.2-11.4, p-trend < 0.01) (324). Qui C and colleagues (323) reported that leptin levels during the first trimester of pregnancy were positively associated with the risk of developing GDM when adjusted for parity, family history of type 2 diabetes, and pre-pregnancy BMI (T3:T1, OR: 4.90, 95% CI: 1.40-17.5, p-trend < 0.02) (325).

The positive association between CRP and risk of metabolic syndrome was found in studies in the United States (326), Spain (327), Korea (328), India (329), and Iran (330). A study in Poland (331) reported the increased hs-CRP, IL-6, and TNF- $\alpha$  increased a number of metabolic syndrome components. This study also presented a negative association between adiponectin and a quantity of metabolic syndrome parameters. The Indian Atherosclerosis Research Study (IARS) (332) found that hs-CRP levels was associated with risk of developing metabolic syndrome (OR: 1.49, 95% CI: 1.14-1.95), but the association between IL-6 and metabolic syndrome was not found. A study in Japan by Matsushita et al. presented that increased TNF- $\alpha$  and CRP, including decreased adiponectin were associated with metabolic syndrome (OR: 1.27; 95% CI: 1.00-1.60, OR: 1.49; 95% CI: 1.19-1.87, and OR: 2.03, 95% CI: 1.55-2.66, respectively) (333). A negative association of adiponectin and positive associations of leptin and CRP on metabolic syndrome were reported in a population-based cross-sectional study in Kuwait (334) and Spain (335). However, the Framingham Heart Study (336) reported no association between risk of metabolic syndrome and inflammatory biomarkers (CRP, IL-6, and TNF- $\alpha$ ).

#### e) Liver enzymes

Liver functions maintain glucose levels during fasting and in the postprandial period. The liver enzymes are used to evaluate of liver function: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-

glutamyltransferase (GGT). AST and ALT are considered as hepatocellular health markers while GGT can indicate biliary tract function (337).

The correlation between liver enzymes and risk of type 2 diabetes has been reported for many decades, but the results are still inconclusive. The Insulin Resistance Atherosclerosis Study, a multicenter observational epidemiologic study (338) showed that AST had a significant positive association with the risk of type 2 diabetes (OR: 1.98, 95% CI: 1.23–3.17; Q4 vs Q1–Q3). The association between AST and incidence of type 2 diabetes was reported in the Mexico City Diabetes Study (339) and the cohort study in China (340), but not in a Korean (337), Japanese (341), or English (342) study. The Namwon study (337) presented that after adjusting for diabetes risk factors, serum ALT concentration were associated with type 2 diabetes in both males (OR: 1.95, 95% CI: 1.18-3.21) and females (OR: 1.49, 95% CI: 1.03-2.16) when comparing the highest quartile ( $ALT \geq 30$  units/l) to the lowest quartile ( $ALT \leq 20$  units/l). Many studies also reported the association between ALT and incidence of type 2 diabetes (338, 340, 342-345). However, studies of middle-aged Japanese men (341) and a Mexican population (339) reported no correlation between ALT and risk of type 2 diabetes after adjusting for other diabetes risk factors. The Mexico City Diabetes Study (339) reported that GGT was an independent predictor for type 2 diabetes when controlling for diabetes risk factors, plasma proinsulin and 2 hour glucose levels (OR:1.62, 95% CI:1.08–2.42). The study in Korea (337) also reported that females in the highest GGT quartile ( $GGT \geq 55$  units/l) had a significantly

increased risk of type 2 diabetes (OR: 1.85, 95% CI: 1.23-2.79) when compared with the lowest quartile (GGT  $\leq$  19 units/l). Many studies (342, 344-346) confirmed that increased GGT increased the risk of type 2 diabetes. The study in middle-aged Japanese men (341) reported that increased serum alkaline phosphatase significantly raised the risk of type 2 diabetes after adjustment for all diabetes risk factors (OR: 2.04, 95% CI: 1.39-3.00 for Q4 vs Q1, p-trend  $<0.001$ ).

A 5-year follow-up study in the United States (326) presented the association between incidence of metabolic syndrome and ALT and AST/ALT ratio (OR: 1.43, 95% CI: 1.15-1.77 and OR: 0.72, 95% CI: 0.57-0.90, respectively). Meta-analysis studies (347, 348) considering prospective cohort studies, reported that the highest category of GGT and ALT increased risk of metabolic syndrome when compared with the lowest category (RR: 1.63, 95% CI: 1.43-1.82 and RR: 1.81, 95% CI: 1.49-2.14, respectively). These studies also found that risk of metabolic syndrome was 1.09 per 5U/l increment of GGT levels (95% CI: 1.06-1.13) and 1.13 per 5U/l increment of ALT levels (95% CI: 1.11-1.16).

#### **f) Iron status**

Body iron stores have been reported as an independent predictor of the risk for type 2 diabetes. The EPIC (European Prospective Investigation of Cancer)-Norfolk Cohort Study (349) and a study in China (350) reported that a raised ferritin level increased the risk of type 2 diabetes during 5 years of follow-up. Montonen et al. examined the association between body iron stores and risk of

type 2 diabetes among 27,548 participants during a 7 year follow-up. This study found that serum ferritin concentrations were positively associated with the risk of type 2 diabetes (RR: 1.73, 95% CI: 1.15- 2.6, *p-trend* 0.002), while there was no correlation between transferrin receptors and the risk of type 2 diabetes (351). The Camden study reported that first trimester serum ferritin levels were positively associated with the risk of GDM (OR: 2.35, 95% CI: 1.06- 5.22, *p-trend* <0.05) (322).

#### **g) Amino acid profiles**

The population-based Metabolic Syndrome in Men (METSIM) Study (4.7-year follow-up) reported that alanine (OR 1.02, 95% CI: 1.01-1.04), leucine (OR 1.05, 95% CI: 1.01-1.08), Phenylalanine (OR 1.06, 95% CI: 1.00-1.13), isoleucine (OR 1.10, 95% CI: 1.05-1.15), tyrosine (OR 1.12, 95% CI: 1.05-1.19), and glutamine (OR 0.97, 95% CI: 0.96-0.99) predicted the incidence of type 2 diabetes (352). Moreover, the increased level of alanine, leucine, isoleucine, valine, phenylalanine, and tyrosine and decreased level of glutamine and histidine increased fasting plasma glucose and 2 hour postprandial glucose, and decreased insulin sensitivity.

#### **2.3.6 Genes related to obesity and diabetes mellitus**

Four hundred and sixteen genes were found that were associated with type 2 diabetes or GDM or obesity. These genes can be divided into two priority groups. Twenty-eight genes classified into the first group having association between OFCs and type 2 diabetes or GDM or obesity (Table 2.2). In the second group, three hundred and

eighty-eight genes have association with significant association with type 2 diabetes or GDM or obesity in human studies (Table 2.3).

The association between gene polymorphisms and the risk of diabetes differs among populations. Polymorphisms in genes related to  $\beta$ -cell function, insulin sensitivity, glucose transport, glucose homeostasis, cytokine, and obesity have been found to be associated with the incidence of diabetes. Genes related to obesity were associated with body mass index, waist circumference, waist-to-hip ratio, and fat distribution. Genes classified in first priority group were described more in term of mechanisms and the association with diabetes mellitus and obesity.

**a) ABCC8 (ATP-binding cassette, sub-family C (CFTR/MRP), member 8)**

ABCC8 influences the K-ATP channel function, which causes increased insulin secretion by pancreatic  $\beta$ -cells. Elbein et al. reported a ABCC8 gene mutation that decreased pancreatic  $\beta$ -cell compensation to reduced insulin sensitivity (353).

A candidate gene study in the United States reported that the polymorphism of rs4148643 and rs1799854 was associated with the risk of both type 2 diabetes and GDM (354). A study in Turkish (355) and Finnish (354) populations found that the variation at rs1799854 and rs1799859 in ABCC8 were associated with type 2 diabetes. Additionally, the association between rs1799854 polymorphism and type 2 diabetes mellitus was confirmed in a Japanese population (356).

**b) ADIPOQ (adiponectin, C1Q and collagen domain containing)**

ADIPOQ gene has an influence on adiponectin concentration, which is involved in increased glucose uptake via glucose transporter 4 (GLUT-4), and increased fatty acid uptake and oxidation (357). Yamauchi reported that adiponectin stimulated phosphorylation of acetyl coenzyme A carboxylase, glucose uptake, lactate production, and fatty acid oxidation through activated 5-prime-AMP-activated protein kinase (358). A human study found that low plasma adiponectin levels was associated with hyperinsulinemia and insulin resistance (359), and increased adiposity in children decreased insulin sensitivity (360).

The Chennai Urban Rural Epidemiology Study (CURES) in India reported that ADIPOQ variation (rs17846866) was associated with type 2 diabetes (361). The correlation between two SNPs in ADIPOQ (rs1063537 and rs16861194) and the risk of type 2 diabetes was reported in a Chinese Han population (362, 363). The meta-analysis from Western Australian cohort studies provided additional SNPs (rs12637534, rs16861209, and rs17366568) associated with type 2 diabetes (364). The genetic studies in Chinese Han and Japanese populations reported that the SNPs rs2241766 and rs1501299 increased the risk of type 2 diabetes (365, 366). However, the former study in China reported no correlation between the polymorphism in ADIPOQ (rs16861194, rs26672, rs12495941, rs2241766, rs1501299, rs12629945, rs6444175, rs267729, rs2275738, rs1342387, rs1029629, rs11061971, rs12342, and rs1044471) and the incidence of type 2 diabetes mellitus. The study in Korea presented no association between type 2 diabetes and

rs2241766 and rs1501299 (367). The association between type 2 diabetes and the polymorphism at rs2241766, rs1501299, and rs822396 in ADIPOQ was controversial in a Japanese population (368). However, the study in Bulgaria (369) reported the association between GDM and ADIPOQ variant at rs266729, rs2241766 and rs1501299. On the contrary, the study in Malaysia (370), China (371) and Iran (372) reported that rs2241766 had correlation with GDM.

The genetic association study in Indians presented that ADIPOQ variants at rs1501299, rs822396, and 2241767 had significant correlations with obesity (373). A study in France reported that obesity was related to rs266729 and rs1501299, but no association between obesity and rs17300539 and rs2241766 was found (374). A meta-analysis study reported that ADIPOQ polymorphism (rs1501299, rs2241766, and rs17300539) had no correlation with obesity (375).

### c) **ADRB3 (Adrenoceptor beta 3)**

ADRB3 is a member of beta adrenergic receptor family. Adrenergic receptors mediate catecholamine-induced activation of adenylate cyclase through G protein-coupled receptors (376). ADRB3 regulates energy balance through lipolysis in adipocyte, free fatty acid mobilization from adipose cells to portal vein and thermogenesis in skeletal muscle (377, 378). The mutation of ADRB3 gene is associated with decreased resting metabolic rate, obesity, obesity-related diseases (diabetes and hypertension), calorogenic dysfunction, early onset of diabetes mellitus, and increased body weight with aging (378, 379).



The association of ADRB3 (rs4994) polymorphism on the risk of type 2 diabetes was presented in the Chinese Han (365) and Japanese populations (380). Furthermore, the association between the similar SNP (rs4994) and GDM was reported in the study in Austria (381). A study in Chinese population (382) reported additional SNPs (rs72655364 and rs72655365) in ADRBP3 associated with type 2 diabetes. However, the study in Taiwanese (383) and Italian (384) populations reported that rs4994 variant had no correlation with GDM. In Asian populations (385-387) found that SNP (rs4994) was associated with obesity. Gagnon et al. analyzing the data from the Quebec Family Study (QFS) and the Swedish Obese Subjects (SOS) reported that rs4994 mutation had no association with obesity (377).

**d) CDKAL1 (CDK5 regulatory subunit associated protein 1-like 1)**

CDKAL1 is a marker of impaired insulin secretion, and increases risk of type 2 diabetes. The role of CDKAL1 gene in the function of pancreatic  $\beta$ -cells is unknown. A mouse study showed that CDKAL1 knockout mice impaired conversion of proinsulin to insulin and decreased ATP generation in mitochondria after glucose stimulation (388).

A genetic study in the Chinese Han population reported the association between type 2 diabetes and CDKAL1 variants at rs10946398, but there was no association at rs736425 and rs4712527 variant (389). The meta-analysis study (390) presented the significant correlation between type 2 diabetes and CDKAL1 variant at rs7754840 and rs7756992 in Asian, Caucasian, African, and Arab

populations, which is similar to the study in Japanese and Lebanese populations (391-395). The association between rs10916398 and the diabetes in Asian, Caucasian, and African populations was also reported in the meta-analysis study (390). In addition, the study in a Caucasian population (the Wellcome Trust Case Control Consortium (WTCCC) and Finland-United States Investigation of NIDDM genetics (FUSION)) reported that the CDKAL1 variant (rs10916398) increased the incidence of type 2 diabetes (396, 397). Additional SNPs (rs4712524, rs9295475, and rs9460546) associated with type 2 diabetes were reported in East Asian and European populations (398). A GWAS of a Japanese population reported the association between type 2 diabetes and rs2237892 (399). Moreover, a GWAS of a Caucasian population found that rs7754840 variant increased the risk of type 2 diabetes (400).

The polymorphisms in CDKAL1 at rs7756992 and rs7754840 increase risk of GDM (401). The association between the SNP in CDKAL1 (rs7754840) and GDM was confirmed in GWAS in a Korean population (402). However, the study in the Chinese population reported that the SNP rs7754840 had no association with GDM (403). In addition, the study in Danish populations added more SNP (rs7756992) which have a correlation with GDM (404).

The GWA study in a Japanese population reported the association between CDKAL1 (rs2206734) and BMI (405). The study in a Chinese population found that the polymorphism at rs10946398 was not associated with BMI (406).

**e) CDKN2A/2B (Cyclin-dependent kinase inhibitor 2A/B)**

CDKN2A/2B gene is located on chromosome 9p2, which is located between CDKN2A and CDKN2B. CDKN2A and CDKN2B genes are involved in cell cycling control in tumor of lung, breast, brain, bone, skin, bladder, kidney, ovary, and lymphocyte (407, 408). The function of CDKN2A/2B on diabetes has not reported.

A study of a Dutch population reported the association between the risk of type 2 diabetes and rs1412829 in CDKN2A/2B (409). A study of a Japanese and Malay populations (395, 410) reported an additional SNP (rs10811661) associated with type 2 diabetes. The association between type 2 diabetes and rs10811661 mutation was found in the Chinese, Indian, Korean, and Han Chinese populations (394, 411-413), but not in the African American and Lebanese populations (392, 414). Both WTCCC and FUSION studies reported that variation at rs10811661 and rs564398 increased the risk of type 2 diabetes (396, 397). Moreover, the meta-analysis study also reported an association between type 2 diabetes and rs10811661 in Asian and Caucasian populations, and rs564398 in Caucasian populations (390). In GWA study, the association between type 2 diabetes and rs10811661 was found in Caucasian populations (400).

Wang Y. reported the association of the CDKN2A/2B variant at rs2383208 with GDM in a Chinese population (403). The study found CDKN2A/2B variation (rs10811661) associated with GDM, which was confirmed by a study in a Korean population (401). However, no association

between GDM and rs10811661 (Danish population) (404) and rs10757261 (Korean population) (402) was reported.

**f) FTO (Fat mass and obesity associated)**

Gerken et al. (415) reported that FTO shared sequence with iron- and 2-oxoglutarate-dependent oxygenases, and FTO mRNA level found in hypothalamus was regulated by feeding and fasting. A mouse study by Gao et al. (416) found that mice with FTO mutation had postnatal growth retardation (lower body weight, shorter body length, and lower bone mineral density) and decreased insulin-like growth factor 1 (IGF-1) levels. FTO variants disrupt AT rich interactive domain 5B (MRF1-like) (ARID5B)-mediated repression of iroquois homeobox 3 (IRX3) and iroquois homeobox 5 (IRX5). The depression of IRX3 and IRX5 leads to a cell-autonomous shift from white adipocyte browning and mitochondrial thermogenesis, which result in increased fat storage and body weight (417).

The Wellcome Trust Case Control Consortium (WTCCC) and Finland-United States Investigation of NIDDM Genetics (FUSION) reported that the variation in FTO (rs8050136) was associated with the risk of type 2 diabetes in European populations (396, 397). The correlation between type 2 diabetes and FTO polymorphism (rs8050136 and rs17817449, except rs1121980) was reported in a Lebanese Arab population (418). The meta-analysis study based on European and East Asian populations (419) found the association between type 2 diabetes and variation in FTO at rs9939609, which is similar to the study in Norwegian

and Swedish populations after adjusting for age, sex, and BMI (420). In addition, polymorphism at rs9939609 increased the risk of GDM in the study in Spain (421). The study in a Chinese population reported additional SNPs associated with type 2 diabetes, namely rs6499640 and rs3751812 (422). However, the genetic studies reported no association between the diabetic risk and rs9939609 in a Japanese population (391), including rs8050136 in African American and Chinese populations (411, 414). The study in Denmark found that there was no association between the SNP rs9939609 and GDM (404).

A large prospective study in the United States found the association between obesity and rs9939609 in white-Americans and rs1421085 in African-Americans (423). A meta-analysis confirmed that polymorphism in the FTO gene at rs9939609 increased the risk of both overweight and obese subjects (424-426). The GWAS study of Scuteri A and colleagues found the correlation between obesity and many SNPs in FTO namely rs9930506, rs8050136, rs1121980, rs7193144, rs9939609, rs9926289, rs6602024, rs7907949, rs965670, rs1188445, and rs6965526 (427). Later GWAS in a European population provided another FTO variant (rs1421085) that was associated with obesity (428).

#### **g) GCK (Glucokinase)**

GCK gene has an important role in glucose homeostasis by censoring insulin release in pancreatic  $\beta$  cells (429). In mouse study, mice with isolated pancreatic islets of heterozygous GCK knockout had impaired glucose sensitivity

and impaired ability of  $\beta$  cells to secrete insulin for maintaining glucose homeostasis (430, 431).

The association between rs2284779 variant and the risk of type 2 diabetes was stated in the genetic study of a population with Caucasian ancestry (432). The study of Finns reported other GCK polymorphism correlated with type 2 diabetes (rs2244164, rs12534623, rs2268573, and rs882020) (433).

Moreover, studies in Sweden (434) and China (435) presented the association of the variant rs1799884 with risk of GDM. The HAPO (Hyperglycemia and Adverse Pregnancy Outcome) study, a collaboration among United Kingdoms, Australia and Thailand, confirmed the association between the SNP rs1799884 and GDM (436). However, studies in the United States (437) and the United Kingdom (438), found no significant association between rs1799884 mutation and GDM. The variation at rs4607517 has no association with GDM in a study of a Chinese population (403).

#### **h) GNPDA2 (Glucosamine-6-phosphate deaminase 2)**

GNPDA2 gene encodes an enzyme catalyzing the reversible action: converting D-glucosamine-6-phosphate into D-fructose-6-phosphate and ammonium (439). GNPDA2 is involved in nucleotide metabolic process of nucleotide sugar, amino sugar, carbohydrate, and N-acetylglucosamine (439).

The association between GNPDA2 variant at rs10938397 and type 2 diabetes was reported in the meta-analysis study in European and East Asian

population (419) and the study in a Chinese population (422). The association of GNPDA2 on the risk of GDM has not been presented.

The candidate gene study in Chinese proposed the rs10938397 variant has significant association with central obesity (426). The meta-analysis of GWA data reported that GNPDA2 (rs10938397) was associated with BMI (425). The meta-analysis in African ancestry found the additional SNP (rs348465) to be associated with body mass index (440).

**i) HHEX (Haematopoietically expressed homeobox)**

HHEX gene encodes a transcription factor related to Wnt signaling for cell growth and development. A mouse study found that HHEX knockout mice had impaired forebrain, cardiovascular, thyroid, and liver development (441, 442).

The variation at rs1111875, rs7923837, and rs5015480 in HHEX associated with both type 2 diabetes in Han Chinese, Korean, Chinese, and Japanese populations (389, 391, 394, 395, 410), and GDM in a Korean population (401). A genetic study in India confirmed that rs1111875 variant increased the risk of type 2 diabetes (413). Moreover, the WTCC study reported that the SNP rs5015480 was associated with type 2 diabetes (396). Nevertheless, no correlation between HHEX (rs1111875) variation and type 2 diabetes was reported by the study in the Netherlands (409). The GWA study in French and Finnish, Caucasian populations reported the association between type 2 diabetes and the polymorphism in HHEX (rs1111875 and rs7923837) (397, 400, 443).

**j) HNF1A (Hepatocyte Nuclear Factor 1 homeobox A)**

HNF1A, a homeodomain containing transcription factor, is expressed in liver, pancreas, intestine, and kidney (444). Mutation of HNF1A gene related to hepatic adenomas familial (HEPAF), maturity-onset diabetes of the young 3 (MODY3), and insulin-dependent diabetes mellitus (IDDM). According to HNF1A mutation,  $\beta$ -cell dysfunction in MODY3 results from impaired DNA binding, reduced transcriptional activation, and impaired subcellular localization of pancreatic  $\beta$ -cells (444, 445).

HNF1A (rs1169288) variant was associated with both type 2 diabetes and GDM (434, 446, 447). The GWA study in a Hispanic population reported that two SNPs in HNF1A (rs7305618 and rs21573907) were associated with type 2 diabetes (448). The Finnish case-control study reported additional SNP (rs2701175) variant increased the risk of type 2 diabetes (449). A study combining GWA data of European population found that that variant rs7957197 increased the incidence of type 2 diabetes (450).

**k) HNF1B (Hepatocyte nuclear factor 1-beta)**

HNF1B, a member of homeodomain containing transcription factor, is expressed in liver, pancreas, bile ducts, thymus, genital tract, lung, and gut (451). Many studies reported functions of HNF1B including epithelial differentiation during human organogenesis (452), renal tubulogenesis regulation (453), hepatic insulin sensitivity control (454), and pancreatic endocrine cell generation (455).



Moreover, HNF1B gene is also associated with pancreatic  $\beta$  cell dysfunction and insulin resistance (454).

The study in Caucasian reported the association between type 2 diabetes and five SNPs in HNF1B (rs6422978, rs11263755, rs2285741, rs10962, and rs3110641) (432). The genetic study in the United States found that the polymorphism in HNF1B (rs12450628 and rs1008284) was associated with type 2 diabetes (433). The study in a Japanese population presented the association between type 2 diabetes and the SNPs rs1016991 and rs2688, not at rs757210, rs757211, rs718960, and rs2689 (356). The cohort study in the United States present no association between type 2 diabetes and the variation in HNF1B (rs11649743, rs4430796, and rs7501939) (456). However, the association between the SNP rs4430796 and type 2 diabetes was reported in the GWAS of a European population (450). Only polymorphism in rs7903146 is associated with GDM in a Danish population (404).

#### **1) IGF2BP2 (Insulin-like growth factor 2mRNA binding protein 2)**

IGF2BP2 is a family of mRNA-binding protein (IMP1, IMP2, and IMP3), which relates to RNA stability, localization, and translation. IMPs are expressed in developing cells, especially in neuronal and epithelial cells in mid-gestation. IGF2BP2 mRNA is found in many organs (brain, gut, testis, liver, pancreas, bone marrow, kidney, lung, and muscle) during perinatal period and in adult tissues (457). IGF2BP2 variant was associated with impaired pancreatic  $\beta$  cell function (458, 459), and impaired insulin sensitivity (460).

Genetic studies presented the association between type 2 diabetes and the polymorphism in IGF2BP2 at rs4402960 and rs1470579 in Japanese, Chinese, Korean and Indian populations (391, 394, 395, 410, 461), and at rs7651090 in a Chinese population (411). The genetic study in East Asian and European populations found that the IGF2BP2 variation (rs4376068 and rs6769511) increased the risk of type 2 diabetes (398). However, The WTCC and FUSION study (396, 397) and the genetic studies in Dutch, Chinese Han, and Japanese populations (368, 389, 409) reported no association between type 2 diabetes and the SNP rs4402960. The correlation between the variant rs1470579 in IGF2BP2 and type 2 diabetes was confirmed by the GWAS in a Japanese population (399). The GWAS in a Caucasian population also reported that type 2 diabetes was associated with the SNPs rs1470579 and rs4402960 (400).

The association between the rs4402960 polymorphism and GDM was reported in the candidate gene approach in China (403) and Korea (462). The GWAS in the Korean population reported that the SNP in IGF2BP2 (rs1470579) was correlated with GDM (402). The association between rs4402960 and GDM was not significant in the Danish population (404).

#### **m) IL-10 (Interleukin-10)**

IL-10 gene encoded anti-inflammatory cytokine, a T helper 2 mediated cytokine, which inhibits cytokine production by t helper 1 cells (463). Il-10 gene expression is important for inflammatory response and disease progression.

Dysregulation of IL-10 increased inflammatory response and risk of developing

autoimmune diseases such as Crohn's disease, hepatitis, Systemic Lupus Erythematosus (SLE), and allergic asthma (464). A mouse study by Pennline et al. (465) found that IL-10 can prevent the onset of diabetes in non-obese diabetic (NOD) mice by inhibiting interferon- $\gamma$  synthesis. This study reported that NOD mice receiving IL-10 treatment reduce the severity of insulinitis, prevent pancreatic islet cell infiltration, and promote normal insulin production. Esposito et al. (466) found that low IL-10 levels were associated with metabolic syndromes while high IL-10 concentration was associated with obesity.

A meta-analysis study, based on Asian and European populations, presented that the variation at rs1800872 was associated with type 2 diabetes (467). The association between GDM and the polymorphism in IL-10 (rs1800872) was reported in a Malaysian population (468). However, the genetic study in North Indian and Taiwanese population reported no correlation between type 2 diabetes and two SNPs in IL-10 (rs1800872 and rs1800871) (469, 470).

A candidate gene study in Caucasians reported that the variant in IL-10 at rs1800872 was associated with increased BMI and waist-to-hip ratio (471).

#### **n) IRS (Insulin receptor substrate-1)**

IRS1 gene, the insulin receptor substrate protein family, encoded a signaling adapter protein. IRS1 has a key role in transmitting signals from the insulin and insulin-like growth factor 1 receptors to intracellular phosphoinositide 3-kinase/protein kinase B pathway and extracellular signal-regulated kinases mitogen-activated protein kinase pathway (472). A mouse study found that IRS

knockout mice reduced insulin content of  $\beta$ -cells and decreased glucose-stimulated insulin secretion leading to glucose intolerance (473).

The genetic studies in Mexican (474), Indian (475), and Dutch (476) populations reported the variant rs1801278 in IRS1 was associated with type 2 diabetes. A meta-analysis study analyzing data from ten articles (3428 GDM and 4637 controls) showed that the polymorphism in IRS1 (rs1801278) also related to GDM (477). This was also reported in Greek (478), Italian (384), and Saudi (479) populations. Moreover, the meta-analysis study in French found that the rs2943641 variant increased the risk of type 2 diabetes (480). However, there is no association between the IRS1 variation (rs1801278) and type 2 diabetes in the African-American population (481), and GDM in the Scandinavian population (482). A case-control study in the African-American population reported that the polymorphism in IRS1 at rs1801278 was associated with higher BMI (481).

**o) KCNJ11 (Potassium channel, inwardly rectifying subfamily J, member 11)**

KCNJ11 gene is a family of the potassium channel gene. KCNJ11 gene encodes an inward-rectifier potassium ion channel (Kir6.2) protein, which is subunit of the ATP-sensitive potassium (KATP) channel. Increased glucose concentration increases potassium flow into cell through the KATP channel, and ATP binds to Kir6.2 in order to increase intracellular potassium ion concentration. Increased intracellular potassium concentration activating calcium ion channel leads to increase intracellular free calcium ion levels which trigger other

components of insulin secretion pathway. KCNJ11 gene mutation can lead to diabetes by disrupting Kir6.2 protein activity, reducing ATP sensitivity of KATP channel activity and suppression of insulin secretion (483).

The association between type 2 diabetes and KCNJ11 polymorphism at rs5215 was reported in the Chinese Han population (389) and at rs5219 in Chinese (411), Japanese (410), and Indian (461) populations. However, the study in Turkey reported no association between type 2 diabetes and the SNPs in KCNJ11 (rs5215, rs5219, rs5218, rs5216, and rs1800467) (355). The GWA study in a Caucasian population found that the variant rs5219 in KCNJ11 increased the incidence of type 2 diabetes (400). In addition, the GWA study in a Finnish population (397) confirmed the correlation between type 2 diabetes and the SNP rs5215.

The variation at rs5219 also has a correlation with GDM in a Scandinavian population (482). However, the studies in Korea (401) and Denmark (404) presented no significant association between GDM and two SNPs in KCNJ11 (rs5215 and rs5219).

**p) KCNQ1 ((Potassium channel, voltage gated KQT-like subfamily Q, member 1)**

KCNQ1 encodes a pore-forming alpha-subunit of the voltage-gated K channel (KvLQT1). KCNQ1 is expressed in a wide variety of tissue including: heart, lung, liver, kidney, adipose tissue, brain, skeleton muscle and pancreas (484). KCNQ1 controls ventricular repolarization process, which lead to cardiac

conduction abnormality (485). A complex interaction between ATP-sensitive  $K^+$  ( $K_{ATP}$ ) channels and voltage-dependent  $K^+$  ( $K_v$ ) channels regulated insulin secretion from pancreas. The Electronic mechanism at  $K_{ATP}$  and  $K_v$  channels triggers and maintains glucose-stimulated insulin secretion. This effect may lead to impair pancreatic  $\beta$ -cell function (486).

A meta-analysis of GWAS in African American population reported the association between two SNP (rs231356 and rs2283228) in *KCNQ1* and type 2 diabetes (487). A meta-analysis in Mexican-American population (448) and a GWAS in Japanese population (488) found that rs2237892 variation increased risk of type 2 diabetes. Another meta-analysis study including Japanese, Chinese, and Korean population also reported that rs2074196, rs2237892 and rs2237895 polymorphisms (SNP) was significantly associated with type 2 diabetes (489). The association between rs2237895 and type 2 diabetes was reported in a GWAS in Han Chinese population (490) and the Punjabi cohort study (Indians living in India and the United States) (484). A case-control study in Lebanese population also found that *KCNQ1* variation (rs2237892 and rs2237895) was associated with type 2 diabetes (418).

A meta-analysis study from multi-ethnic population (Korean, Chinese, French, Greek, Swede, Brazilian, Dane, Turkish, and American), reported that rs2237892 associated with GDM (491). A candidate gene study in Chinese population found that *KCNQ1* polymorphism at rs2237895 and 2237896 increased risk of GDM but there is no association between rs2237892 had GDM

(492). Candidate gene studies in Korean population presented that the KCNQ1 polymorphism at rs2074196 and rs2237892 and rs2237895 increased risk of GDM (493, 494).

**q) LEP (Leptin)**

LEP gene encodes leptin hormone regulating body weight through leptin receptors. The more fat accumulates, the more leptin is produced because fat cells release leptin in proportion to their size. Leptin is involved in food intake inhibition, energy expenditure regulatory, energy and glucose homeostasis, bone formation, immune and inflammatory response, angiogenesis, hematopoiesis, and wound healing. The activation of leptin receptor mediates transcriptional regulation of the melanocortin pathway in hypothalamus and downregulates endocannabinoid expression in order to control food intake and energy balance (495). The peripheral actions of leptin are inhibition of insulin synthesis and secretion in pancreatic  $\beta$ -cell insulin biosynthesis (496). A study found that obesity may result from downregulation of leptin receptor expression and unresponse of leptin signal. A mouse study reported that leptin deficient mice (obese mice and lipodystrophic mice) presented hyperinsulinemia leading to downregulate insulin receptor in liver and adipose tissue (497).

LEP gene has correlation with obesity and BMI. Leptin regulates glucose uptake and fatty acid oxidation and inhibits insulin secretion. The study in Korean women showed that the polymorphism of LEP at rs10954173 and rs11761556 increased the risk of type 2 diabetes (498). The LEP variation (rs7799039) had no

association with type 2 diabetes in a Chinese population (499). Enquobahrie DA and colleagues, analyzing genotype from placenta, reported the LEP expression in GDM group was significantly different from the control (500).

A candidate gene study in South Africans found the association between BMI and LEP polymorphism (rs10954174 and rs6966536) (501). However, the study in Finland (502), and Italy (503) presented no association between rs2167270 and obesity. A meta-analysis study reported no association between obesity and rs2167270 and rs7799039 (375).

#### **r) LEPR (Leptin receptor)**

LEPR encoded a single transmembrane protein mediating the action of leptin. LEPR gene mutation causes impaired receptor signaling of leptin, and related to obesity, hyperleptinemia, and atherogenic lipid profiles (504). A study found that LEPR mutation in obese patients resulted in severe obesity, immune dysfunction, pituitary dysfunction, hyperphagia, and delayed puberty (505, 506).

The meta-analysis study, analyzing data from 16 studies, presented the significant correlation between polymorphism at rs1137101 in LEPR and the risk of type 2 diabetes (507). The polymorphisms at rs1892534 and rs2211651 are associated with early onset type 2 diabetes mellitus in a Taiwanese population (508). No association between LEPR polymorphism was reported in Korean or Chinese Han populations (362, 498). Moreover, the correlation between LEPR and GDM has not been reported.



The gene association study found that BMI was significantly related to the polymorphism in LEPR at rs1137100, rs1137101, rs12033452, rs3790419, and rs7518632 (498). Other candidate gene studies in Caucasians presented the correlation between rs1137101 and rs9436746 and BMI (509, 510). Meta-analysis studies found that the LEPR gene (rs1137101, rs1137100, rs8179183, and rs62589000, rs10889567, rs3790437) was not associated with obesity (375, 510).

**s) MTNR1B (Melatonin receptor 1B)**

MTNR1B encodes melatonin 2 protein (MT2), a receptor for melatonin, which is expressed in  $\beta$ -cells. MT2 is involved in insulin secretion in  $\beta$ -cells because melatonin inhibits adenylate cyclase/cyclic the guanylate cyclase/cyclic adenosine monophosphate (AC/cAMP), guanosine monophosphate (GC/cGMP), and 1,4,5-trisphosphate (IP3) signal pathways (511). Many studies reported that MT2 receptor was associated with decreased glucagon secretion and alternative glucose metabolism (512-515). Therefore, *MTNR1B* polymorphisms affect pancreatic glucose sensing, insulin secretion, and glucose tolerance (513, 514). MTNR1B is associated with fasting plasma glucose levels in type 2 diabetes (514).

A meta-analysis study reported the MTNR1B (rs10830963) increases the risk of type 2 diabetes (514). This SNP is also associated with GDM in a Czech Republic study (516). Other candidate gene approaches in Han Chinese (517) and Greek (518) populations found similar SNP results to the study from the Czech Republic. In addition, the study in the Korean population (519) showed that the

variation at rs10830963 and rs1387153 in MTNR1B was associated with GDM. This was supported by the results of a meta-analysis of ten articles (3428 GDM and 4637 controls) with the same SNPs (477). The GWAS in the Korean population proposed the new SNP (rs10830962) associated with GDM (402). However, Wang Y presented no association between MTNR1B (rs10830963) and GDM (403).

**t) PPARG (Peroxisome proliferator-activated receptor gamma)**

The PPARG gene is associated with insulin action, adipocyte differentiation, lipid storage, and fat-specific gene expression (520). Kim et al. (521) reported that PPARG activates glucose transporter 2 and glucokinase in liver and pancreatic  $\beta$ -cells, which improves glucose homeostasis. Moreover, PPARG increases insulin sensitive in peripheral tissue and glucose sensitivity of liver and pancreatic  $\beta$ -cells. In a mouse study, PPARG knockout mice higher insulin-induced increase in glucose disposal rate and greater insulin-induced suppression of hepatic glucose production than in wildtype mice (522).

The candidate gene studies in Chinese (411), Japanese (523), and Indian (461) populations and the GWAS in Finnish (397) and Caucasian populations (400) found that the PPARG variant (rs1801282) increased the risk of type 2 diabetes. However, a genetic study in the Chinese Han population reported no association between type 2 diabetes and PPARG variants at rs1801282, rs12636454, and rs11128597 (389). The WTCCC study in England, reported a controversial association between rs1801282 and type 2 diabetes mellitus (396).

The association between GDM and PPARG variant (Both rs1801282 and rs3856806) was found in the study in French (524). However, a candidate gene approach in Sweden, Denmark and Korea did not report the association between PPARG variation (rs3856806 and rs1801282) and GDM (401, 404, 525). The association between rs1801282 and total body fat mass was proposed in menopause women (526).

**u) SLC30A8 (Solute carrier family 30 (zinc transporter), member 8)**

SLC30A8, a member of zinc transporter (ZNT) family, encodes zinc transporter (ZnT8). ZnT proteins transport zinc out of cells when zinc is excess, and sequester cytoplasmic zinc into cell when zinc is replete. Zinc facilitates the formation of dense core granules for insulin crystallization in pancreatic  $\beta$ -cell and has a positive influence on insulin gene transcription (527). A mouse study (528) showed that ZnT8 knockout mice reduced zinc content in pancreatic  $\beta$ -cell insulin-secretory granules affecting insulin processing and crystallization. Reduced zinc concentration in the secretory granules leads to increased proinsulin to insulin ratio in blood circulation and decreased glucose-induced insulin secretion (527).

Studies in Lebanese (418), Japanese (391) and Dutch (409) populations reported no association between type 2 diabetes and the polymorphism in SLC30A8 (rs3802177 and rs13266634). Studies of Chinese, British and Indian populations (396, 411, 461) including the GWAS in French and Caucasian populations (400, 443) found that the polymorphism at rs13266634 was

associated with type 2 diabetes. This SNP was also associated with GDM in a Korean population (401). In addition, the genetic study of the Han Chinese population found an association between type 2 diabetes and SLC30A8 at rs3802177 rs11558471, and rs13266634 (389). However, the rs13266634 mutation had no significant association with type 2 diabetes in an African American population (414).

**v) TCF7L2 (Transcription factor 7-like 2)**

The TCF7L2 gene encodes a high mobility group box-containing transcription factor related to blood glucose homeostasis. Yi et al. reported that TCF7L2 regulated proglucagon by repressing the glucagon gene in enteroendocrine cells via Wnt signaling pathway (529). TCF7L2 gene is related to pancreatic cells development and glucose-induced insulin secretion (530). A study in human found that patients with type 2 diabetes decreased TCF7L2 protein levels in pancreas when compared with healthy controls. This study proposed that interaction between TCF7L2 and glucagon-like peptide-1 (GLP1R) and glucose-dependent insulintropic polypeptide (GIPR) may regulate pancreatic  $\beta$ -cell function and survival (531).

In Dutch, Han Chinese, British, Korean, Chinese, African American, Arabic and Indian populations, the polymorphism in TCF7L2 (rs7903146) increases the risk of type 2 diabetes (394, 396, 409, 412, 414, 461, 532) and increases in risk of GDM in Scandinavian (525), Korean (401), Danish (404), and Czech (533) women. The study in China (389) also reported an association

between type 2 diabetes and two SNP (rs7903146 and rs6585205). The association between the SNP rs10885409 and type 2 diabetes was reported in a North Indian population (413). However, a study in Netherland reported no correlation between the variant rs4430796 in TCF7L2 gene (450). The association between type 2 diabetes and rs7903146 was confirmed in the GWAS in French, Finnish and Caucasian population (397, 400, 443). Moreover, the Japanese GWAS reported that additional SNP rs7901695 increased the incidence of type 2 diabetes (399). A Meta-analysis of GWAS in African American population found additional SNP (rs114748339) associated with type 2 diabetes (487).

Candidate gene studies in Austria (534), Spain (421), and the Czech Republic (533) reported additional SNPs (rs12255372, rs4506565, and rs7901695, respectively) related to GDM. However, the same study in Korea (401) and Denmark (404) also showed that variation at rs12255372 in TCF7L2 had no correlation with GDM.

#### **w) TNF- $\alpha$ (Tumor necrosis factor alpha)**

TNF- $\alpha$  encoded a cell signaling protein produced at inflammatory site. TNF- $\alpha$  also activates multiple protein kinases and phosphoprotein phosphatases. A mouse study found that TNF- $\alpha$  caused insulin resistance because TNF- $\alpha$  infusion activated protein kinase A, which inhibited the tyrosine kinase activity of insulin receptor (535). TNF- $\alpha$  is interferes insulin signaling in adipose cell, muscle, and liver. TNF- $\alpha$  inhibit glucose-induced insulin secretion (536). A cell study by Tsiotra et al. (537) found that TNF- $\alpha$  suppressed both basal and glucose-

induced insulin secretion and proinsulin mRNA transcription. Hotamisligil et al. (535) described the mechanism of TNF- $\alpha$ -induced insulin resistance, TNF- $\alpha$  reduced GLUT4 mRNA levels in adipocyte and myocyte and inhibited insulin-stimulated glucose transport.

The study in a Finnish population reported that the polymorphism in TNF- $\alpha$  at rs1800610 increased the risk of type 2 diabetes (449). Additional SNP (rs361525) associated with type 2 diabetes was found in a Mexican population (538), but not in Taiwanese and British populations (539, 540). The study in China (541) and Mexico (542) presented that GDM was associated with the variant rs1800629 in TNF- $\alpha$ . However, the study in Malaysia (468) and Brazil (543), reported no significant association between TNF- $\alpha$  variation (rs1800629) and GDM. Additionally, the study in Tunisia, Taiwan, and Mexico presented no association between the variant rs1800629 in TNF- $\alpha$  and type 2 diabetes mellitus (538, 539, 544). A meta-analysis study based on Asian and Caucasian population and the large-scale study in a British population confirmed the association between the SNP rs1800629 and the diabetes (540, 545).

The study in a European population (Ireland and France) reported that the TNF- $\alpha$  polymorphism (rs1800629) increased the risk of obesity (BMI >30 kg/m<sup>2</sup>) (546). The association between rs1800629 and obesity was confirmed with a meta-analysis study (375). However, other studies in Caucasian population presented no correlation between TNF- $\alpha$  variation and obesity (544, 547-549).

## **2.4 Genetic Study in Epidemiology**

### **2.4.1 Overview**

Genetic epidemiology aims to explain the role of genetic variation leading to disease risk in populations. The study of genetic epidemiology relies on biometrical work and statistical methods (segregation analysis, linkage analysis, association analysis, and simulation methods). Genetically influenced diseases can be divided into monogenic diseases (diseases resulting from a single gene mutation) and complex or multifactorial diseases (diseases occurring with genetic and environmental factors) and each uses a different method of analysis.

When information about DNA was unavailable, scientists used the Mendelian laws of inheritance to study the correlation between genetic variation and diseases. Nowadays, with extensive data on the human genome, the genome scan and candidate genes approach have been used to analyze the association between complex diseases and genes.

### **2.4.2 Genetic epidemiology approaches**

#### **a) Candidate gene approaches**

##### **i) Segregation analysis**

Segregation analysis is useful in the study of monogenic diseases.

This approach uses phenotypic data within pedigrees to determine the pattern of inheritance of major genes influencing a phenotype and to estimate the parameters of the genetic model (recessive, dominant, co-dominant), which is based on a probability calculation to observe the

phenotype on parameters in genetic models and on family structure. The most likely model nested within a general model is selected by likelihood-ratio test. Moreover, complex segregation analysis may be used for multifactorial disease. However, segregation analysis is not useful in the case of non-major genes and a few moderately influenced genes (550).

**ii) Linkage analysis**

Linkage studies assess on which part of the chromosome a disease gene is located. Linkage analysis investigates the association between a marker and a disease in related individuals. This approach aims to examine the linkage between a susceptible locus and disease, and estimates the recombination rate. There are two methods for linkage analysis namely model-based (parametric or “lod score”) and model free (nonparametric). The lod score method is based on the likelihood of the observed marker and disease in a family under the model for distribution of the unobserved disease gene. Nonparametric methods compare the proportion of alleles shared IBD (identical by descent) by pairs of affected relatives with the proportion expected based on their relationship. The degree of complexity of the disease has an effect on the genetic model assumption (550).

**iii) Linkage disequilibrium (genetic association methods)**

Association studies investigate which allele or genotype of genes are associated with disease. The case-control study design compares allele



or genotype frequencies in a group of unrelated affected individuals with a group of unrelated unaffected individuals. However, populations consisting of two or more subgroups with different allele frequencies, different confounding factors, and different baseline rates of disease, may cause increased false positive associations and biased relative risk estimation. Therefore, matching or adjusting for race can be used to control for the problems arising from a variety in a population stratification.

The family-based study design was created to determine the association between suspected alleles of diseases with internal control of confounding. There are two types of controls in family-based study designs; sibling controls or case-parent-trios (parental control or pseudo-siblings). The data analysis is based on a standard conditional logistic regression model. The case-sibling study design asserts that affected individuals are cases and their unaffected siblings are controls. In a case-parent-trio study design, only cases and their parents are genotyped and form the set of hypothetical pseudo-sibling consisting of the three other genotypes which could have been transmitted from the parents. The case-pseudo sibling sets are analyzed as 1:3 matched case-control design. The internal control in the model comes from the two parental alleles that are not transmitted to the affected child. The common test for case-parent-trio study design is the Transmission Disequilibrium test (TDT), which

compares the transmitted alleles with non-transmitted alleles from heterozygous parents to child. The TDT is a test of both linkage and association together. This design avoids possible bias from inadequate controls and population stratifications (550).

#### **b) Genome-Wide Association Studies (GWAS)**

GWAS is a standard approach for exploring the basis of complex genetic diseases from hundreds of thousands of single-nucleotide polymorphisms (SNPs) by the case-control, population-based prospective and cross-sectional study designs. Due to the ability to assay more than a million SNPs, GWAS has become a new and powerful tool in searching for novel biological insights to explain susceptibility for diseases (550).

The power of GWAS to identify true genetic associations depends on the quality of the database. In the beginning of GWAS, the most associations discovered in GWAS were indirectly causal because a small fraction of the genetic variation in the genome was detected. With the extensive availability of genome variation data, GWAS can infer the genotypes for most of the common variants in the genome by using an imputation technique. However, many associations between SNPs and diseases are weak with the odd ratios around 1.2-1.6. In order to avoid false positive claims, the genome-wide level of significance ( $p\text{-value} < 5 \times 10^{-8}$ ) was calculated from the Bonferroni correction for one million SNPs. Moreover, ethnic variation in a population also may result in a biased interpretation. To help deal with this problem the STRUCTURE program (the

adjustment for an estimate of global ancestry from a finite set of founding populations using ancestry informative marker), and the EIGENSTRAT program (applying the principal components from all or subset of the markers) have been proposed to control for false positives in a heterogeneous population. Due to a limited genome database and statistic dependency, GWAS requires a large sample size and multiple replications of similarly large samples in order to effectively provide evidence of genetic association (215).

The associations between genes and disease from GWAS explain a small proportion of the genetic causes of disease. Therefore, secondary analysis of GWAS data including targeted hypothesis testing, gene-gene interaction, and gene-environment interaction analysis may further our understanding of the etiology of genotype and phenotype and improve the ability to detect relevant genetic polymorphisms.

### c) **Gene-Environment interaction**

The effects of genetic variants may depends on environment exposures or the effect of an environment factors depends on an individual genotype. G-E interaction model may increase the power to detect novel genes or environmental factors that influence the trait through the interaction. In term of statistics, G-E interaction is defined as a deviation from a model on a particular scale by using linear regression models for quantitative traits and logistic regression models for binary traits (551).

#### d) Gene-Gene (G-G) interaction

G-G interaction may be analyzed by pathway-based approaches. Pathway analysis determines association between phenotype and sets of genes corresponding to biological pathways, which provide larger effects, the greater power of discovery, and natural connections to biological mechanisms. Pathway-based approaches may determine environmental factors interacting with the set of genes, which leads to a complex phenotype (552).

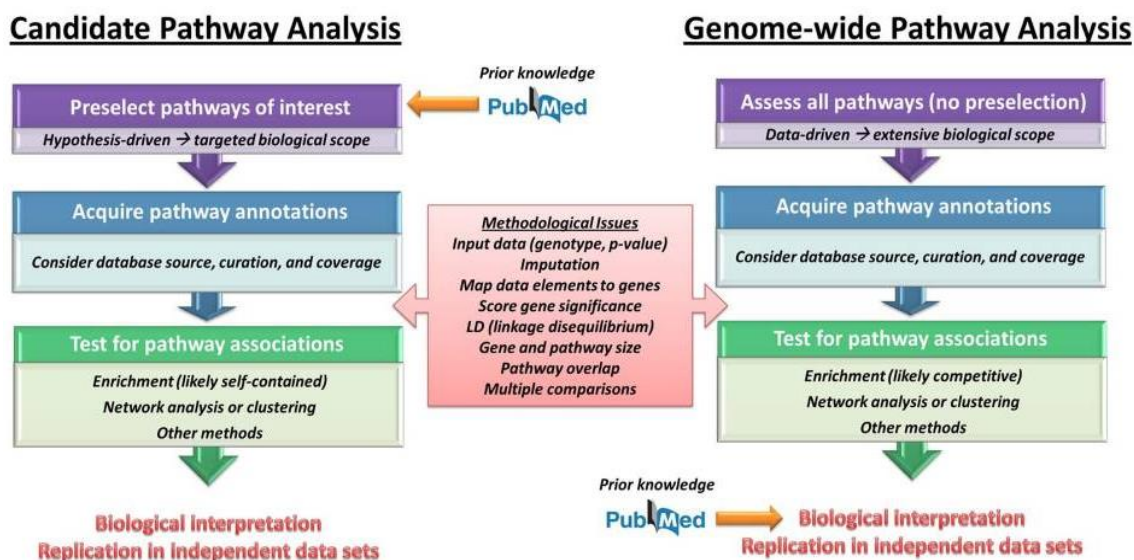


Figure 2.3 Guideline for pathway analysis (552).

Table 2.2 Summary of candidate genes with established associations with orofacial clefts found in dissertation literature review to have published links with type 2 diabetes, gestational diabetes mellitus, or obesity.

Gene ID	Gene name	Chromosome	OFCs <sup>1</sup>	T2DM <sup>2</sup>	GDM <sup>3</sup>	Obesity
ADAMTS9	ADAM metalloproteinase with thrombospondin type 1 motif, 9	3p14.1	(553) <sup>a</sup>	(409, 418, 554)	-	(555)
CBS	Cystathionine-beta-synthase	21q22.3	(556, 557) <sup>b</sup>	(558)	-	(559)
COMT	Catechol-O-methyltransferase	22q11.21	(560, 561) <sup>b</sup>	-	-	(562)
CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1	2p22.2	(563) <sup>b</sup>	-	-	(564)
ESR1	Estrogen receptor 1	6q25.1	(565) <sup>b</sup>	(566)	-	-
ETV5	Ets variant 5	3q28	(422, 567, 568) <sup>b</sup>	-	-	(569)
F13A1	Coagulation factor XIII, A1 polypeptide	6p25.3-p24.3	(570-572) <sup>b</sup>	-	-	(573)
FAF1	Fas (TNFRSF6) associated factor 1	1p33	(574) <sup>a</sup>	(575)	-	-
GDF15	Growth differentiation factor 15	19p13.11	(576) <sup>b</sup>	-	(500)	-
GSTT1	Glutathione S-transferase theta 1	22q11.23	(134, 577) <sup>b</sup>	(578)	-	-
HLA-B	Major histocompatibility complex, class I, B	6p21.3	(579) <sup>b</sup>	(487)	-	-
KLF9	Kruppel-like factor 9	9q13	(580) <sup>b</sup>	-	-	(405)
LMX1B	LIM homeobox transcription factor 1, beta	9q33.3	(581) <sup>a</sup>	-	-	(582)
MAF	V-maf avian musculoaponeurotic fibrosarcoma oncogene homolog	16q22-q23	(139) <sup>b</sup>	-	-	(428)
MTHFR	Methylenetetrahydrofolate reductase (NAD(P)H)	1p36.3	(583-586) <sup>b</sup>	(558, 587)	-	(559)

Table 2.2 Summary of candidate genes with established associations with orofacial clefts found in dissertation literature review to have published links with type 2 diabetes, gestational diabetes mellitus, or obesity (Cont.)

Gene ID	Gene name	Chromosome	OFCs <sup>1</sup>	T2DM <sup>2</sup>	GDM <sup>3</sup>	Obesity
MTR	5-methyltetrahydrofolate-homocysteine methyltransferase	1q43	(588, 589) <sup>b</sup>	(558)	-	(558, 559)
PAX6	Paired box 6	11p13	(590) <sup>b</sup>	-	-	(569, 591)
PCYT1A	Phosphate cytidyltransferase 1, choline, alpha	3q29	(592, 593) <sup>b</sup>	-	-	(594)
PEMT	Phosphatidylethanolamine N-methyltransferase	17p11.2	(592) <sup>b</sup>	(587)	-	(594)
RBP4	Retinol binding protein 4, plasma	10q23.33	(595) <sup>b</sup>	-	(596)	-
RPS7	Ribosomal protein S7	2p25	(597) <sup>b</sup>	-	(500)	-
SLC8A1	Solute carrier family 8 (sodium/calcium exchanger), member 1	2p22.1	(598) <sup>b</sup>	(599)	-	-
STK11	Serine/threonine kinase 11	19p13.3	(600) <sup>b</sup>	(601)	-	-
TCN2	Transcobalamin II	22q12.2	(602) <sup>b</sup>	(558, 559)	-	(558, 559)
TFAP2B	Transcription factor AP-2 beta (activating enhancer binding protein 2 beta)	6p12	(603) <sup>b</sup>	-	-	(569, 591)
TGFB1	Transforming growth factor, beta 1	19q13.1	(604-606) <sup>b</sup>	-	(541)	(607)
THADA	Thyroid adenoma associated	2p21	(139, 608) <sup>b</sup>	(609-611)		
VEGFA	Vascular endothelial growth factor A	6p12	(612) <sup>a</sup>	-	-	(555)

<sup>1</sup>OFCs Orofacial clefts; <sup>2</sup>T2DM, type 2 diabetes mellitus; <sup>3</sup>GDM, gestational diabetes mellitus

Genes associated with OFCs: <sup>a</sup>, animal study; <sup>b</sup>, human study

Genes associated with T2DM, GDM, and obesity base on human study

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus.

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
AATF	Apoptosis antagonizing transcription factor	17q12	-	-	(573)
ABCC1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	16p13.1	-	-	(573)
ABCC8	ATP-binding cassette, sub-family C (CFTR/MRP), member 8	11p15.1	(354-356)	(354)	
ACE	Angiotensin I converting enzyme	17q23.3	(613)	-	(614, 615)
ACTN2	Actinin, alpha 2	1q42-q43	(616)	-	-
ADAM12	ADAM metalloproteinase domain 12	10q26	-	(500)	(573)
ADCY10	Adenylate cyclase 10 (soluble)	1q24	(611, 617)	-	-
ADCY3	Adenylate cyclase 3	2p23.3	-	-	(440)
ADCY5	Adenylate cyclase 5	3q21.1	(618)	-	-
ADD2	Adducin 2 (beta)	2p13.3	-	(500)	-
ADIPOR1	Adiponectin receptor 1	1q32.1	(619)	-	-
ADIPOQ	Adiponectin, C1Q and collagen domain containing	3q27	(361-366)	(369-372)	(373, 374)
ADRB2	Adrenoceptor beta 2, surface	5q31-q32	(620)	-	(385)
ADRB3	Adrenoceptor beta 3, surface	8p12	(365, 380, 382)	(381)	(385, 386)
AGPAT4	1-acylglycerol-3-phosphate O-acyltransferase 4	6q26	-	(500)	-
AGRP	Agouti related neuropeptide	16q22	-	-	(621)
AGT	Angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	14q22	(622)	-	(623)

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.)

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
AGTR1	Angiotensin II receptor, type 1	3q24	(622)	-	
AKAP1	A kinase (PRKA) anchor protein 1	17q22	-	-	(582, 624)
ALX4	ALX homeobox 4	11p11.2	(418)	-	
ANAPC13	Anaphase promoting complex subunit 13	3q22.2	-	-	(582)
ANGPTL6	Angiopoietin-like 6	19p13.2	-	-	(625)
ANKRD50	Ankyrin repeat domain 50	4q28.1	(616)	-	-
ANXA4	Annexin A4	2p13	(449)	(500)	-
AP3S2	Adaptor-related protein complex 3, sigma 2 subunit	15q26.1	(626)	-	-
APCS	Amyloid P component, serum	1q21-q23	-	-	(625)
APLP2	Amyloid beta (A4) precursor-like protein 2	11q24	-	-	(582)
APOC1	Apolipoprotein C-I	19q13.2	-	-	(582)
APOE	Apolipoprotein E	19q13.2	(627)	-	(607, 628)
AQP3	Aquaporin 3 (Gill blood group)	9p13	-	(500)	-
ARAP1	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 1	11q13.4	(450)	-	-
ARHGAP44	Rho GTPase activating protein 44	17p12	(599)	-	-
ARID1B	AT rich interactive domain 1B (SWI1-like)	6q25.1		-	(582)
ARL15	ADP-ribosylation factor-like 15	5p15.2	(575)	-	-
AZGP1	alpha-2-glycoprotein 1, zinc-binding	7q22.1	-	-	(629)
BCDIN3D	BCDIN3 domain containing	12q13.12	-	-	(582)
BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)	2p16.1	(450)	-	-
BCL2	B-cell CLL/lymphoma 2	18q21.3	(630)	-	-
BCL2A1	BCL2-related protein A1	15q24.3	-	-	(426)



Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
BDNF	Brain-derived neurotrophic factor	11p13	(419, 631, 632)	-	(426, 591, 633, 634)
BHLHE40	Basic helix-loop-helix family, member e40	3p26	-	(500)	-
C15orf41	Chromosome 15 open reading frame 41	15q14	-	-	(582)
C16orf62	Chromosome 16 open reading frame 62	16p12.3	-	-	(573)
C1D	C1D nuclear receptor corepressor	2p13-p12	-	(500)	-
C2CD4A/4B	C2 calcium-dependent domain containing 4A/4B	15q22.2	(450)	-	-
CADM2	Cell adhesion molecule 2	3p12.1	-	-	(569)
CALM1	Calmodulin 1 (phosphorylase kinase, delta)	14q32.11	-	(500)	(569, 591)
CAPN10	Calpain 10	2q37.3	(475, 635)	-	(636)
CARHSP1	Calcium regulated heat stable protein 1, 24kDa	16p13.2	-	(500)	-
CASP9	Caspase 9, apoptosis-related cysteine peptidase	1p36.21	(617)	-	-
CCDC102B	Coiled-coil domain containing 102B	18q22.1	(617)	-	-
CCDC80	Coiled-coil domain containing 80	3q13.2	-	-	(573)
CCL2	CD276 molecule	15q23-q24	-	-	(573)
CD63	CD63 molecule	12q12-q13	-	(500)	-
CD93	CD93 molecule	20p11.21	-	(500)	-
CD96	CD96 molecule	3q13.13-q13.2	(599)	-	-
CDH12	Cadherin 12, type 2 (N-cadherin 2)	5p14.3	-	-	(582)
CDKAL1	CDK5 regulatory subunit associated protein 1-like 1	6p22.3	(389-398, 400, 405)	(401, 402, 404)	
CDKN2A	Cyclin-dependent kinase inhibitor 2A	9p21	(461)	-	-

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
CDKN2A/2B	Cyclin-dependent kinase inhibitor 2A/2B	9p21	(390, 394-397, 400, 409-413)	(401, 403)	-
CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha	19q13.1	-	(500)	-
CES1	Carboxylesterase 1	16q22.2	-	-	(624)
CFB	Complement factor B	6p21.3	-	-	(637)
CHCHD2P9	Coiled-coil-helix-coiled-coil-helix domain containing 2 pseudogene 9	9q21.31	(450)	-	-
CHDH	Choline dehydrogenase	3p21.1	(558)	-	(558)
CHRNA5	Cholinergic receptor, nicotinic, alpha 5 (neuronal)	15q24	(638)	-	-
CHRNA2	Cholinergic receptor, nicotinic, beta 2	1q21.3	(638)	-	-
CHRNA4	Cholinergic receptor, nicotinic, beta 4	15q24	(638)	-	-
CIDEA	Cell death-inducing DFFA-like effector a	18p11.21; 18	-	-	(573)
CLDN7	Claudin 7	17p13.1	-	(500)	-
CLMN	Calmin (calponin-like, transmembrane)	14q32.13	-	-	(569, 573)
CMIP	C-Maf inducing protein	16q23	(639)	-	-
CNGB3	Cyclic nucleotide gated channel beta 3	8q21.3	-	-	(582)
CNPY2	Canopy FGF signaling regulator 2	12q15	-	(500)	-
CNTN1	Contactin 1	12q11-q12	(599)	-	-
CNTNAP4	contactin associated protein-like 4	16q23.1	-	-	(582)
COL13A1	Collagen, type XIII, alpha 1	10q22	(599)	-	-
COL17A1	Collagen, type XVII, alpha 1	10q24.3	-	(500)	-
COL8A1	Collagen, type VIII, alpha 1	3q12.3	(418)	-	-
CPE	Carboxypeptidase E	4q32.3	-	-	(621)

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
CPEB4	Cytoplasmic polyadenylation element binding protein 4	5q21	-	-	(555)
CPVL	Carboxypeptidase, vitellogenic-like	7p15.1	-	(500)	(573)
CRTC2	CREB regulated transcription coactivator 2	1q21.3	(601)	-	-
CSN3	Casein kappa	4q21.1	(599, 616)	-	-
CTSA	Cathepsin A	20q13.1	-	-	(629)
CWC22	CWC22 spliceosome-associated protein	2q31.3	(599)	-	-
CXCL12	Chemokine (C-X-C motif) ligand 12	10q11.1	-	-	(564)
DAPK2	Death-associated protein kinase 2	15q22.31	-	-	(573)
DARS	Aspartyl-tRNA synthetase	2q21.3	-	-	(582)
DBC1	Deleted in bladder cancer 1	chromosome: 15	(616)	-	(582)
DBI	Diazepam binding inhibitor (GABA receptor modulator, acyl-CoA binding protein)	2q12-q21	-	-	(564)
DNAJC15	DnaJ (Hsp40) homolog, subfamily C, member 15	13q14.1	-	-	(582)
DNAJC27	DnaJ (Hsp40) homolog, subfamily C, member 27	2p23.3	-	-	(569)
DRD2	Dopamine receptor D2	11q23	-	-	(640, 641)
DUSP9	Dual specificity phosphatase 9	Xq28	(450)	-	-
DYNLL1	Dynein, light chain, LC8-type 1	12q24.23	-	(500)	-
DYT7	Dystonia 7, torsion (autosomal dominant)	18p	(642)	-	-
EFNA5	Ephrin-A5	5q21	-	-	(582)
EGR2	Early growth response 2	10q21.1	(616)	-	-
EIF4B	Eukaryotic translation initiation factor 4B	12q13.13	-	(500)	-

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
EIF4E3	Eukaryotic translation initiation factor 4E family member 3	3p14	-	-	(582)
EIF4EBP1	Eukaryotic translation initiation factor 4E binding protein 1	8p12	-	-	(573)
ENPP1	Ectonucleotide pyrophosphatase/ phosphodiesterase 1	6q22-q23	(365, 449, 643)	-	-
EPB41L3	Erythrocyte membrane protein band 4.1-like 3	18p11.32	(616)	-	-
ESRRG	Estrogen-related receptor gamma	1q41	(599)	-	-
ETV5	Ets variant 5	3q28	-	-	(569)
EXT2	Exostosin glycosyltransferase 2	11p12-p11	(644)	-	-
FABP2	Fatty acid binding protein 2, intestinal	4q28-q31	(645)	-	(628)
FAIM2	Fas apoptotic inhibitory molecule 2	12q13	(419)	-	(569, 582, 646)
FAM129A	Family with sequence similarity 129, member A	1q25	(599)	-	-
FAM19A5	Family with sequence similarity 19 (chemokine (C-C motif)-like), member A5	22q13.32	-	-	(582)
FANCL	Fanconi anemia, complementation group L	2p16.1	-	-	(569)
FBXL17	F-box and leucine-rich repeat protein 17	5q21.3	(599)	-	-
FERMT1	Fermitin family member 1	20p12.3	-	(500)	-
FHIT	Fragile histidine triad	3p14.2	(599)	-	-
FHOD3	Formin homology 2 domain containing 3	18q12	-	-	(582)
FLT1	Fms-related tyrosine kinase 1	13q12	-	(500)	-
FOSL2	FOS-like antigen 2	2p23.3	-	(500)	-
FOXA2	Forkhead box A2	20p11	-	-	(582)
FOXP3	Forkhead box N3	14q31.3	(397)	-	(582)

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
FTO	Fat mass and obesity associated	16q12.2	(396, 397, 418-420, 422, 423)	(421)	(423-428)
FXRD5	FXRD domain containing ion transport regulator 5	19q13.12	-	(500)	-
GABRA4	Gamma-aminobutyric acid (GABA) A receptor, alpha 4	4p12	(647)	-	-
GALNT10	Polypeptide N-acetylgalactosaminyltransferase 10	5q33.2	-	-	(440)
GALNT2	Polypeptide N-acetylgalactosaminyltransferase 2	1q41-q42	-	(500)	(440)
GAS6	Growth arrest-specific 6	13q34	-	-	(564)
GATAD2A	GATA zinc finger domain containing 2A	19p13.11	(630)	-	-
GBA	Glucosidase, beta, acid	1q21	-	(500)	-
GCK	Glucokinase	7p15.3-p15.1	(432, 433)	(434-436)	-
GCKR	Glucokinase (hexokinase 4) regulator	2p23	(611, 618)	-	-
GDNF	Glial cell derived neurotrophic factor	5p13.1-p12	(616)	-	-
GFM1	G elongation factor, mitochondrial 1	3q25	(599)	-	-
GHRL	Ghrelin/obestatin prepropeptide	3p26-p25	-	-	(621, 648)
GIPR	Gastric inhibitory polypeptide receptor	19q13.3	-	-	(405, 591)
GLIS3	GLIS family zinc finger 3	9p24.2	(356, 639)	-	-
GLP1R	Glucagon-like peptide 1 receptor	6p21	-	-	(621)
GNG7	Guanine nucleotide binding protein (G protein), gamma 7	19p13.3	-	(500)	-
GNPDA2	Glucosamine-6-phosphate deaminase 2	4p12	(419, 422)	-	(425, 426, 440)
GP2	Glycoprotein 2 (zymogen granule membrane)	16p12	-	-	(591)

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
GPC5	Glypican 5	13q32	(599)	-	-
GPRC5B	G protein-coupled receptor, class C, group 5, member B	16p12	-	-	(569)
GRB10	Growth factor receptor-bound protein 10	7p12.2	(599)	-	-
GRB14	Growth factor receptor-bound protein 14	2q22-q24	(626)	-	(555)
GRIK1	Glutamate receptor, ionotropic, kainate 1	21q22.11	(616)	-	-
GRIN2B	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B	12p12	-	-	(582)
GRK5	G protein-coupled receptor kinase 5	10q26.11	(649)	-	-
GRM3	Glutamate receptor, metabotropic 3	7q21.1-q21.2	-	-	(582)
GYPC	Glycophorin C (Gerbich blood group)	2q14-q21	(616)	-	-
HADHA	Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), alpha subunit	2p23	-	-	(564)
HCN4	Hyperpolarization activated cyclic nucleotide gated potassium channel 4	15q24.1	-	-	(582)
HDGF	Hepatoma-derived growth factor	1q23.1	-	(500)	-
HES1	Hes family bHLH transcription factor 1	3q28-q29	-	-	(564)
HHEX	Haematopoietically expressed homeobox	10q23.33	(389, 391, 394-397, 400, 410, 413, 443)	(401)	-
HMG20A	High mobility group 20A	15q24	(626)	-	-
HMGA2	High mobility group AT-hook 2	12q15	(450, 487)	-	-
HNF1A	HNF1 homeobox A	12q24.2	(446-450)	(434)	-

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
HNF1B	Hepatocyte nuclear factor 1-beta	17q12	(356, 432, 433, 450)	(404)	-
HNF4A	Hepatocyte nuclear factor 4-alpha	20q13.12	(450, 626)	-	-
HOXB1	Homeobox B1	17q21.3	-	-	(582)
HOXB5	Homeobox B5	17q21.3	-	-	(650)
HOXC13	Homeobox C13	12q13.3	-	-	(555)
HPSE2	Heparanase 2 (inactive)	10q23-q24	(616)	-	
HSPA1B	Heat shock 70kDa protein 1B	6p21.3	-	-	(547)
HTR2A	5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled	13q14-q21	-	-	(621)
IDE	Insulin-degrading enzyme	10q23-q25	(651)	(402)	-
IFI30	Interferon, gamma-inducible protein 30	19p13.1	-	(500)	-
IGKC	Immunoglobulin kappa constant	2p12	(652)	-	-
IGF2BP2	Insulin-like growth factor 2mRNA binding protein 2	3q27.2	(391, 394, 395, 398-400, 410, 411, 461)	(402, 403, 462)	-
IL10	Interleukin-10	1q31-q32	(467)	(468)	(471)
IL18RAP	Interleukin 18 receptor accessory protein	2q12	-	-	(564)
IL1 $\beta$	Interleukin-1 beta	2q14	(653, 654)	-	-
IL1R1	Interleukin 1 receptor, type I	2q12	-	-	(564)
IL1RN	Interleukin-1 receptor antagonist	2q14.2	(654, 655)	-	-
IL4	Interleukin-4	5q31.1	(655, 656)	-	-
IL6	Interleukin-6	7p21	(449, 544, 657, 658)	-	(375, 544, 659)

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
IL6ST	Interleukin 6 signal transducer	5q11.2	-	-	(564)
INHA	Inhibin, alpha	2q35	-	(500)	-
INS	Insulin	11p15.5	-	(384)	-
IRF5	Interferon regulatory factor 5	7q32	-	-	(629)
IRS1	Insulin receptor substrate-1	2q36	(474, 475, 480)	(384, 477-479)	(481)
IVNS1ABP	Influenza virus NS1A binding protein	1q25.1-q31.1	-	-	(573)
JAZF1	JAZF zinc finger 1	7p15.2-p15.1	(409, 609, 651)	-	-
JUN	Jun proto-oncogene	1p32-p31	-	-	(564)
KCND2	Potassium channel, voltage gated Shal related subfamily D, member 2	7q31	(599)	-	-
KCNIP3	Kv channel interacting protein 3, calsenilin	2q21.1	(651)	(500)	-
KCNJ11	Potassium channel, inwardly rectifying subfamily J, member 11	11p15.1	(389, 397, 400, 410, 411, 461)	(482)	-
KCNK16	Potassium channel, two pore domain subfamily K, member 16	6p21.2-p21.1	(356, 639)	-	-
KCNQ1	Potassium channel, voltage gated KQT-like subfamily Q, member 1	11p15.5-p15.4	(489)	(491-494)	-
KCTD15	Potassium channel tetramerization domain containing 15	19q13.11	-	-	(569)
KCTD8	Potassium channel tetramerization domain containing 8	4p13	(611, 647)	-	-
KIF1C	Kinesin family member 1C	17p13.2	-	(500)	-



Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
KLF9	Kruppel-like factor 9	9q13	-	-	(405)
KLF14	Kruppel-like factor 14	7q32.3	(450)	-	-
KLHL32	Kelch-like family member 32	6q16.1	-	-	(440)
LEP	Leptin	7q31.3	(498)	(660, 661)	(501-503)
LEPR	Leptin receptor	1p31	(507, 508)	-	(498, 509, 510)
LIMCH1	LIM and calponin homology domains 1	4p13	-	-	(582)
LINGO2	Leucine rich repeat and Ig domain containing 2	9p21.2	(616)	-	(569)
LPIN1	Lipin 1	2p25.1	-	-	(625)
LPL	Lipoprotein lipase	8p22	-	-	(628)
LPP	LIM domain containing preferred translocation partner in lipoma	3q28	(575)	-	-
LRP1B	Low density lipoprotein receptor-related protein 1B	2q21.2	-	-	(569, 646)
LTA	Lymphotoxin alpha	6p21.3	(397, 662)	-	-
LY86	Lymphocyte antigen 86	6p25.1	-	-	(555)
LYPLAL1	Lysophospholipase-like 1	1q41	-	-	(555)
MARCH4	Membrane-associated ring finger (C3HC4) 4, E3 ubiquitin protein ligase	2q35	-	-	(582)
MAEA	Macrophage erythroblast attacher	4p16.3	(663)	-	-
MAGEA9	Melanoma antigen family A9	Xq28	-	(500)	-
MAP2K5	Mitogen-activated protein kinase kinase 5	15q23	-	-	(569, 591)
MAPRE2	Microtubule-associated protein, RP/EB family, member 2	18q12.1	(664)	-	-
MATN3	Matrilin 3	2p24-p23	-	-	(582, 591)

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
MBL2	Mannose-binding lectin (protein C) 2, soluble	10q11.2	-	(665)	-
MCF2L2	MCF.2 cell line derived transforming sequence-like 2	3q27.1	(368)	-	-
MC4R	Melanocortin 4 receptor	18q22	(611)	-	(405, 428, 440, 569, 591, 666)
MC5R	Melanocortin 5 receptor	18p11.2	-	-	(667)
MIF	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	22q11.23	-	(500)	-
MINA	MYC induced nuclear antigen	3q11.2	-	-	(582)
MMADHC	Methylmalonic aciduria (cobalamin deficiency) cbID type, with homocystinuria	2q23.2	(611)	-	-
MPHOSPH9	M-phase phosphoprotein 9	12q24.31	(575)	-	-
MRC1	Mannose receptor, C type 1	10p12.33	-	-	(573, 629)
MSH6	MutS homolog 6	2p16	(599)	-	-
MSRA	Methionine sulfoxide reductase A	8p23.1	(478, 668)	-	-
MT1A	Metallothionein 1A	16q13	(669)	-	-
MTCH2	Mitochondrial carrier 2	11p11.2	-	-	(569)
MTHFD1L	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like	6q25.1	(558)	-	(558)
MTIF3	Mitochondrial translational initiation factor 3	13q12.2	-	-	(569)
MTHFSD	Methenyltetrahydrofolate synthetase domain containing	16q24.1	(599)	-	-
MTNR1A	Melatonin receptor 1A	4q35.1	-	(517)	-

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
MTNR1B	Melatonin receptor 1B	11q21-q22	(514, 517, 518)	(402, 477, 516, 519)	-
MYT1L	Myelin transcription factor 1-like	2p25.3	-	-	(582)
NAA25	N(alpha)-acetyltransferase 25, NatB auxiliary subunit	12q24.13	-	-	(573)
NAP5	Non-intrinsic ABC protein 5	chromosome: 1	-	-	(582)
NCKAP5	NCK-associated protein 5	2q21.2	(599)	-	-
NCOA1	Nuclear receptor coactivator 1	2p23	-	-	(564)
NEGR1	Neuronal growth regulator 1	1p31.1	-	-	(569, 634)
NEUROD1	Neuronal differentiation 1	2q32	(433, 449)	-	-
NEUROG3	Neurogenin 3	10q21.3	(670)	-	-
NFE2L3	Nuclear factor, erythroid 2-like 3	7p15.2	-	-	(555)
NKX2-2	NK2 homeobox 2	20p11.22	(356)	-	-
NKX6-1	NK6 homeobox 1	4q21.33	(356)	-	-
NOTCH2	Notch 2	1p13-p11	(609, 610)	-	-
NPC1	Niemann-Pick disease, type C1	18q11.2	-	-	(428)
NPY	Neuropeptide Y	7p15.1	-	-	(509, 623)
NPY1R	Neuropeptide Y receptor Y1	4q32.2	-	-	(621, 624)
NPY2R	Neuropeptide Y receptor Y2	4q31	-	-	(386, 671)
NPY5R	Neuropeptide Y receptor Y5	4q32.2	-	-	(621)
NRF1	Nuclear respiratory factor 1	7q32	(672)	-	-
NRP1	Neuropilin 1	10p12	(599)	-	-
NRXN1	Neurexin 1	2p16.3	-	-	(582)
NRXN3	Neurexin 3	14q31	-	-	(569, 582)

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
NUDT3	Nudix (nucleoside diphosphate linked moiety X)-type motif 3	6p21.2	-	-	(569)
OLFM4	Olfactomedin 4	13q14.3	-	-	(646, 650)
OR13D1	Olfactory receptor, family 13, subfamily D, member 1	9q31.1	(616)	-	(440)
OSTF1	Osteoclast stimulating factor 1	9q13-q21.2	-	-	(582)
OVCH2	Ovochymase 2 (gene/pseudogene)	11p15.4	-	-	(582)
PALLD	Palladin, cytoskeletal associated protein	4q32.3	-	-	(573)
P2RX4	Purinergic receptor P2X, ligand gated ion channel, 4	12q24.32	-	-	(673)
PANK4	Pantothenate kinase 4	1p36.32	(617)	-	-
PAR3B	Par-3 family cell polarity regulator beta	2q33.3	-	-	(582)
PCDH20	Protocadherin 20	13q21	-	-	(582)
PCK1	Phosphoenolpyruvate carboxykinase 1 (soluble)	20q13.31	(449)	-	-
PCSK1	Proprotein convertase subtilisin/kexin type 1	5q15-q21	-	-	(591, 674, 675)
PCSK2	Proprotein convertase subtilisin/kexin type 2	20p11.2	(676)	-	-
PDX1	Pancreatic and duodenal homeobox 1	13q12.1	(432)	-	-
PEPD	Peptidase D	19q13.11	(356, 639)	-	-
PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	10p15.1	-	-	(629)
PHLDB1	Pleckstrin homology-like domain, family B, member 1	11q23.3	(616)	-	-
PKHD1	Polycystic kidney and hepatic disease 1	6p12.2	-	-	(582)
PKP1	Plakophilin 1	1q32	-	-	(582)

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
PLA2G16	Phospholipase A2, group XVI	11q12.3	-	(500)	-
PLA2G1B	Phospholipase A2, group IB (pancreas)	12q24.31	-	-	(673)
PLIN2	Perilipin 2	9p22.1	-	(500)	-
PMEPA1	Prostate transmembrane protein, androgen induced 1	20q13.31-q13.33	-	-	(629)
PMS2P3	PMS1 homolog 2, mismatch repair system component pseudogene 3	7q11.23	-	-	(637)
POC5	POC5 centriolar protein	5q13.3	-	-	(569, 582)
POLG2	Polymerase (DNA directed), gamma 2, accessory subunit	17q	-	(500)	-
PON1	Paraoxonase 1	7q21.3	(559)	-	(559)
PPARA	Peroxisome proliferator-activated receptor alpha	22q13.31	-	(462)	-
PPARD	Peroxisome proliferator-activated receptor delta	6p21.2	-	-	(677)
PPARG	Peroxisome proliferator-activated receptor gamma	3p25	(396, 397, 400, 411, 461, 523)	(524)	(526)
PPARGC1A	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	4p15.1	(365)	-	(625)
PPIB	Peptidylprolyl isomerase B (cyclophilin B)	15q21-q22	-	(500)	-
PPP1R3A	Protein phosphatase 1, regulatory subunit 3A	7q31.1	(652)	-	-
PPP1R3B	Protein phosphatase 1, regulatory subunit 3B	8p23.1	(652)	-	-
PRC1	Protein regulator of cytokinesis 1	15q26.1	(450)	-	-
PRKD1	Protein kinase D1	14q11	(647)	-	(569, 646)
PRKG2	Protein kinase, cGMP-dependent, type II	4q13.1-q21.1	(599)	-	-
PRLR	Prolactin receptor	5p13.2	-	(678)	-

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
PROCR	Protein C receptor, endothelial	20q11.2	-	(500)	-
PROX1	Prospero homeobox 1	1q41	(618)	-	-
PSMD6	Proteasome 26S subunit, non-ATPase 6	3p14.1	(356, 449, 639)	-	-
PTBP2	Polypyrimidine tract binding protein 2	1p21.3	-	-	(569)
PTDSS2	Phosphatidylserine synthase 2	11p15.5	-	-	(594)
PTER	Phosphotriesterase related	10p12	-	-	(428)
PTPRD	Protein tyrosine phosphatase, receptor type, D	9p23-p24.3	(490)	-	-
PYY	Peptide YY	17q21.1	-	-	(671)
QPCTL	Glutaminyl-peptide cyclotransferase-like	19q13.32	-	-	(569)
RALGPS2	Ral GEF with PH domain and SH3 binding motif 2	1q25.2	(616)	-	-
RASGRP1	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	15q14	(649)	-	-
RAPGEF1	Rap guanine nucleotide exchange factor (GEF) 1	9q34.3	(672)	-	-
RBFOX1	RNA binding protein, fox-1 homolog (C. elegans) 1	16p13.3	-	-	(679)
RBMS1	RNA binding motif, single stranded interacting protein 1	2q24.2	(680)	-	-
RFNG	RFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase	17q25	-	(500)	-
RIMS1	Regulating synaptic membrane exocytosis 1	6q12-q13	(599)	-	-
RNASE4	Ribonuclease, RNase A family, 4	14q11	-	(500)	(573)
RNF13	Ring finger protein 13	3q25.1	-	-	(629)
RNF138	Ring finger protein 138, E3 ubiquitin protein ligase	18q12.1	(433)	-	(582)
RNLS	Renalase, FAD-dependent amine oxidase	10q23.31	(599)	-	-

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
RORA	RAR-related orphan receptor A	15q22.2	(616)	-	-
RPL27A	Ribosomal protein L27a	11p15	-	-	(569)
RPS29	Ribosomal protein S29	14q	-	(500)	-
RSPO3	R-spondin 3	6q22.33	-	-	(555)
SASH1	SAM and SH3 domain containing 1	6q24.3	(664)	-	-
SAMD12	Sterile alpha motif domain containing 12	8q24.12	(599)	-	-
SCG3	Secretogranin III	15q21	-	-	(637)
SDCCAG8	Serologically defined colon cancer antigen	81q43	-	-	(666)
SDF2L1	Stromal cell-derived factor 2-like 1	22q11.21	(616)	-	-
SEC16B	SEC16 homolog B, endoplasmic reticulum export factor	1q25.2	-	-	(405, 440, 569, 591, 646)
SGCG	Sarcoglycan, gamma (35kDa dystrophin-associated glycoprotein)	13q12	(681)	-	-
SH2B1	SH2B adaptor protein 1	16p11.2	(419)	-	(569, 634, 682)
SH3BGRL	SH3 domain binding glutamate-rich protein like	Xq13.3	-	-	(573)
SIDT1	SID1 transmembrane family, member 1	3q13.2	(599)	-	-
SLC15A4	Solute carrier family 15 (oligopeptide transporter), member 4	12q24.32	(368)	-	-
SLC24A3	Solute carrier family 24 (sodium/potassium/calcium exchanger), member 3	20p13	(616)	-	-
SLC2A1	Solute carrier family 2 (facilitated glucose transporter), member 1	1p34.2	(573, 683)	-	-

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
SLC2A2	Solute carrier family 2 (facilitated glucose transporter), member 2	3q26.1-q26.2	(449, 672)	-	-
SLC30A8	Solute carrier family 30 (zinc transporter), member 8	8q24.11	(389, 391, 396, 400, 409, 411, 418, 443, 461)	(401)	-
SLC39A8	Solute carrier family 39 (zinc transporter), member 8	4q24	-	-	(569, 646)
SLC44A3	Solute carrier family 44, member 3	1p21.3	(664)	-	-
SMPD1	Sphingomyelin phosphodiesterase 1, acid lysosomal	11p15.4-p15.1	-	(500)	-
SNHG11	Small nucleolar RNA host gene 11	20q11.23	-	-	(573)
SOCS3	Suppressor of cytokine signaling 3	17q25.3	-	-	(621)
SOD1	Superoxide dismutase 1, soluble	21q22.11	(684)	-	-
SORBS1	Sorbin and SH3 domain containing 1	10q23.33	(616)	-	-
SPCS3	Signal peptidase complex subunit 3	4q34.2	-	(500)	-
SPECC1	Sperm antigen with calponin homology and coiled-coil domains 1	17p11.2	(599, 611)	-	-
SPSB3	SplA/ryanodine receptor domain and SOCS box containing 3	16p13.3	-	-	(573)
SREBF1	Sterol regulatory element binding transcription factor 1	17p11.2	-	-	(685)
SRI	Sorcin	7q21.1	-	(500)	-
SRR	Serine racemase	17p13	(490)	(403)	-
SRRT	Serrate, RNA effector molecule	7q21	-	(500)	-



Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
ST3GAL6	ST3 beta-galactoside alpha-2,3-sialyltransferase 6	3q12.1	-	-	(573)
ST6GAL1	ST6 beta-galactosamide alpha-2,6-sialyltransferase 1	3q27.3	(626)	-	-
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)	17q21.31	-	-	(621)
STEAP4	STEAP family member 4	7q21.12	-	(500)	-
STRIP1	Striatin interacting protein 1	1p13.3	-	-	(582)
SV2C	Synaptic vesicle glycoprotein 2C	5q13.3	-	-	(582)
TAZ	Tafazzin	Xq28	-	-	(629)
TCEB1	Transcription elongation factor B (SIII), polypeptide 1 (15kDa, elongin C)	8q21.11	(599)	-	(607)
TCF7L2	Transcription factor 7-like 2	10q25.3	(389, 394, 396, 397, 399, 400, 409, 412-414, 443, 461, 487, 532)	(401, 404, 421, 525, 533, 534)	
TFAP2B	Transcription factor AP-2 beta (activating enhancer binding protein 2 beta)	6p12	-	-	(569, 591, 646)
THSD7B	Thrombospondin, type I, domain containing 7B	2q22.1	-	-	-
TLE4	Transducin-like enhancer of split 4	9q21.31	(450)	-	-
TMBIM6	Transmembrane BAX inhibitor motif containing 6	12q13.12	-	-	(436, 634)
TMEFF2	Transmembrane protein with EGF-like and two follistatin-like domains 2	2q32.3	(397, 616)	-	-
TMEM101	Transmembrane protein 101	17q21.31	-	-	(573)

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
TMEM154	Transmembrane protein 154	4q31.3	(575)	-	-
TMEM160	Transmembrane protein 160	19q13.32	-	-	(569)
TMEM163	Transmembrane protein 163	2q21.3	(686)	-	(582)
TMEM18	Transmembrane protein 18	2p25.3	(419)	-	(569, 634, 646, 666, 687)
TNF- $\alpha$	Tumor necrosis factor alpha	6p21.3	(449, 538, 540, 545)	(541, 542)	(375, 546)
TNNI3K	TNNI3 interacting kinase	1p31.1	-	-	(569, 646)
TP53	Tumor protein p53	17p13.1	(672)	-	-
TP53INP1	Tumor protein p53 inducible nuclear protein 1	8q22	(450)	-	-
TRPS1	Trichorhinophalangeal syndrome I	8q24.12	(599)	-	-
TSPAN8	Tetraspanin 8	12q14.1-q21.1	(610)	-	-
TUSC3	Tumor suppressor candidate 3	8p22	-	(500)	-
UBE2E2	Ubiquitin-conjugating enzyme E2E 2	3p24.2	(399)	(688)	-
UCK2	Uridine-cytidine kinase 2	1q23	-	(500)	-
UCK3	None	3p24.2	-	(500)	-
UCP1	Uncoupling protein 1	4q28-q31	-	-	(625, 689)
UCP2	Uncoupling protein 2 (mitochondrial, proton carrier)	11q13	-	-	(385, 628, 685, 690)
UTRN	Utrophin	6q24	(616)	-	-
UCP3	Uncoupling protein 3 (mitochondrial, proton carrier)	11q13.4	(691)	-	(385, 628)
UTS2	Urotensin 2	1p36	-	(500, 692)	-
VLDLR	very low density lipoprotein receptor	9p24	-	-	(624)

Table 2.3 Summary 416 genes found in dissertation literature review to be associated with type 2 diabetes, gestational diabetes mellitus, or obesity related to obesity and/or diabetes mellitus (Cont.).

Gene ID	Gene name	Chromosome	T2DM <sup>1</sup>	GDM <sup>2</sup>	Obesity
VPS13B	Vacuolar protein sorting 13 homolog B (yeast)	8q22.2	-	-	(637)
VPS26A	VPS26 retromer complex component A	10q21.1	(626)	-	-
WFS1	Wolfram syndrome 1	4p16.1	(409, 418, 693)	-	-
WWOX	WW domain containing oxidoreductase	16q23	(639)	-	-
ZBED3	Zinc finger, BED-type containing 3	5q13.3	(450)	-	-
ZC3H4	Zinc finger CCCH-type containing 4	19q13.32	-	-	(582)
ZFAND3	Zinc finger, AN1-type domain 3	6p21.2	(356, 639)	-	-
ZFAND6	Zinc finger, AN1-type domain 6	15q25.1	(450)	-	-
ZNF169	Zinc finger protein 169	9q22.32	-	-	(405)
ZNF608	Zinc finger protein 608	5q23.2	-	-	(569)
ZNF718	Zinc finger protein 718	4p16.3	-	-	(582)

<sup>1</sup>T2DM, type 2 diabetes mellitus

<sup>2</sup>GDM, gestational diabetes mellitus

Genes associated with T2DM, GDM, and obesity base on human study

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## CHAPTER 3

## THE ASSOCIATION BETWEEN MATERNAL PRE-PREGNANCY WEIGHT AND MATERNAL DIABETES AND OROFACIAL CLEFTS IN THE UTAH POPULATION

**3.1 Abstract**

**Background:** The inconsistent association between pre-pregnancy weight and maternal diabetes and risk of orofacial clefts has been reported in a few studies.

**Objective:** To determine the association between maternal pre-pregnancy weight and maternal diabetes mellitus and the risk of orofacial clefts (OFCs) in a population-based case-control study of birth certificate data in Utah.

**Methods:** Cases of OFCs during 1995-2011 were ascertained by the state-wide Utah birth defects registry. Controls were randomly selected from Utah birth certificates at a ratio of 4:1 to cases, matched by birth month and year. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the relative risk for cleft subtypes associated with maternal pre-pregnancy weight and maternal diabetes. Multiple logistic regression analysis was used to adjust for the potential confounding effects of maternal age, education, body mass index (BMI), depression, and maternal diabetes. Pre-existing diabetes, gestational diabetes mellitus (GDM), and all diabetes were evaluated as mediating variables in the association between maternal obesity and risk of OFCs.

**Results:** Results are based on 1,451 live-born cases with registry diagnoses. Obesity increased risk of both non-isolated and isolated OFCs (adjusted odds ratios (aOR): 1.41, 95% CI: 1.07-1.87 and aOR: 1.23, 95% confidence interval (CI): 1.01-1.50, respectively). Underweight mothers had a reduced risk of cleft lip only (CLO) (aOR:

0.46, 95%CI: 0.24-0.88), and an increased risk of cleft palate only (CPO) (aOR: 1.50, 95%CI: 1.04-2.16). Maternal depression increased risk of all OFCs (aOR: 1.31, 95%CI: 1.00-1.73). Pre-existing diabetes increased risk of all OFCs (aOR: 2.19, 95% CI: 1.18-4.09) with a larger effect for non-isolated OFCs (aOR: 3.83, 95%CI: 1.71-8.58) vs isolated OFCs (aOR: 1.54, 95%CI: 0.70-3.41). GDM mothers had an increased risk of all OFCs (aOR: 1.48, 95%CI: 1.05-2.09) with a larger effect for non-isolated OFCs (aOR: 2.29, 95%CI: 1.41-3.72) vs isolated OFCs (aOR: 1.15, 95%CI: 0.75-1.77). Mediation analysis indicated that obesity had a direct effect of increasing the risk of OFCs without the mediating effect of known maternal diabetes.

**Conclusion:** Extremes of maternal pre-pregnancy weight were associated with risk of OFCs in Utah with obese mothers having an increased risk of all types of OFCs, while underweight mothers having a decreased risk of CLO and increased risk of CPO. Maternal depression was also associated with OFC risk. Both pre-existing and gestational diabetes were associated with OFC risk, with strongest effects for non-isolated OFCs. Both stratification and mediation analyses suggest an effect of obesity on OFCs apart from known maternal diabetes. The growing epidemics of obesity and diabetes and the challenge of early detection and treatment of GDM underscore the public health importance of further research in this area.

### 3.2 Introduction

Orofacial clefts (OFCs) are congenital deformations of the lip, palate, or both. Genetic and environmental factors and interaction between both have been reported as the causes of OFCs. OFCs are an important health issue in both developed and

developing countries. The global incidence of OFCs is one in every 500-2500 births depending on geographic location, racial and ethnic groups, maternal age, environmental exposures, and socioeconomic status (1). OFCs affect on average one in every 750 births in the United States and the highest incidence among states with state-wide birth defect registers is in Utah (1 in 450 births) (2).

Maternal obesity increases health risks for both mother and child (3, 4). Maternal obesity has been associated with fetal malformations such as neural tube defects, congenital heart defects, and orofacial clefts (4). Obesity-related birth defect mechanisms include hyperglycemia, insulin resistance, and poor folate status (4, 5). Case-control studies (6-8) and a meta-analysis study (9) reported a positive association between maternal obesity and the occurrence of OFCs while other studies have not (10-12). However, the associations with various cleft phenotypes and the role of maternal underweight are less well studied. An international consortium of OFC case-control studies found associations between both maternal obesity and underweight risk of cleft palate with or without cleft lip, but not with cleft lip alone (13).

Diabetes mellitus is a disorder of the regulation of blood glucose. Hyperglycemia is a characteristic of diabetes mellitus resulting from a defect in insulin secretion or insulin action or both (14). Diabetes mellitus also stimulates the production of adverse metabolic factors including ketone bodies, branched chain amino acid and advanced glycation end products and these factors may disrupt normal embryonic development (15, 16). Maternal diabetes leads to altered expression levels of specific genes and increases

the variation of gene expression levels which may also disrupt embryonic development (17, 18).

Human studies have reported associations between maternal diabetes and the risk of OFCs. Data from a large international consortium from the U.S., Denmark, and Norway confirmed that maternal diabetes increased the incidence of OFCs after adjusting for maternal age, education levels, multivitamin use, maternal BMI categories, and history of smoking (19). The Atlanta Birth Defects Case-Control Study (20) showed that being an insulin-dependent diabetic mother increased the risk of cleft palate. The data from the National Birth Defect Prevention Study (NBDPS) found that both pre-gestational (type 1 or 2) and gestational diabetes mellitus increased the risk of isolated cleft lip with or without cleft palate (CL/P) and cleft palate only (CPO) (21). Moreover, two large studies reported that maternal diabetes increased the incidence of OFCs (19, 22). However, another study from NBDPS did not find an association between gestation diabetes mellitus (GDM) and risk of cleft lip with cleft palate or cleft lip only (23).

The inconsistent association between pre-pregnancy weight and maternal diabetes and risk of OFCs has been reported in a few studies. Few studies have presented the association between maternal obesity and diabetes and risk of OFCs based on subtypes of clefts (non-isolated vs isolated and combinations of lip and palate). Moreover, there is no previous study that has reported the effect of maternal obesity on risk of OFCs mediated by maternal diabetes. The investigation of maternal weight and diabetes may confirm or provide additional information regarding OFC associations. Thus, this study aims to determine the association between pre-pregnancy weight and

maternal diabetes and risk of OFCs in the Utah population using a complete sample from a state-wide birth defects registry linked to birth certificates.

### **3.3 Subjects and Methods**

#### **3.3.1 Study design, population, and data sources**

The study design is a case-control study. The study protocol was reviewed and approved by the institutional review boards of Utah State University (USU), the University of Utah, and the Utah Department of Health.

Cases of OFCs were ascertained by the Utah Birth Defect Network (UBDN), operated by the Utah Department of Health (UDOH). UBDN, a statewide population-based surveillance system, identifies all prenatal or postnatal major structural birth defects in fetuses and neonates (24). The OFC classifications used in the data analyses were based on the final UBDN diagnoses based on a review by a medical geneticist. OFC cases resulting from pregnancy outcomes (live birth, stillbirth, or pregnancy termination) were divided into cleft lip alone, cleft palate alone, cleft lip without cleft palate, and cleft lip with cleft palate and classified as isolated, syndromic, or multiple birth defect cases. The case mothers of a child with an OFC during 1995-2011 were linked to the Utah Population database (UPDB) (24). The UPDB provides information for research on genetics, epidemiology, demography, and public health. The database represents Utah's population appearing in administrative records from the late 18<sup>th</sup> century to the present and receives annual updates from birth and death certificates, hospitalization and ambulatory surgery records, and driver licenses. Controls were randomly selected from Utah birth certificates at a ratio of 4:1 to live-born cases matched by birth month and

year. The anonymized identification numbers of cases and controls from UPDB were linked to the Utah Birth Certificate database. In addition, the UPDB provided information on OFC cases noted in fetal and neonatal death records.

Utah birth certificate forms were revised in 1991, 1997, 2003, and 2009 and these were used to collect data on cases and controls born during 1995-2011. The birth certificate data consists of information on newborns (sex, date of birth, birth weight and length, and birthplaces) and their parents (race/ethnicity, ages, and marital status, and the number of children born to the mother, etc.), including delivery complications, maternal medical risk factors, congenital malformations, and birth injuries.

Relevant changes in birth certificates were in the classification of maternal and paternal education, maternal alcohol consumption, maternal smoking, maternal medical risk factors, and notation of congenital anomalies. In the years of 1991- 2008, maternal and paternal education levels were recorded as the number of highest grades completed, and changed to be eight categories of the highest degree of school completed in 2009 (8<sup>th</sup> grade or less, 9<sup>th</sup>-12<sup>th</sup> grade, high school graduate, some college credit but no degree, associate degree, Bachelor's degree, Master's degree, and Doctorate or professional degree). Maternal smoking and alcohol consumption were recorded as average number of cigarettes per day and average number of drinks per week in each trimester of gestation. In 2009, a question about maternal tobacco use three months before pregnancy was added and a question on maternal alcohol use was deleted. Questions on history of gestational diabetes mellitus and pre-existing diabetes were added in 1997 in order to replace a more general question on unspecified diabetes. Many medical risk factors were added in 2009

including depression. Cleft lip, cleft palate, and cleft lip and palate were separately coded after 2008 on birth certificate instead of cleft lip/palate in previous forms under the question of congenital anomalies of child. Medical risk factors were noted primarily by text fields and ICD-9-CM codes. Due to the non-specific question on diabetes mellitus on birth certificate forms during 1995-1996, these years were excluded from analyses.

### **3.3.2 Statistical analysis**

The Statistical Package for Social Sciences (IBM SPSS) statistics version 22 for Windows was used for data analyses. Descriptive analyses were conducted to determine the distribution of maternal characteristics (age, body mass index (BMI), smoking status, education level, and diabetic status) by OFC case-control status. Chi-square testing was used to assess the association between OFCs and categorical variables. Multivariable logistic regression was used to determine the odds ratio estimating the association between maternal BMI categories, diabetes mellitus, gestational diabetes mellitus, and depression and the risk of orofacial clefts with adjustment for potentially confounding variables. Stratified analyses were conducted to evaluate effect measure modification.

Mediation analysis was conducted using the approach introduced by Vanderweele et al. (25). This approach is based on the counterfactual framework of mediation analysis (26), and extends mediation analysis for a dichotomous outcome with exposure-outcome confounders, mediator-outcome confounders, and exposure-mediator interaction. Pre-gestational diabetes mellitus, GDM and hyperglycemia combined during pregnancy were considered as mediators of the association between maternal overweight and obesity and risk of OFCs.

### 3.4 Results

A total of 1,750 cases of OFCs were ascertained from the UBDN including live births (n=1596, 91.2%), stillbirths (n=59, 3.4%), terminations (n=81, 4.6%), and spontaneous abortions (n=14, 0.8%). 1,611 OFC live-born cases were linked to the birth certificate records; 139 cases were excluded from the case-control study because they were not live-births (n=136) or non-Utah maternal residences (n=3). Overall prevalence of OFCs based on the UBDN registry data, trended to decline over the period 2000-2011 (average 2.09 per 1000 live births) and the decline appeared to be due to a decline in isolated OFCs while the prevalence of non-isolated OFCs appeared relatively constant (Figure 3.1). Birth certificates reported 77.6% OFC cases (n=1,250) determined by the UBDN registry (n=1,611) and the completeness of reporting improved from 71.1% during 1995 to 91.9% during 2011.

Among OFC cases born during 1997-2011, non-isolated OFCs accounted for 29.8% (n=432), isolated OFCs accounted for 70.2% (n=1091) of all OFC cases; 34.3% were cleft palate only (CPO) (n=497), 25.3% were cleft lip only (CLO) (n=367), and 40.5% were cleft lip with cleft palate (CLP) (n=587).

Mean of maternal age at birth of non-isolated cases but not isolated cases was higher than controls (27.6±5.9 years vs 27.1±5.5 years). There was no significant difference between controls and all OFCs cases in the mean of paternal age at birth of index child or parity. Maternal age greater than 35 years increased risk of non-isolated OFCs when compared with mother age of 20-35 years (OR: 1.62, 95% CI: 1.18-2.23). The association between paternal age and risk of OFCs was not found. There was no



association between mothers and fathers of age nineteen or younger and risk of OFCs (Table 3.2). Mothers of all OFCs and isolated OFCs had higher mean BMI than control mothers (Table 3.1). The prevalence of depression, pre-existing DM and GDM in both non-isolated and isolated OFCs were higher than controls.

Higher maternal and paternal education levels (higher than bachelor degree vs high school diploma or less) were associated with a decreased risk of both all and isolated OFCs (maternal education; OR: 0.85, 95% CI: 0.73-0.98, p-trend = 0.03 and OR: 0.82, 95% CI: 0.69-0.98, p-trend = 0.03 respectively and paternal education: OR: 0.76, 95% CI: 0.63-0.92, p-trend = 0.01 and OR: 0.75, 95% CI: 0.60-0.95, p-trend = 0.01 respectively) (Table 3.2). The education associations appeared stronger for isolated OFCs compared to non-isolated OFCs.

The birth certificate data for maternal smoking and alcohol consumption after 1999 had missing rates over than 90%, thus, these data after 1999 were excluded from analysis. Mothers with depression had an increased risk of all OFCs (OR: 1.38; 95% CI: 1.06-1.80) and the estimates were similar, but with wider confidence intervals for the sub-groups of non-isolated OFCs (OR: 1.60; 95% CI: 1.60 -2.43) (Table 3.2).

Obesity (BMI > 30 kg/m<sup>2</sup>) compared to normal weight increased risk of all OFCs, non-isolated, and isolated OFCs with p-trends < 0.0001, 0.01, and 0.01, respectively (Table 3.2). The adjusted odds ratio for obese mothers (BMI ≥ 30 kg/m<sup>2</sup>) was 1.29 (95% CI: 1.09-1.53) for all OFCs, 1.41 (95% CI: 1.07-1.87) for non-isolated OFCs, and 1.23 (95% CI: 1.01-1.50) for isolated OFCs when compared to mothers with normal BMI. Maternal underweight was associated with a decreased risk of isolated CLO (aOR

(adjusted odds ratio): 0.42; 95% CI: 0.20-0.85) and increased risk of all CPO (aOR: 1.50; 95% CI: 1.04-2.16) after adjustment for maternal age, education, depression, and all diabetes (Table 3.3). The associations between maternal obesity and all, non-isolated, and isolated OFCs were only slightly less after adjustment for maternal age, education, depression, and all diabetes (Table 3.4). Linear regression analysis showed that the slope of prevalence of maternal obesity during 1997-2011 increased every year for both OFC groups and controls. The slope of the regression line of case mothers was significantly higher than the slope of control mothers (p-value = 0.028) (Figure 3.2).

Mother with pre-existing diabetes had an increased risk of all OFCs (aOR: 2.19; 95% CI: 1.18-4.09) with a stronger effect for non-isolated OFCs (aOR: 3.83; 95% CI: 1.71-8.58) vs isolated OFCs (aOR: 1.54; 95% CI: 0.70-3.41) when adjustment for maternal age, maternal education, depression, and BMI (Table 3.5). Based on cleft subtypes, pre-existing diabetes increased risk of non-isolated CLP, CL/P (cleft lip with/without cleft palate) and cleft palate with or without cleft lip (CP/L) (aOR: 6.23; 95% CI: 2.31-16.81, aOR: 4.64; 95% CI: 1.74-12.40, and aOR: 4.41; 95% CI: 1.96-9.90, respectively). GDM mothers had an increased risk of all OFCs (aOR: 1.48, 95%CI: 1.05-2.09) with a larger effect for non-isolated OFCs (aOR: 2.29, 95%CI: 1.41-3.72) vs isolated OFCs (aOR: 1.15, 95%CI: 0.75-1.77) (Table 3.6). Associations between GDM and risk of non-isolated CPO and CP/L were found (aOR: 2.91; 95% CI: 1.59-5.32, and aOR: 2.22; 95% CI: 1.32-3.73, respectively). All diabetes had an increased risk of all OFCs (aOR: 1.52; 95% CI: 1.14-2.04) with a stronger effect for non-isolated OFCs (aOR: 2.38; 95% CI: 1.58-3.59) vs isolated OFCs (aOR: 1.18; 95% CI: 0.82-1.71) (Table 3.7).

In addition, all diabetes increased risk of non-isolated CPO, CLP, CL/P, and CP/L (aOR: 2.71; 95% CI: 1.59-4.62, aOR: 2.08; 95% CI: 1.06-4.07, aOR: 2.05; 95% CI: 1.13-3.72 and aOR: 2.43; 95% CI: 1.58-3.74, respectively)

Mediation analyses adjusting for maternal age and education indicated that there are direct effects of maternal obesity on all, non-isolated, and isolated OFCs not mediated through maternal diabetes (OR: 1.31; 95% CI: 1.11-1.55, OR: 1.45; 95% CI: 1.09-1.92, OR: 1.25; 95% CI: 1.03-1.53, respectively) (Table 3.8). There was no apparent interaction between obesity and maternal diabetes. The odds ratio for the indirect (mediation) effect of pre-existing diabetes was 1.02 (95% CI: 1.00-1.04) for all OFCs and 1.06 (95% CI: 1.01-1.12) for non-isolated OFCs. However, there was no apparent mediation effect of GDM and all DM on risk of OFCs (all, non-isolated, and non-isolated OFCs). Stratified analyses based on logistic regression with the same covariates revealed similar associations between obesity and OFC risk for both non-diabetic (aOR: 1.33; 95% CI: 1.12-1.57) and diabetic (aOR: 1.38; 95% CI: 0.67-2.85). Moreover, maternal diabetes increased risk of OFCs in both normal weight and obese mothers (aOR: 1.45; 95% CI: 0.83-2.52 and aOR: 1.37; 95% CI: 0.86-2.19, respectively)

### 3.5 Discussion

This study found that obese mothers had an increased risk of both non-isolated and isolated OFCs, while underweight mothers had a decreased risk of CLO and an increased risk of CPO. An association between maternal depression and risk of OFCs was also found in this study. In addition, mothers with pre-existing diabetes had an increased

risk for having a child with non-isolated CLP, CL/P, and CP/L, and GDM mothers were associated with non-isolated CPO and CP/L.

The study was population-based and relatively robust against selection bias. OFC cases were drawn from the state-wide Utah birth defects registry, which provided exhaustive case-finding from multiple sources and determination of OFC diagnosis by medical geneticist review. Birth certificates were obtained for 99.7 percent of live-born registry cases. OFC cases that were not live-born were excluded from the case-control analysis because of the difficulty of selecting appropriate controls but were included in the estimates of overall rates of OFCs in Utah. Cleft diagnoses from birth certificate records included 77.6% of cases found by the registry over the entire study period, however the completeness of cleft diagnoses from birth certificate records tended to improve over time thus birth certificate diagnoses may be used to analyze OFC occurrence in areas that lack registries but that have high-quality completion of birth certificates. Data on maternal medical conditions (diabetes mellitus, GDM, depression, and hypertension) and potential confounders were obtained from birth certificate records, and these conditions are likely under-reported. We found a large amount of missing data on smoking and alcohol use, thus we were unable to control for the potential confounding by these factors. The prevalence of OFCs has been decreasing, yet the rate of OFC risk factors including obesity, and diabetes have been increasing. This indicates that only one environmental risk factor cannot explore or predict the OFC occurrence.

This study found that underweight mothers had a significantly decreased risk of CLO, but increased risk of CPO. To our knowledge the protective effect of maternal

underweight on risk of CLO has not been previously reported and is inconsistent with the previous study by Kutbi et al (27), which reported that maternal underweight marginally increased risk of isolated CLO and CPO. The characteristic of underweight mothers in OFCs requires further study. Moreover, we found an increased risk of CPO, CLP, CL/P, and CP/L with maternal obesity, which is consistent with a previous study by Kutbi et al suggesting that obesity has a specific effect on palate formation but not lip formation (27). A recent meta-analysis by Blanco et al also presented that maternal obesity increased risk of CPO and CL/P (28).

The association between maternal diabetes and risk of non-isolated OFCs is consistent with limited previous studies (19, 21). Correa et al. using the data from the National Birth Defect Prevention Study (NBDPS) during 1997-2003 showed an association between both pre-existing diabetes (type 1 or 2) and GDM and risk isolated OFCs, while only pre-existing diabetes was associated with syndromic OFCs (21). Correa et al. also reported higher estimates of odds ratios and wider confidence intervals than our Utah study (non-isolated CPO, OR: 10.73, 95% CI: 3.99-28.86 and non-isolated CL/P, OR: 8.07, 95% CI: 3.05-21.39). Data from a large international consortium of case-control studies from the U.S., Denmark, and Norway (19) found an association between GDM and syndromic OFCs and CPO. A prospective study by Moore et al (10) reported suggestive associations between pre-existing (type 1 or 2) and GDM and risk of OFCs but also with very wide confidence intervals that included 1.0 (prevalence ratios (PR): 8.9, 95% CI: 0.85-46.5; and PR: 2.6, 95% CI: 0.82-8.5, respectively).

The data on pre-existing diabetes and GDM in our Utah study are based on the birth certificate record and may be underreported. A study by DeSisto et al. (29) comparing prevalence of GDM in the United States from birth certificates and the Pregnancy Risk Assessment Monitoring System (PRAMS) reported that prevalence of GDM in Utah from PRAMS (6.4%) was higher than the prevalence from birth certificates (4.0%) in 2010. A study by Owen-Gary and War reported that only 44.8% of the confirmed GDM cases had a GDM diagnosis in their medical records (30). The discrepancies among birth certificates, PRAMS, and medical records may result from under diagnosis and from poor quality of transcription or documenting medical record data on birth certificates. The current gold standard for GDM screening, oral glucose tolerance tests (OGTT), is applied during 24<sup>th</sup> -28<sup>th</sup> week of gestation. However, there is no clear agreement on the diagnostic criteria for GDM using oral glucose tolerance tests (OGTT) because different organizations have provided different guidelines and thresholds (31). These reasons lead to under-diagnosing and unclear-documenting of GDM on birth certificates. Therefore, the association between GDM and risk of non-syndromic OFCs may be underestimated because of underreported GDM on birth certificates.

We found a direct effect of obesity and small indirect (mediating) effect of pre-existing diabetes on risk of OFCs. Both stratification and mediation analyses indicated that the effect of obesity on increasing risk of OFCs apart from the presence of known maternal diabetes. However, mediation analysis may not be a powerful method when

there is a small sample size of pregnancies exposed to diabetes and the presence of undetected diabetes in the population.

Based on stratified analysis, diabetic mothers with normal BMI (18.5-24.9 kg/m<sup>2</sup>) also have an increased risk of OFCs. This relevance of this further finding can be appreciated by considering studies of non-obese diabetics (BMI 18-24.9 kg/m<sup>2</sup>). A cohort study in the United States minority populations (32) found that about 13% of participants were normal weight diabetics (BMI 17-25 kg/m<sup>2</sup>); Asians had a five-times higher prevalence of diabetes in the normal weight group when compared to the obese group (17% vs 4%). This study also reported that normal weight diabetics had more rapid pancreatic beta cell failure than obese diabetics, which is also supported by the observation of impaired pancreatic insulin secretion in normal weight diabetics from a prospective study in the United Kingdom (33). Moreover, a case-control study in Portugal (34) reported that normal weight diabetic patients had higher chemerin levels than controls. Chemerin is an adipokine regulating adipocyte development and differentiation and glucose metabolism in liver and skeletal muscle tissue. The pathophysiology of normal weight diabetes and the association between chemerin and other metabolic factors require further study.

Due to the complex relationship between BMI and diabetes it is difficult to establish that BMI and diabetes are entirely independent causes of effects on OFCs. From stratified analysis, maternal underweight had a protective effect on the formation of lip alone which happens during 4<sup>th</sup>-8<sup>th</sup> week of gestation, while both maternal diabetes and obesity had an effect of an increased risk of cleft palate which occurs during 6<sup>th</sup>-12<sup>th</sup> week

of gestation. BMI and maternal diabetes might have effects on facial formation in a variety of pathways. BMI is a rather crude measurement of body composition and is not a precise indicator of fat mass, fat distribution, or metabolic status. Abdominal obesity has a higher lipolysis rate than peripheral fat depots and is associated with adverse conditions of the metabolic syndrome including elevated free fatty acids (FFA), cytokines, and adipokines (35, 36). In addition, obesity increases activity of cytochrome P450 (CYP) 2E1, which may lead to poor folate status (37). Several epidemiological studies have reported that obesity increases the risk of inadequate folate status (38-41); BMI may have an adverse effect on cellular uptake and tissue distribution of folate. Obesity leads to insulin resistance, the impairment of insulin sensitivity in sites of glucose disposal, which can develop to type 2 diabetes mellitus and GDM (42). Many studies (43-45) reported that obese women increased risk of developing GDM.

The mechanisms explaining the relationship between maternal diabetes and orofacial clefts are unknown. Elevated blood glucose and insulin stimulates the production of many adverse metabolic factors including ketone bodies, branched chain amino acid, inflammatory markers, advanced glycation end products, altered expression levels of specific genes, and increase the variation of gene expression levels (15-18). These factors may disrupt normal embryonic development.

Our results suggest that in Utah maternal obesity, depression, and diabetes are associated with increased risk of OFCs, and underweight mothers had a decreased risk of CLO. Mechanistic studies are needed to understand the causal effects of maternal obesity, underweight, depression, metabolic abnormalities, and diabetes on OFCs risk. Effective



interventions are needed for promoting healthy body weight and metabolic status in reproductive age women in order to reduce the risk of OFCs. Effective early screening for GDM risk is needed for the periconceptional period and in the first month of gestation in order to allow early interventions for controlling hyperglycemia, hyperinsulinemia and other associated metabolic abnormalities to prevent OFCs and other congenital malformations.

Table 3.1 Demographic characteristics of orofacial cleft cases and controls (1997-2011)

Parameters	Control N = 5804	All_OFCs n = 1451	Non-isolated OFCs n = 432	Isolated OFCs n = 1091
<b>Age of father</b>	29.6±6.0	29.6±6.2	29.7±5.9	29.5±6.2
<b>Age of mother</b>	27.1±5.5	27.2±5.6	27.6±5.9*	27.0±5.5
<b>Number of previous live births now still live</b>	1.4±1.4	1.4±1.5	1.4±1.6	1.3±1.4
<b>Maternal BMI<sup>a</sup></b>	24.5±5.5	25.0±5.7**	25.0±5.9	24.9±5.6**
<b>Maternal BMI<sup>a</sup> category</b>				
Underweight (<18.5 kg/m <sup>2</sup> )	348 (6.2%)	78 (5.7%)	27 (6.7%)	51 (5.2%)
Normal (18.5-24.9 kg/m <sup>2</sup> )	3252 (58.2%)	757 (55.1%)	219 (54.8%)	538 (55.3%)
Overweight (25-29.9 kg/m <sup>2</sup> )	1212 (21.7%)	293 (21.3%)	73 (18.3%)	220 (22.6%)
Obese (≥30 kg/m <sup>2</sup> )	777 (13.9%)	245 (17.8%)	81 (20.3%)	164 (16.9%)
<b>Maternal Race/ethnicity</b>				
White, non-Hispanic	4668 (80.4%)	1150 (79.3%)	330 (76.4%)	820 (75.2%)
Black, non-Hispanic	45 (0.8%)	6 (0.4%)	2 (0.5%)	4 (0.4%)
Asian or Pacific Islander	118 (2.0%)	28 (1.9%)	8 (1.9%)	20 (1.8%)
American Indian	71 (1.2%)	29 (2.0%)	12 (2.8%)	17 (1.6%)
Hispanic	833 (14.4%)	210 (14.5%)	69 (16.0%)	141 (12.9%)
Unknown	58 (1.0%)	17 (1.2%)	8 (1.9%)	9 (0.8%)
<b>Paternal Race/ethnicity</b>				
White, non-Hispanic	4392 (75.7%)	1089 (75.1%)	308 (71.3%)	781 (71.6%)
Black, non-Hispanic	56 (1.0%)	7 (0.5%)	1 (0.2%)	6 (0.5%)
Asian or Pacific Islander	89 (1.5%)	20 (1.4%)	8 (1.9%)	12 (1.1%)
American Indian	38 (0.7%)	15 (1.0%)	7 (1.6%)	8 (0.7%)
Hispanic	683 (11.8%)	168 (11.6%)	59 (13.7%)	109 (10.0%)
Unknown	155 (2.7%)	31 (2.1%)	11 (2.5%)	20 (1.8%)

Table 3.1 Demographic characteristics of orofacial cleft cases and controls (1997-2011) (Cont.)

Parameters	Control N = 5804	All_OFCs n = 1451	Non-isolated OFCs n = 432	Isolated OFCs n = 1091
<b>Maternal education</b>				
High school or less	2402 (41.9%)	633 (44.6%)	184 (43.9%)	449 (44.9%)
Some college or bachelor	1812 (31.6%)	447 (31.5%)	129 (30.8%)	318 (31.8%)
Higher than bachelor	1520 (26.5%)	340 (23.9%)	106 (25.3%)	234 (23.4%)
<b>Paternal education</b>				
High school or less	1924 (36.4%)	502 (38.9%)	149 (39.0%)	353 (38.9%)
Some college or bachelor	2526 (47.8%)	623 (48.3%)	183 (47.9%)	440 (48.5%)
Higher than bachelor	831 (15.7%)	165 (12.8%)	50 (13.1%)	115 (12.7%)
<b>Hypertension<sup>a</sup></b>	373 (6.4%)	93 (6.4%)	31 (7.2%)	62 (6.1%)
<b>Depression</b>	224 (3.9%)	76 (5.2%)	26 (6.0%)	50 (4.9%)
<b>Pre-existing diabetes mellitus<sup>b</sup></b>	29 (0.5%)	16 (1.1%)	8 (2.0%)	8 (0.8%)
<b>Gestational diabetes mellitus<sup>b</sup></b>	133 (2.3%)	49 (3.4%)	22 (5.2%)	27 (2.7%)
<b>All diabetes mellitus<sup>c</sup></b>	181 (2.8%)	69 (4.3%)	31 (6.5%)	38 (3.4%)

<sup>a</sup> Hypertension refers to chronic hypertension, pregnancy induced hypertension, pre-eclampsia, eclampsia, and toxemia

<sup>b</sup> Based on data during 1997-2011

<sup>c</sup> All diabetes mellitus refers to combination between pre-existing DM and GDM

Table 3.2 Association between demographic factors and risk of OFCs

Characteristic	All OFCs (N=1451)		Non-isolated OFCs (N=432)		Isolated OFCs (N=1091)	
	OR (95%CI)	p-value	OR (95%CI)	p-value	OR (95%CI)	p-value
<b>Maternal age</b>						
Less than 19 years	0.89 [0.70-1.11]	0.29	0.78 [0.52-1.19]	0.25	0.93 [0.72-1.20]	0.56
20-35 years	1 [Reference]		1 [Reference]		1 [Reference]	
More than 35	1.21 [0.98-1.50]	0.08	1.62 [1.18-2.23]	0.003	1.04 [0.81-1.34]	0.77
<b>Paternal age</b>						
Less than 19 years	0.68 [0.44-1.06]	0.09	0.39 [0.14-1.07]	0.07	0.81 [0.50-1.31]	0.38
20-35 years	1 [Reference]		1 [Reference]		1 [Reference]	
More than 35	1.11 [0.94-1.30]	0.22	1.06 [0.80-1.40]	0.68	1.13 [0.94-1.36]	0.21
<b>Maternal BMI<sup>a</sup></b>						
Underweight (<18.5 kg/m <sup>2</sup> )	0.96 [0.74-1.25]	0.77	1.15 [0.76-1.74]	0.50	0.89 [0.65-1.21]	0.44
Normal (18.5-24.9 kg/m <sup>2</sup> )	1 [Reference]		1 [Reference]		1 [Reference]	
Overweight (25-29.9 kg/m <sup>2</sup> )	1.04 [0.89-1.21]	0.62	0.89 [0.68-1.18]	0.42	1.10 [0.93-1.30]	0.29
Obese (≥30 kg/m <sup>2</sup> )	1.36 [1.15-1.60]	<0.001	1.55 [1.19-2.02]	0.001	1.28 [1.05-1.56]	0.01
	p-trend <sup>1</sup> 0.001		p-trend <sup>1</sup> 0.01		p-trend <sup>1</sup> 0.01	
<b>Maternal education</b>						
High school or less	1 [Reference]		1 [Reference]		1 [Reference]	
Some college or bachelor	0.94 [0.82-1.07]	0.34	0.93 [0.74-1.17]	0.54	0.94 [0.80-1.10]	0.43
Higher than bachelor	0.85 [0.73-0.98]	0.03	0.91 [0.71-1.17]	0.46	0.82 [0.69-0.98]	0.03
	p-trend 0.03		p-trend 0.43		p-trend 0.03	
<b>Father education</b>						
High school or less	1 [Reference]		1 [Reference]		1 [Reference]	
Some College or bachelor	0.95 [0.83-1.08]	0.40	0.94 [0.75-1.17]	0.56	0.95 [0.82-1.11]	0.50
Higher than bachelor	0.76 [0.63-0.92]	0.01	0.78 [0.56-1.08]	0.13	0.75 [0.60-0.95]	0.01
	p-trend 0.01		p-trend 0.15		p-trend 0.03	

Table 3.2 Association between demographic factors and risk of OFCs (Cont.).

Characteristic	All OFCs (N=1451)		Non-isolated OFCs (N=432)		Isolated OFCs (N=1091)	
	OR (95%CI)	p-value	OR (95%CI)	p-value	OR (95%CI)	p-value
<b>Hypertension</b>	1.00 [0.79-1.26]	0.97	1.13 [0.77-1.65]	0.66	0.95 [0.72-1.25]	0.69
<b>Depression</b>	1.38 [1.06-1.80]	0.02	1.60 [1.05-2.43]	0.03	1.29 [0.94-1.76]	0.11

<sup>a</sup> BMI (Body mass index) calculated as body weight (kg)/height (m<sup>2</sup>)

<sup>1</sup> excluding underweight

Control = 5804

Table 3.3 Association between maternal underweight and risk of orofacial clefts (1997-2011)<sup>a</sup>

Cleft Group		Odds Ratios and 95% Confidence Intervals by Cleft Subtypes					
		OFCs	CLO	CPO	CLP	CL/P	CP/L
<b>All</b>	Crude	0.96 [0.74-1.25]	0.50 [0.27-0.92]	1.45 [1.01-2.08]	0.89 [0.60-1.32]	0.73 [0.52-1.03]	1.14 [0.86-1.50]
	Model1	0.96 [0.74-1.24]	0.46 [0.24-0.88]	1.50 [1.04-2.16]	0.86 [0.58-1.29]	0.71 [0.50-1.00]	1.14 [0.86-1.50]
	Model2	0.95 [0.74-1.24]	0.46 [0.24-0.88]	1.50 [1.04-2.16]	0.86 [0.58-1.29]	0.71 [0.50-1.00]	1.14 [0.86-1.50]
<b>Non-isolated</b>	Crude	1.15 [0.76-1.74]	0.67 [0.16-2.81]	1.46 [0.86-2.46]	0.91 [0.44-1.90]	0.85 [0.44-1.64]	1.22 [0.80-1.88]
	Model1	1.21 [0.80-1.85]	0.80 [0.19-3.42]	1.54 [0.91-2.62]	0.91 [0.44-1.91]	0.89 [0.46-1.73]	1.26 [0.82-1.95]
	Model2	1.22 [0.80-1.85]	0.80 [0.19-3.41]	1.54 [0.91-2.63]	0.92 [0.44-1.92]	0.89 [0.46-1.73]	1.27 [0.82-1.96]
<b>Isolated</b>	Crude	0.89 [0.65-1.21]	0.47 [0.24-0.93]	1.44 [0.90-2.32]	0.88 [0.56-1.40]	0.70 [0.47-1.03]	1.09 [0.78-1.53]
	Model1	0.86 [0.63-1.17]	0.42 [0.20-0.85]	1.46 [0.91-2.36]	0.84 [0.53-1.34]	0.66 [0.44-0.98]	1.07 [0.76-1.51]
	Model2	0.86 [0.63-1.17]	0.42 [0.20-0.85]	1.47 [0.91-2.36]	0.84 [0.53-1.34]	0.66 [0.44-0.98]	1.07 [0.76-1.51]

OFCs: orofacial clefts; CL: cleft lip only; CP: cleft palate only; CLP: cleft lip with cleft palate; CL/P: cleft lip with/without cleft palate; CP/L: cleft palates with or without cleft lip.

<sup>a</sup> All diabetes refers to combination between pre-existing DM and GDM

Model1: Covariates in multiple logistic regression models included maternal age, maternal education levels, and depression.

Model2: Covariates in multiple logistic regression models included maternal age, maternal education levels, depression, and all diabetes.

Table 3.4 Association between maternal obesity and risk of orofacial clefts (1997-2011)<sup>a</sup>

Cleft Group		Odds Ratios and 95% Confidence Intervals by Cleft Subtypes					
		OFCs	CLO	CPO	CLP	CL/P	CP/L
<b>All</b>	Crude	1.36 [1.15-1.60]	1.25 [0.93-1.68]	1.37 [1.05-1.78]	1.41 [1.12-1.79]	1.35 [1.11-1.63]	1.39 [1.16-1.68]
	Model1	1.33 [1.12-1.57]	1.27 [0.94-1.72]	1.33 [1.01-1.74]	1.36 [1.07-1.73]	1.33 [1.09-1.61]	1.35 [1.12-1.63]
	Model2	1.29 [1.09-1.53]	1.26 [0.93-1.70]	1.24 [0.94-1.64]	1.34 [1.05-1.71]	1.31 [1.07-1.59]	1.30 [1.07-1.57]
<b>Non-isolated</b>	Crude	1.55 [1.19-2.02]	1.35 [0.63-2.86]	1.57 [1.09-2.27]	1.58 [1.04-2.41]	1.52 [1.05-2.20]	1.58 [1.19-2.09]
	Model1	1.55 [1.18-2.03]	1.47 [0.68-3.17]	1.59 [1.09-2.31]	1.51 [0.99-2.32]	1.50 [1.03-2.19]	1.56 [1.17-2.07]
	Model2	1.41 [1.07-1.87]	1.42 [0.65-3.11]	1.42 [0.96-2.08]	1.40 [0.91-2.17]	1.41 [0.96-2.07]	1.41 [1.05-1.90]
<b>Isolated</b>	Crude	1.28 [1.05-1.55]	1.24 [0.90-1.70]	1.20 [0.83-1.73]	1.35 [1.02-1.78]	1.30 [1.05-1.61]	1.29 [1.03-1.62]
	Model1	1.24 [1.02-1.51]	1.25 [0.90-1.72]	1.13 [0.78-1.64]	1.30 [0.98-1.73]	1.28 [1.03-1.60]	1.23 [0.98-1.56]
	Model2	1.23 [1.01-1.50]	1.23 [0.89-1.71]	1.09 [0.75-1.59]	1.31 [0.99-1.74]	1.28 [1.02-1.60]	1.23 [0.97-1.55]

OFCs: orofacial clefts; CL: cleft lip only; CP: cleft palate only; CLP: cleft lip with cleft palate; CL/P: cleft lip with/without cleft palate; CP/L: cleft palates with or without cleft lip

<sup>a</sup> All diabetes refers to combination between pre-existing DM and GDM

Model1: Covariates in multiple logistic regression models included maternal age, maternal education levels, and depression.

Model2: Covariates in multiple logistic regression models included maternal age, maternal education levels, depression, and all diabetes.

Table 3.5 Association between pre-existing diabetes and risk of orofacial clefts (1997-2011)

Cleft Group		Odds Ratios and 95% Confidence Intervals by Cleft Subtypes					
		OFCs	CLO	CPO	CLP	CL/P	CP/L
<b>All</b>	Crude	2.25 [1.22-4.15]	1.10 [0.26-4.63]	2.50 [1.03-6.06]	2.76 [1.26-6.07]	2.12 [1.03-4.37]	1.68 [1.24-2.29]
	Model1	2.24 [1.21-4.15]	1.07 [0.25-4.52]	2.49 [1.03-6.05]	2.76 [1.25-6.09]	2.11 [1.02-4.35]	2.63 [1.02-4.35]
	Model2	2.19 [1.18-4.09]	1.02 [0.24-4.34]	2.54 [1.04-6.22]	2.64 [1.19-5.88]	2.03 [0.97-4.21]	2.59 [1.35-4.97]
<b>Non-isolated</b>	Crude	3.89 [1.77-8.56]	N/A	2.89 [0.87-9.55]	6.48 [2.47-16.97]	4.91 [1.88-12.81]	4.42 [2.00-9.73]
	Model1	3.81 [1.72-8.42]	N/A	2.88 [0.87-9.58]	6.30 [2.39-16.64]	4.75 [1.81-12.47]	4.34 [1.96-9.62]
	Model2	3.83 [1.71-8.58]	N/A	2.99 [0.89-10.10]	6.23 [2.31-16.81]	4.64 [1.74-12.40]	4.41 [1.96-9.90]
<b>Isolated</b>	Crude	1.58 [0.72-3.47]	1.27 [0.30-5.37]	2.21 [0.67-7.30]	1.41 [0.43-4.65]	1.35 [0.52-3.51]	1.72 [0.71-4.16]
	Model1	1.59 [0.72-3.49]	1.24 [0.30-5.25]	2.20 [0.66-7.26]	1.43 [0.43-4.74]	1.36 [0.52-3.53]	1.73 [0.72-4.19]
	Model2	1.54 [0.70-3.41]	1.19 [0.28-5.06]	2.21 [0.65-7.40]	1.35 [0.41-4.50]	1.30 [0.50-3.38]	1.68 [0.69-4.09]

OFCs: orofacial clefts; CL: cleft lip only; CP: cleft palate only; CLP: cleft lip with cleft palate; CL/P: cleft lip with/without cleft palate; CP/L: cleft palates with or without cleft lip.

Model1: Covariates in multiple logistic regression models included maternal age, maternal education levels, and depression.

Model2: Covariates in multiple logistic regression models included maternal age, maternal education levels, depression, and body mass index.



Table 3.6 Association between gestational diabetes mellitus and risk of orofacial clefts (1997-2011)

Cleft Group		Odds Ratios and 95% Confidence Intervals by Cleft Subtypes					
		OFCs	CLO	CPO	CLP	CL/P	CP/L
<b>All</b>	Crude	1.50 [1.08-2.10]	1.32 [0.71-2.47]	2.09 [1.33-3.29]	1.13 [0.66-1.94]	1.20 [0.79-1.84]	1.57 [1.09-2.26]
	Model1	1.45 [1.04-2.04]	1.35 [0.72-2.53]	1.92 [1.20-3.05]	1.11 [0.64-1.91]	1.20 [0.78-1.85]	1.48 [1.02-2.15]
	Model2	1.48 [1.05-2.09]	1.41 [0.75-2.65]	2.07 [1.29-3.32]	1.04 [0.59-1.83]	1.18 [0.76-1.83]	1.50 [1.02-2.20]
<b>Non-isolated</b>	Crude	2.33 [1.47-3.70]	2.65 [0.82-8.61]	2.94 [1.66-5.19]	1.41 [0.57-3.50]	1.71 [0.83-3.54]	2.29 [1.40-3.74]
	Model1	2.15 [1.33-3.45]	2.64 [0.80-8.69]	2.67 [1.47-4.82]	1.31 [0.53-3.27]	1.62 [0.78-3.37]	2.08 [1.25-3.45]
	Model2	2.29 [1.41-3.72]	2.78 [0.83-9.32]	2.91 [1.59-5.32]	1.37 [0.55-3.46]	1.70 [0.81-3.57]	2.22 [1.32-3.73]
<b>Isolated</b>	Crude	1.17 [0.77-1.77]	1.11 [0.54-2.29]	1.45 [0.73-2.87]	1.03 [0.54-1.97]	1.06 [0.65-1.75]	1.19 [0.73-1.94]
	Model1	1.16 [0.76-1.77]	1.14 [0.55-2.36]	1.36 [0.68-2.72]	1.03 [0.53-1.98]	1.08 [0.65-1.78]	1.17 [0.71-1.90]
	Model2	1.15 [0.75-1.77]	1.18 [0.73-2.94]	1.46 [0.73-2.94]	0.92 [0.61-1.72]	1.03 [0.61-1.72]	1.13 [0.68-1.88]

OFCs: orofacial clefts; CL: cleft lip only; CP: cleft palate only; CLP: cleft lip with cleft palate; CL/P: cleft lip with/without cleft palate; CP/L: cleft palates with or without cleft lip

<sup>1</sup> based on data during 1997-2011

Model1: Covariates in multiple logistic regression models included maternal age, maternal education levels, and depression.

Model2: Covariates in multiple logistic regression models included maternal age, maternal education levels, depression, and body mass index.

Table 3.7 Association between all diabetes and risk of orofacial clefts (1997-2011)<sup>a</sup>

Cleft Group		Odds Ratios and 95% Confidence Intervals by Cleft Subtypes					
		OFCs	CLO	CPO	CLP	CL/P	CP/L
<b>All</b>	Crude	1.55 [1.17-2.06]	1.16 [0.65-2.05]	2.09 [1.41-3.09]	1.36 [0.89-2.08]	1.28 [0.90-1.83]	1.68 [1.24-2.29]
	Model1	1.52 [1.14-2.02]	1.18 [0.66-2.10]	1.95 [1.31-2.91]	1.35 [0.88-2.08]	1.29 [0.90-1.85]	1.63 [1.19-2.22]
	Model2	1.52 [1.14-2.04]	1.19 [0.67-2.13]	2.05 [1.36-3.08]	1.30 [0.83-2.02]	1.26 [0.87-1.82]	1.63 [1.19-2.24]
<b>Non-isolated</b>	Crude	2.40 [1.62-3.56]	1.99 [0.62-6.45]	2.77 [1.67-4.57]	2.03 [1.06-3.91]	2.02 [1.14-3.61]	2.45 [1.63-3.70]
	Model1	2.27 [1.52-3.39]	1.98 [0.61-6.46]	2.55 [1.52-4.29]	1.97 [1.02-3.81]	1.97 [1.10-3.53]	2.30 [1.51-3.50]
	Model2	2.38 [1.58-3.59]	1.97 [0.59-6.54]	2.71 [1.59-4.62]	2.08 [1.06-4.07]	2.05 [1.13-3.72]	2.43 [1.58-3.74]
<b>Isolated</b>	Crude	1.20 [0.85-1.72]	1.03 [0.54-1.96]	1.56 [0.88-2.77]	1.11 [0.65-1.90]	1.08 [0.70-1.65]	1.28 [0.86-1.92]
	Model1	1.21 [0.84-1.73]	1.05 [0.55-2.01]	1.49 [0.84-2.66]	1.12 [0.66-1.92]	1.10 [0.72-1.68]	1.27 [0.84-1.91]
	Model2	1.18 [0.82-1.71]	1.06 [0.55-2.05]	1.56 [0.87-2.80]	1.03 [0.59-1.80]	1.04 [0.67-1.62]	1.23 [0.81-1.87]

OFCs: orofacial clefts; CL: cleft lip only; CP: cleft palate only; CLP: cleft lip with cleft palate; CL/P: cleft lip with/without cleft palate; CP/L: cleft palates with or without cleft lip

<sup>a</sup> All diabetes refers to combination between pre-existing DM and GDM

Model1: Covariates in multiple logistic regression models included maternal age, maternal education levels, and depression.

Model2: Covariates in multiple logistic regression models included maternal age, maternal education levels, depression, and body mass index.

Table 3.8 Total, direct, and indirect effects with 95% CI of the association between maternal obesity and risk of orofacial clefts mediated through maternal diabetes

	<b>Pre-existing diabetes</b>	<b>GDM</b>	<b>All diabetes<sup>a</sup></b>
	OR [95% CIs]	OR [95% CIs]	OR [95% CIs]
<b>All OFCs</b>			
Direct effect	1.33 [1.12-1.58]	1.30 [1.09-1.54]	1.31 [1.11-1.55]
Indirect effect	1.02 [1.00-1.04]	1.00 [0.98-1.03]	1.00 [0.97-1.04]
Total effect	1.35 [1.14-1.61]	1.30 [1.10-1.54]	1.32 [1.11-1.56]
<b>Non-isolated OFCs</b>			
Direct effect	1.44 [1.08-1.92]	1.42 [1.07-1.89]	1.45 [1.09-1.92]
Indirect effect	1.06 [1.01-1.12]	1.00 [0.95-1.06]	1.01 [0.95-1.08]
Total effect	1.53 [1.15-2.04]	1.43 [1.08-1.89]	1.46 [1.11-1.93]
<b>Isolated OFCs</b>			
Direct effect	1.28 [1.05-1.57]	1.25 [1.02-1.53]	1.25 [1.03-1.53]
Indirect effect	1.00 [0.98-1.02]	1.00 [0.98-1.03]	1.00 [0.97-1.03]
Total effect	1.28 [1.05-1.57]	1.25 [1.03-1.52]	1.25 [1.03-1.52]

<sup>a</sup> All diabetes refers to combination between pre-existing DM and GDM

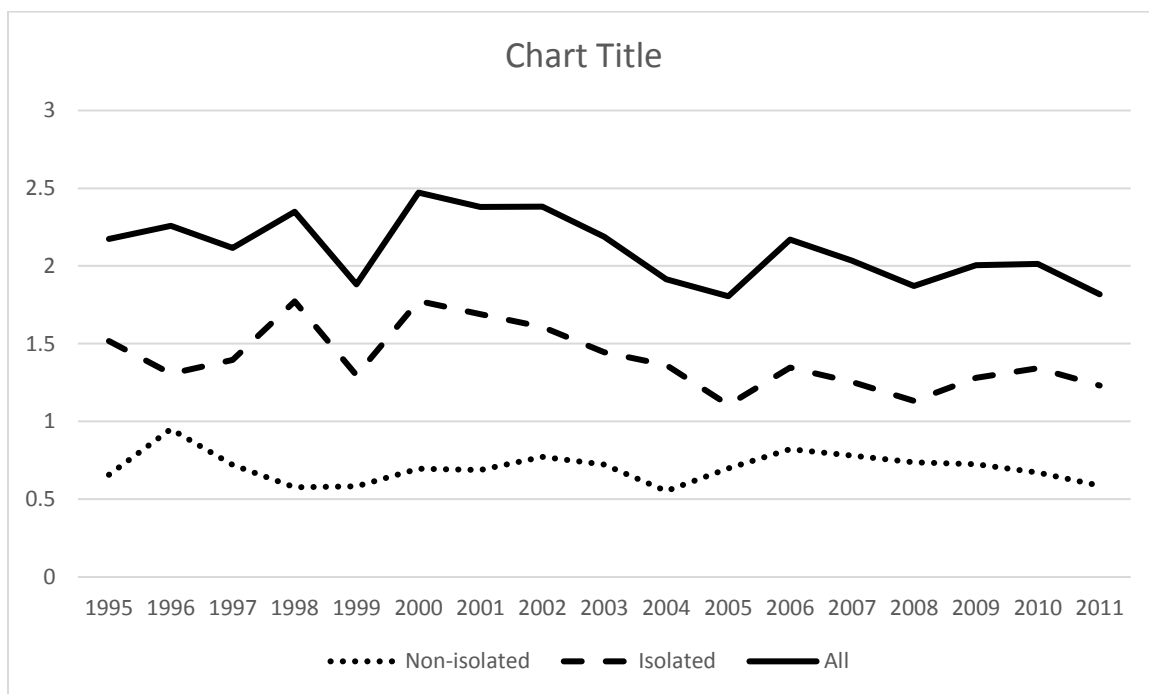


Figure 3.1 Prevalence of OFCs during 1995-2011 (per 1000 live birth)

All: coefficient = -0.023; 95%confidence interval = -0.043, -0.004; p-value= 0.02

Non-isolated: coefficient = -0.002; 95%confidence interval = -0.013, 0.009; p-value= 0.73

Isolated: coefficient = -0.02; 95%confidence interval = -0.04, -0.003; p-value= 0.03

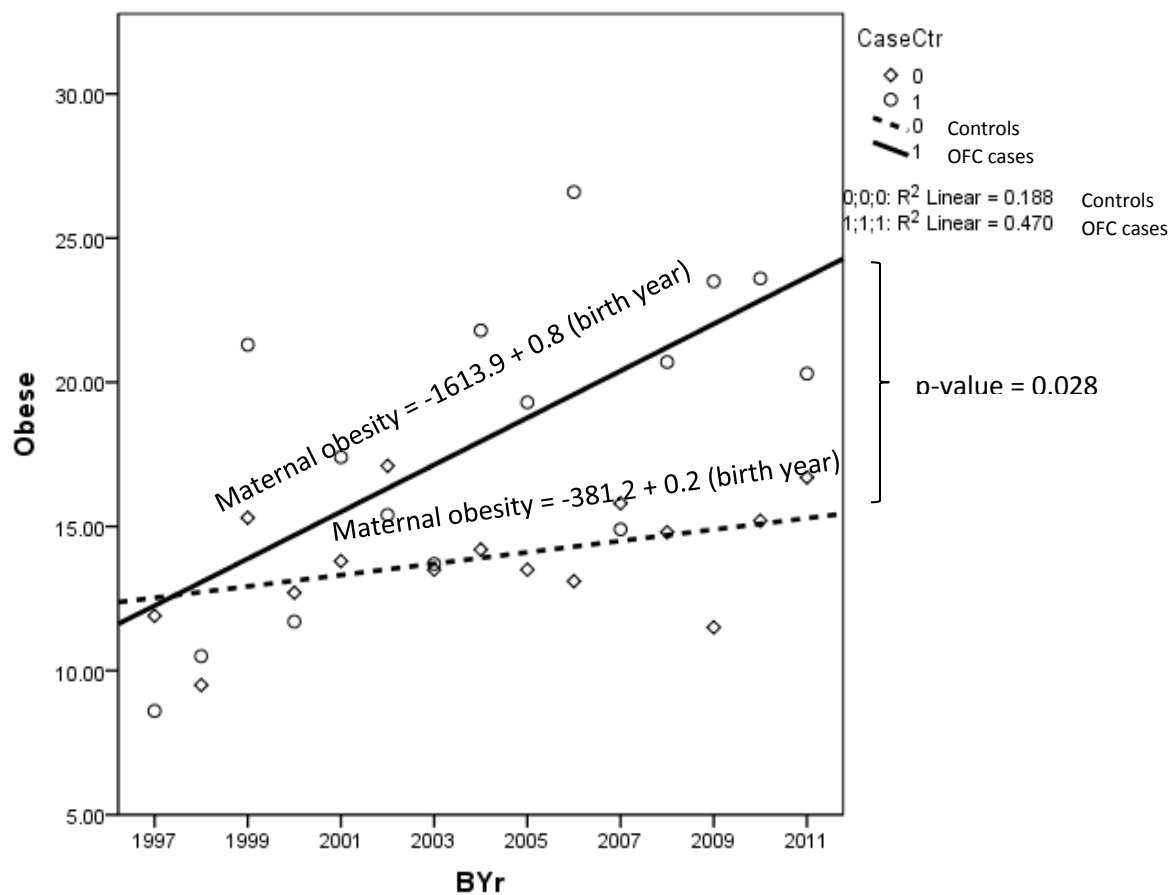


Figure 3.2 The regression line of maternal obesity ( $\text{BMI} \geq 30 \text{ kg/m}^2$ ) prevalence by year divided by OFC cases and controls.

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## CHAPTER 4

THE ASSOCIATION BETWEEN MATERNAL DIABETES AND BIOMARKERS  
OF METABOLIC SYNDROME AND OROFACIAL CLEFTS**4.1 Abstract**

**Background:** The evidence of the association between mothers having a congenitally malformed offspring and risk of developing diabetes and metabolic syndrome later in life is limited. This approach however may provide clues to the presence of undetected metabolic abnormalities involved in teratogenesis early in pregnancy.

**Objective:** to determine the association between the occurrence of maternal diabetes and maternal biomarkers of metabolic syndrome and isolated orofacial clefts (OFCs) using data from the Utah Cleft 2 study.

**Methods:** Cases and controls were selected from the participants in the Utah Cleft 1 study (1995-2005) and the National Birth Defects Prevention Study (NBDPS) in Utah (2005-2011). Participants were interviewed by telephone, and physical examination and blood collection was completed in clinical visits. Laboratory assays of metabolic syndrome-related biomarkers were performed by Laboratory Corporation of America (Labcorp), Utah and Quansys Bioscience, Logan, Utah. Independent t-test and chi-square tests were used to assess the association between OFCs and continuous and categorical variables. Two-way ANOVA was performed to assess the differences of adipokines and cytokines. Multivariable logistic regression was used to estimate risk for OFCs while adjusting for the potential confounding effects of maternal age and smoking.

**Results:** Mothers having GDM in any pregnancy had an increased risk of OFCs (OR: 3.05, 95% CI: 1.61-5.80) and this was consistent for each type of OFC. OFC case-mothers tended to be more obese than controls (OR: 1.45, 95% CI 0.99-2.13). Mothers of children with cleft palate, compared to controls, had higher mean levels of plasma glucose, insulin, triglycerides, waist circumference and systolic blood pressure, and lower HDL; these associations were not seen for mothers of children with cleft lip only. Similarly, mean plasma IL-8 and leptin levels were associated with cleft palate but not with cleft lip; risk analysis by tertile revealed a weaker association for IL-8 and cleft palates with or without cleft lip (CP/L) (OR for highest vs lowest tertile: 1.36, 95% CI 0.81-2.30; p-trend 0.24) and a stronger association for leptin and CP/L (OR for highest vs. lowest tertile: 2.21, 95% CI 1.28-3.81; p-trend 0.004) Metabolic syndrome indices were associated with cleft palate (NCEP/ATP III score OR: 1.60, 95% CI 1.00-2.56; IDF score OR: 1.64, 95% CI 1.04-2.59); these scores were not associated with cleft lip alone.

**Conclusion:** Gestational diabetes mellitus was associated with risk of OFCs and this association may reflect a progression of metabolic syndrome abnormalities that are teratogenic yet undetected in the periconceptual period. Individual metabolic syndrome biomarkers and indices were associated with cleft palate, but were not associated with cleft lip alone, this may reflect specific vulnerabilities of palate development which occur later than lip development. IL-8 and leptin levels were also associated with cleft palate and not cleft lip alone and suggest that further studies of novel metabolic syndrome-related biomarkers in the periconceptual period may be useful in understanding the causes and prevention of OFCs.

## 4.2 Introduction

OFCs occur in every 0.4 to 2 per 1000 births depending on geographic location, racial and ethnic groups, maternal age, environmental exposures, and socioeconomic status (1, 2). The U.S. National Birth Defects Prevention Network reported the prevalence of OFCs during 2002-2006 of CL/P was 1.33 per 1000 live births, and 0.73 per 1000 live births for CP (3). The prevalence of OFCs in Utah is higher than the overall prevalence in the United States (2.25 per 1000 births) (4)

Metabolic syndrome is the grouping of visceral obesity, insulin resistance, hyperglycemia, dyslipidemia (hypertriglyceridemia and hypo-HDL cholesterolemia), and hypertension (5). In addition, metabolic syndrome increases the risk of cardiovascular diseases (CVD), type 2 diabetes mellitus, and stroke. Both type 2 diabetes mellitus and gestational diabetes mellitus, risk factors of OFCs, have associations with metabolic syndrome. Many studies have reported that metabolic syndrome increases the risk of type 2 diabetes mellitus (6-9). A meta-analysis study showed that the subsequent risk of metabolic syndrome increased in women with a history of GDM (10). On the other hand, metabolic syndrome in early pregnancy increases the incidence of GDM (11).

Biochemical markers are hormones, enzymes, antibodies, or other substances in urine, blood, tissue, or other body fluids. These biomarkers have been used to detect abnormality or disease. Besides HDL and triglyceride, liver function tests (ALT: alanine aminotransferase and GGT: gamma-glutamyltransferase) (12-14), cytokines (CRP: C-reactive protein, IL-6: interleukin-6, and TNF- $\alpha$ : tumor necrosis factor alpha) (15-18), and adipokines (leptin and adiponectin) (16-18) also have association with metabolic



syndrome. The biomarkers associated with metabolic syndrome have been correlated with the risk of developing diabetes mellitus (13, 19-21).

A few studies have reported that mothers having a congenitally malformed child, including OFCs increased risk of developing diabetes many years later (22, 23). Thus, we hypothesize that Utah mothers giving birth to children with orofacial clefts have an enhanced risk of diabetes and abnormal biomarkers associated with metabolic syndrome. This study aims to analyze biomarkers related to metabolic syndrome in mothers after giving birth to an OFC child compared to control-mothers of unaffected children.

### **4.3 Subjects and Methods**

#### **4.3.1 Study Design**

The Utah Cleft 2 study is a case-control interview and clinical study of orofacial clefts in Utah. The study protocol was reviewed and approved by the institutional review boards of Utah State University (USU), the University of Utah, and the Utah Department of Health (UDOH).

#### **4.3.2 Study Participants**

Cases and controls were selected from the participants in the Utah Cleft 1 case-control study (24) and the National Birth Defects Prevention Study (NBDPS) in Utah (25). In the Utah Cleft 1 study, case-mothers having a child with OFCs between January 1995 and June 2005 were recruited from UDOH, and control mothers were randomly selected, frequency matched by birth month and year, and gender of case child at ratio 1:1 by using Utah birth certificate files. The NBDPS in Utah, also a state-wide population-based case-control study, recruited case mothers having a child with OFCs

between 2005-2011 from the UDOH database, and randomly selected control mothers from birth certificates. The OFC cases were limited to isolated OFCs; cases with multiple birth defects were excluded.

### **4.3.3 Data collection**

Data from the Utah Cleft 2 case-control study were collected by trained interviewers after participants were contacted by either mail or telephone during 2011-2015. Verbal consent was obtained by telephone before interviewing mothers about medical history, nutritional supplements, and prescription medicines. In clinical visits, mothers gave written informed consent and height, weight, waist circumference, and blood pressure were measured and a blood sample was taken.

### **4.3.4 Laboratory analyses of biomarkers**

Blood samples from non-fasting subjects were obtained using Purple top (EDTA) and Blue-top (EDTA) tubes from mothers. Blood samples were centrifuged for 10 min at 3600 rpm and divided into aliquots of plasma, buffy coats, and red blood cells. Aliquots for metabolic biomarkers were analyzed at the Laboratory Corporation of America (Labcorp), Utah. Samples for analyzing cytokines and adipokines were kept at  $-80^{\circ}\text{C}$ , and shipped on dry ice to Quansys Bioscience, Logan, Utah. (Table 4.1)

#### **a) Hematology, metabolic profiles, and lipid profiles b)**

Labcorp (26) methods for metabolic biomarkers included Kinetic for liver function tests, enzymatic for glucose, carbon dioxide, total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), electrochemiluminescence immunoassay (ECLIA) for insulin,

and Roche Tina Quant for hemoglobin A1c. Liver function tests included alanine aminotransferase (ALT/SGPT), aspartate aminotransferase (AST/SGOT), and alkaline phosphatase.

#### **b) Cytokines and adipokines**

Blood samples were collected from non-fasting participants and specimens were placed on wet ice then centrifuged within 2 hours after collection and kept at  $-80^{\circ}\text{C}$  until analysis. Plasma was shipped on dry ice to Quansys Biosciences (27) (Logan, Utah) in order to analyze CRP, cytokines (IL-1a, IL-6, IL-8, and TNFa), and adipokines (adiponectin, leptin, and resistin). The serum specimens were analyzed by the multiplex enzyme-linked, immunosorbent assay (ELISAs) technology, microscaled and multiplexed to simultaneously measure multiple proteins.

#### **4.3.4 Statistical analyses**

Descriptive analyses were conducted to determine the distribution of maternal biomarkers by OFC status. Metabolic syndrome protocols of NCEP/ATP III (28) and IDF (29) were used to classify metabolic syndrome status. Independent-sample t-tests and chi-square tests were used to assess the association between OFCs and continuous and categorical variables respectively. Multivariable linear regression was used to adjust for the potential confounding effects of maternal age and smoking status in the comparison of continuous biomarkers of cases and controls. Multivariable logistic regression analysis was used to estimate odds ratios (ORs) with 95% confident intervals (95% CI) for evaluation of OFC risk. The linear trend test was analyzed to determine the trend of risk across levels of each biomarker. All multivariate analyses were adjusted for maternal age

and smoking. Due to wide range of undetected samples of cytokines and adipokines (0-39.7%), two-way ANOVA was performed to estimate the difference of mean between OFC groups and the control group with models including covariates for detected-undetected status and the measured values for detected samples. All statistical analyses were performed using the Statistical Package for Social Sciences (IBM SPSS) statistics version 22 for Windows.

Table 4.1 Laboratory tests used in the Utah Cleft 2 case-control study

Items	Method	Laboratory
Lipid profiles (total cholesterol, triglyceride, lipoproteins)	Enzymatic	Labcorp <sup>1</sup>
Liver function test (alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase )	Kinetic	Labcorp <sup>1</sup>
Glucose	Enzymatic	Labcorp <sup>1</sup>
Insulin	ECLIA <sup>2</sup>	
Hemoglobin A1c	Roche Tina Quant	Labcorp <sup>1</sup>
C-reactive protein (CRP)	Multiplex ELISAs <sup>3</sup>	Quansys Bioscience
Cytokines and adipokines (interleukin-1 alpha, interleukin-6, interleukin-8, leptin, resistin, tumor necrosis factor alpha, and adiponectin)	Multiplex ELISAs <sup>3</sup>	Quansys Bioscience

<sup>1</sup> Labcorp: Laboratory Corporation of America

<sup>2</sup>ECLIA: Electrochemiluminescence immunoassay

<sup>3</sup> ELISAs: Enzyme-Linked, Immunosorbent Assay

#### 4.4 Results

Efforts were made to recruit 1431 participants from the Utah Cleft 1 case-control study and 456 participants from the NBDPS. A total of 794 participants (435 (30.4%) from the Utah Cleft 1 case-control study and 359 (78.7%) from the NBDPS) were enrolled in this study; 612 participants (77.1%) completed telephone interviews, clinic visits and blood collection, and 157 (19.8%) completed the telephone interview only.

Refusals included 167 participants (116 (12.8%) controls and 51 (6.0%) cases) and 541 participants (189 (20.9%) controls and 352 (41.1%) cases) were unable to be contacted or scheduled (table 4.2).

The mean maternal age at birth and age at clinical visit was not different between cases and controls and the data collection for both groups was an average of 10 years after the index birth. Among 360 isolated OFC cases, cleft palate only (CPO) accounted for 25% (n=90), cleft lip only (CLO) accounted for 31.1% (n=112), and cleft lip with cleft palate (CLP) accounted for 42.5% (n=153). The mean body mass index (BMI) of cases and controls was not different. The rate of maternal smoking in three months before interview was greater in all OFC groups compared to controls (Table 4.3). Alcohol consumption in three months before interview was greater in OFC cases compared to controls (Table 4.3). Type 2 diabetes was less common than GDM and was not associated with OFC risk (Tables 4.3 and 4.4). Gestational diabetes (GDM) was associated with an increased risk of OFCs (OR: 3.05, 95% CI: 1.61-5.80) and this was consistent for each type of OFCs. OFC mothers tended to be more obese than controls (OR: 1.45, 95% CI: 0.99-2.13) (Tables 4.3 and 4.4).

When adjusted for maternal age and smoking, means of waist circumference, insulin, glucose and triglyceride in the CP/L (cleft palate with or without cleft lip) group were significant higher and mean HDL was lower than control mothers (Table 4.5); the mean values for the CLO group were not significantly different from controls. The odds ratios analyzed by tertiles generally showed similar finding, though the 95% CIs for most comparisons included 1.0.

OFC risk by level of the metabolic syndrome scores computed on the basis of NCEP/ATPIII and IDF criteria, and risk by the individual component scores appear in Table 4.8. Metabolic syndrome defined by NCEP/ATPIII criteria ( $\geq 3$ ) was associated with an increased risk of CP/L (OR: 1.60, 95% CI: 1.00-2.56) but no higher risk of CLO (OR: 1.06, 95% CI: 0.54-2.05). Similarly, metabolic syndrome defined by IDF criteria was associated with a greater risk of all CP (OR: 1.64, 95% CI: 1.04-2.59) but no greater risk of CLO (OR: 1.16, 95% CI: 0.62-2.17). Elevated blood pressure, defined as systolic blood pressure  $\geq 130$  mmHg or diastolic blood pressure  $\geq 85$  mmHg was associated with CP/L (OR: 1.77, 95% CI: 1.02-3.09) but not CL only (OR: 1.03, 95% CI: 0.46-2.32). The other individual metabolic syndrome component scores did not appear to be associated with OFCs, underscoring the importance of combined metabolic syndrome indices rather than individual components in association with OFC risk.

Mothers having CLO and cleft lip with/without cleft palate (CL/P) offspring had AST levels higher than mothers in the control group when adjusted for maternal age and smoking (adjusted p-value 0.05 and 0.05, respectively) (Table 4.7).

The mean and median cytokine IL-8 (p-value=0.003) and the adipokine leptin (p-value = 0.006) levels were higher in CP/L cases compared to controls (Table 4.6). The risk analysis by tertile revealed a weaker association for IL-8 (OR for highest vs lowest: 1.36, 95% CI: 0.81-2.30, p-trend 0.24) and a stronger association for leptin (OR for highest vs lowest: 2.21, 95% CI: 1.28-3.81, p-trend 0.004). IL-8 and leptin levels were not associated with CLO. When analyses were stratified by BMI level (normal weight, BMI 18.5 to 24.9 kg/m<sup>2</sup>; overweight, BMI 25 to 29.9 kg/m<sup>2</sup>; obese: BMI more than 30

kg/m<sup>2</sup>), IL-8 and leptin levels generally increased with increasing BMI levels, and significant differences emerged between CP/L vs controls. Based on BMI classification, obese mothers having CPO offspring had higher leptin levels than obese mothers in the control group when adjusted for maternal age and smoking (Figures 4.1).

#### 4.5 Discussion

Mothers having GDM in any pregnancy had an increased risk of all types of OFCs. Mothers having CLO and CL/P offspring were associated with obesity later in life. Mothers having CP/L offspring had an increased risk of developing metabolic syndrome based on both NCEP/ATP III and IDF definitions. Mothers having CP/L offspring had higher IL-8 and leptin levels than control mothers.

The association between gestational diabetes mellitus and risk of non-isolated OFCs is consistent with limited previous studies (30, 31). Correa et al. using the data from the National Birth Defect Prevention Study (NBDPS) during 1997-2003 showed an association between both pre-existing diabetes (type 1 or 2) and GDM and risk isolated OFCs, while only pre-existing diabetes was associated with syndromic OFCs (31). Correa et al. also reported higher estimates of odds ratios and wider confidence intervals than our study (non-isolated CPO, OR: 10.73, 95% CI: 3.99-28.86 and non-isolated CL/P, OR: 8.07, 95% CI: 3.05-21.39). Data from a large international consortium of case-control studies from the U.S., Denmark, and Norway (30) found an association between GDM and syndromic OFCs and CPO. A prospective study by Moore et al (32) reported suggestive associations between pre-existing (type 1 or 2) and GDM and risk of OFCs but also with very wide confidence intervals that included 1.0 (prevalence ratios

(PR): 8.9, 95% CI: 0.85-46.5; and PR: 2.6, 95% CI: 0.82-8.5, respectively). The mechanisms explaining the relationship between maternal diabetes and orofacial clefts are unknown. Elevated blood glucose and insulin stimulates the production of many adverse metabolic factors including ketone bodies, branched chain amino acids, inflammatory markers, advanced glycation end products, altered expression levels of specific genes, and increase the variation of gene expression levels (33-36). These factors may disrupt normal embryonic development.

This study reported associations between CLO and CL/P and obesity, which is consistent with the findings of the Utah birth certificate study of pre-pregnancy weight reported in chapter 3. A large international consortium of U.S. and European population-based studies by Kutbi et al (37) found that pre-pregnancy maternal obesity had an increased risk of CPO, CLP, and CP/L but not CLO; this study suggested that obesity has a specific effect on palate formation but not lip formation. A recent meta-analysis by Blanco et al also presented that maternal obesity increased risk of CPO and CL/P (38).

Metabolic syndrome is a major public health issue because it increases the risk of cardiovascular diseases (CVD), type 2 diabetes mellitus, stroke, etc. We found that mothers of children with CP/L had an increased risk of developing metabolic syndrome later in life. A case-control study in Mexico (23) found that the prevalence of diabetes in mothers delivering a malformed child was higher than control group (controls: 4% and cases: 16.7, 40.4 5, and 53.1% when follow-up 0, 12, and 25 years after the index pregnancy, respectively). Another case-control study in the United Kingdom (22)



reported mothers having CPO or CL/P offspring had a higher prevalence of increased antagonism to insulin than control mothers (CPO: 75%, CL/P: 68.1%, and controls: 28%)

This study found that using the IDF definition identified a higher prevalence of metabolic syndrome than using the NCEP/ATP III definition. Previous studies also reported that using IDF criteria for screening metabolic syndrome provided a higher prevalence than NCEP/ATP III criteria (39-42). The National Health and Nutrition Examination Survey (NHANES) 1999-2002 (39) found that prevalence of metabolic syndrome was  $39.0 \pm 1.1$  % based on IDF criteria, while  $34.5 \pm 0.9$  % based on NCEP/ATP III. Both metabolic syndrome criteria had similar associations with OFCs in the present study.

Previous studies found the association between NCEP/ATP III definition and risk of carotid atherosclerosis (43, 44). However, recent studies suggested that metabolic syndrome definition proposed by IDF was a better predictor for cardiovascular risk than the definition by NCEP/ATP III. A cross sectional study in China presented that IDF definition was more strongly associated with CHD than NCEP/ATP III definition (45). A case-control study in Greece found that IDF definition for metabolic syndrome provided a higher odd ratio for acute coronary syndrome than NCEP/ATP III (OR: 3.26, 95% CI: 2.12-5.00 vs OR: 2.32, 95% CI: 1.53-3.52) (46). A 13-year follow up study in Finns (42) found that metabolic syndrome defined by IDF was associated with risk of coronary heart disease (CHD) mortality (hazard ratio (HR): 1.42, 95% CI: 1.01-1.99), while the association between metabolic syndrome defined by NCEP/ATP III definition and CHD mortality was not significant (HR: 1.30, 95% CI: 0.94-1.81). However, this study also

reported a 1.35 fold (95% CI: 1.05-1.74) risk of CVD mortality was associated with metabolic syndrome according to NCEP/ATP III criteria, which was a bit higher than metabolic syndrome diagnosed by IDF (HR: 1.33, 95% CI: 1.03-1.77). A cross sectional analysis in a Greek population reported that NCEP/ATP III definition was more predictive of cardiovascular disease (CVD) risk than IDF definition (47). A prospective study in Austria (48) found metabolic syndrome defined by NCEP/ATP III definition was strongly predictive of vascular events than the IDF definition (HR: 1.58, 95% CI: 1.10-2.26 vs HR: 1.06, 95% CI: 0.76-1.50).

Both definitions use the same five components, but there are a few differences. The IDF definition requires the presence of central obesity plus two of the rest components while NCEP/ATP III definition makes central obesity one of the five equally weighted criteria. Moreover, the thresholds for waist circumference and fasting blood glucose by the IDF definition are lower than those of the NCEP definition. The thresholds of triglycerides and HDL cholesterol levels, and systolic and diastolic blood pressure are the same. IDF definition emphasizes waist circumference, an index of abdominal obesity which has an effect on metabolic processes by the intra-abdominal visceral fat which has higher lipolysis rate than any other depots. Abdominal adipose tissue elevates free fatty acids (FFA), cytokines (tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6)), adipokines, and angiotensin II. Excess FFA circulation induces insulin resistance, which results from reduced hepatic insulin clearance, decreased skeletal muscle insulin sensitivity, increased hepatic cholesterol production with elevated triglycerides and very low density lipoprotein (VLDL), and altered endothelial function.

Elevated levels of cytokines also impair insulin sensitivity (5, 49). In addition, enlarged fat cells causing fat overflow to muscles, the liver, and the pancreas lead to insulin resistance (50), which plays an important role in the development of metabolic syndrome (51). The IDF consensus group has mentioned other parameters that require further research into metabolic syndrome including tomographic assessment of fat distribution and liver fat, adipose tissue biomarkers (leptin and adiponectin), apolipoprotein B, small LDL particles, insulin resistance parameters (fasting insulin, proinsulin levels, homeostatic model assessment-insulin resistance (HOMA-IR), free fatty acid), oral glucose tolerance test, measurement of endothelial dysfunction, microalbuminuria, inflammatory markers (CRP, TNF- $\alpha$ , IL-6), thrombotic markers (plasminogen activator type 1, fibrinogen), and pituitary-adrenal axis (29).

This study found that mothers having offspring with CP/L had higher insulin levels than control mothers. Insulin resistance is defined as a condition in which normal insulin levels are not adequate at maintaining a normal insulin response in peripheral target tissues (adipose, muscle, and liver), which causes pancreatic beta cells to secrete more insulin (hyperinsulinemia) to overcome the hyperglycemia condition (5). Elevated levels of inflammatory markers also impair insulin sensitivity (5, 49). An *in vitro* study by Hotanisligil et al found that TNF- $\alpha$  inhibits insulin receptor substrate 1 (IRS-1) signaling pathway, which leads to insulin resistance (52). A human study by Bastard et al reported that IL-6 level was associated with insulin resistance (53). This study hypothesized that IL-6 overproduction from adipose tissue activates extracellular signal regulated kinase (ERK) pathway and induces IRS-1, which inhibits insulin signaling. In

addition, insulin resistance is a risk factor for developing GDM. Prospective studies found that pregnant women having high fasting plasma insulin levels (FPI) in the first trimester of gestation had an increased risk of GDM (54-56). The association between insulin and OFCs requires further study.

We found that mothers of children having CPO and CP/L had higher IL-8 and leptin levels than control mothers. IL-8 is a proinflammatory cytokine, which is produced by macrophages and monocytes (57). A case-control study by Bruun et al (58) found that plasma IL-8 levels were 38% higher in obese participants than in lean participants. In addition, in the obesity group, IL-8 levels had a positive correlation with fasting insulin levels and homeostasis model assessment for insulin resistance (HOMA-IR). A case-control study by Zozulinska et al (59) reported that serum IL-8 levels in participants with type 1 and type 2 diabetes mellitus were higher than healthy participants ( $160.29 \pm 34.81$  and  $138.7 \pm 32.8$  pg/ml VS  $39.93 \pm 4.96$  pg/ml).

Serum leptin levels reflect the proportion of adipose tissue in the body, which is highly correlated with fat mass. During pregnancy, leptin is also produced by placenta, which has a pro-angiogenic effect in placental tissue (60) and an anti-apoptotic effect on trophoblast cells (61). A study by Pérez-Pérez et al reported increased leptin and leptin receptor gene expression in placentas from GDM pregnancies compared with normal pregnancies (62). Many studies reported that leptin levels were significantly higher in pregnancies with GDM compared with normal pregnancies (63-65). Studies focusing on the association between either hyperleptinemia or leptin expression and birth defects are limited. A study by Jones et al. (66) found that placental leptin RNA expression was

significantly increased in hypoplastic left heart syndrome (HLHS) cases compared to controls. However, a meta-analysis study reported that SGA (small for gestational age) infants had lower cord blood leptin levels than AGA (appropriate for gestational age) infants (67). An impact of increased leptin levels on placental nutrient transfer was proposed by a cell study by Araujo et al (68). The study showed that there is no significant change of the steady-state intracellular accumulation of folic acid, while diabetic trophoblasts (DTB) cells from gestational diabetic pregnancies had higher rates of inward and outward transport of intracellular folic acid. The higher turnover of intracellular folic acid in DTB cells is required to maintain normal folic acid homeostasis. Moreover, hyperleptinemic condition leads to decreased folic acid uptake. These findings showed that leptin may act as a regulator of placental nutrient transport especially folic acid, and a regulator of fetal growth. However, the study of that effect of leptin on folic acid placental transport is limited.

The leptin gene is transcriptionally activated in response to hypoxia (69). A mouse study by Webster et al. (70) showed that pregnant mice receiving phenytoin during the period of early facial development had cleft lip offspring because phenytoin causes bradycardia leading to a prolonged period of embryonic hypoxia. This study proposed additional exposures increase the risk of cleft lip in humans including maternal cigarette smoking, residence at high altitudes, and exposure to corticosteroids. High leptin circulation decreases folic acid transportation (68), which may relate to not only GDM pregnancies, but also pregnancies exposed to prolonged hypoxia. The association

between increased leptin resulting from hypoxia and risk of birth defect requires further study.

Our results suggest that gestational diabetes, maternal obesity, and metabolic syndrome are associated with an increased risk of OFCs. When compared with control mothers, mothers having cleft palate offspring had higher insulin, IL-8, and leptin levels. Therefore, insulin, IL-8, and leptin levels may be links between GDM, metabolic syndrome, and OFCs. Insulin, IL-8 and leptin might be performed as biomarkers for predicting OFC occurrence. Mechanistic studies are needed to understand the causal effects of maternal obesity, metabolic abnormalities, and gestational diabetes mellitus on OFCs risk. The potential of using insulin, leptin, IL-8 and related biomarkers as predictors of birth defect risk requires further study.

Table 4.2 Tracking status of orofacial cleft (OFC) cases and controls in the Utah Cleft 2 Study.

<b>Status</b>	<b>Controls n (%)</b>	<b>OFC cases n (%)</b>
Completed interview, clinical visit, and blood collection	344 (38.0)	268 (31.3)
Completed interview, clinical visit, and no blood collection	4 (0.4)	2 (0.2)
Completed only interview, no clinical visit, and no blood collection	74 (8.2)	83 (9.7)
Completed clinical visit and blood collection, and no interview	5 (0.6)	3 (0.4)
Completed only blood collection	8 (0.9)	3 (0.4)
Refuse	116 (12.8)	51 (6.0)
Move out of state	135 (14.9)	70 (8.2)
Out of clinic area	25 (2.8)	15 (1.8)
Unable to contact or schedule	189 (20.9)	352 (41.1)
Non-isolated OFCs	0 (0)	1 (0.1)
Spanish speaking	5 (0.6)	8 (0.9)
Deceased	0 (0)	1 (0.1)

OFC: Orofacial cleft

Table 4.3 Demographic characteristics of isolated orofacial cleft case and controls: The Utah Cleft 2 Study

Characteristic	Controls	Orofacial cleft cases					
		All OFCs	CLO	CL/P	CLP	CPO	CP/L
Maternal age at birth of index child (SD)	n= 436 27.8±(5.3)	n=358 27.5±(5.3)	n=112 27.4±(5.0)	n=268 27.5±(5.2)	n=156 27.6±(5.2)	n=90 27.54±(5.70)	n=246 27.6±(5.4)
p-value <sup>1</sup>		0.44	0.40	0.46	0.24	0.27	0.56
Maternal age at blood collection (SD)	n=360 37.7±(7.0)	n=274 37.4±(6.5)	n=90 36.9±(6.3)	n=211 37.4±(6.6)	n=121 37.7±(6.9)	n=63 37.7±(6.2)	n=184 37.7±(6.6)
p-value <sup>1</sup>		0.62	0.33	0.56	0.98	0.97	0.97
Number of past pregnancies (%)	n=423 4.5±(2.21)	n=349 4.2±(1.98)	n=109 4.2± (1.9)	n=261 4.3±(2.0)	n=152 4.3±(2.1)	n=88 3.9±(1.9)	n=240 4.2±(2.0)
p-value <sup>1</sup>		0.04	0.27	0.18	0.34	0.01	0.05
Number of live birth (%)	n=423 4.1±(6.7)	n=349 4.0±(7.4)	n=109 3.5±(1.5)	n=261 3.8±(6.1)	n=152 4.1±(7.9)	n=88 4.4±(10.4)	n=240 4.2±(8.9)
p-value <sup>1</sup>		0.77	0.36	0.59	0.94	0.79	0.93
Current weight (lb)	n=351 163.8±(40.5)	n=269 168.3±(43.9)	n=89 170.4±(44.6)	n=208 168.4±(44.2)	n=119 166.9±(44.1)	n=61 167.9±(43.4)	n=180 167.2±(43.7)
p-value <sup>1</sup>		0.18	0.17	0.20	0.47	0.47	0.36
Current height (cm)	n=351 164.6±(8.5)	n=268 164.4±(7.6)	n=89 164.6±(8.0)	n=207 164.6±(8.0)	n=118 164.6±(8.0)	n=61 163.9±(6.4)	n=179 164.3±(7.50)
p-value <sup>1</sup>		0.75	0.98	0.96	0.96	0.49	0.69
Current BMI (kg/m <sup>2</sup> )	n=351 27.6±7.5	n = 267 28.3±7.5	n = 89 28.5±7.0	n = 207 28.3±7.3	n = 118 28.1±7.6	n = 60 28.3±6.9	n = 178 28.1±7.4
p-value <sup>1</sup>		0.26	0.29	0.30	0.55	0.52	0.43



Table 4.3 Demographic characteristics of isolated orofacial cleft case and controls: The Utah Cleft 2 Study (Cont.)

Characteristic	Control	Orofacial cleft cases					
		All OFCs	CLO	CL/P	CLP	CPO	CP/L
Type 1 diabetes mellitus	n=422 3 (0.7)	n=349 2 (0.6)	n=109 0 (0)	n=261 0 (0)	n=152 0 (0)	n=88 2 (2.3)	n=240 2 (0.8)
p-value <sup>2</sup>		1.00	1.00	0.29	0.57	0.21	1.00
Type 2 diabetes mellitus	n=422 5 (1.2)	n=348 7 (2.0)	n=109 1 (0.9)	n=260 6 (2.3)	n=151 5 (3.3)	n=88 1 (1.1)	n=239 6 (2.5)
p-value <sup>2</sup>		0.39	1.00	0.35	0.14	1.00	0.22
Gestational diabetes mellitus	n=422 14 (3.3)	n=348 33 (9.5)	n=109 10 (9.2)	n=260 24 (9.2)	n=151 14 (9.3)	n=88 9 (10.2)	n=239 23 (9.6)
p-value <sup>2</sup>		<0.001	0.02	0.002	0.007	0.009	0.001
Maternal smoking in past 3 months (%)	n=423 19 (4.5)	n=349 39 (11.2)	n=109 10 (9.2)	n=261 26 (10)	n=152 16 (10.5)	n=88 13 (14.9)	n=240 29 (12.1)
p-value <sup>2</sup>		0.001	0.06	0.007	0.02	0.001	<0.001
Second hand smoking (%)	n=420 17 (4.0)	n=347 29 (8.4)	n=108 7 (6.5)	n=259 22 (8.5)	n=151 15 (9.9)	n=88 7 (8.0)	n=239 22 (9.2)
p-value <sup>2</sup>		0.01	0.30	0.02	0.01	0.16	0.009
Maternal alcohol consumption in past 3 months (%)	n=422 86 (20.4)	n=349 93 (26.7)	n=109 28 (27.5)	n=261 66 (25.3)	n=152 38 (25.0)	n=88 27 (31.0)	n=240 65 (27.2)
p-value <sup>2</sup>		0.04	0.24	0.13	0.25	0.05	0.05

Table 4.3 Demographic characteristics of isolated orofacial cleft case and controls: The Utah Cleft 2 Study (Cont.)

Characteristic	Control	Orofacial cleft cases					
		All OFCs	CLO	CL/P	CLP	CPO	CP/L
Frequency of alcohol consumption in past 3 months (%)							
Never	337 (79.7)	256 (73.6)	61 (70.1)	195 (74.7)	114 (75.0)	81 (74.3)	175 (73.2)
< 1 time/month	30 (7.1)	31 (8.9)	11 (12.6)	20 (7.7)	10 (6.6)	10 (9.2)	21 (8.8)
1-3 times/month	27 (6.4)	36 (10.3)	7 (8.0)	29 (11.1)	20 (13.2)	9 (8.3)	27 (11.3)
1-2 times/week	21 (5.0)	18 (5.2)	5 (5.7)	13 (5.0)	6 (3.9)	7 (6.4)	11 (4.6)
3-4 times/week	8 (1.9)	7 (2.0)	3 (3.4)	4 (1.5)	2 (1.3)	2 (1.8)	5 (2.1)
p-trend		0.12	0.13	0.25	0.39	0.33	0.15

OFCs: orofacial clefts; CPO: cleft palate only; CLO: cleft lip only; CLP: cleft lip with cleft palate; CL/P: cleft lip with or without cleft palate; CP/L: cleft palate with or without cleft lip.

<sup>1</sup> p-value for independent t-test for OFC group compared to controls.

<sup>2</sup> p-value for chi-square for OFC group compared to controls.

Table 4.4 Odds ratio analysis of maternal diabetes history and risk of orofacial clefts

Diabetes history	Odds ratios (95 percent confidence intervals)					
	All OFCs	CLO	CL/P	CLP	CPO	CP/L
Type 2 diabetes mellitus						
No	1 [reference]	1 [reference]	1 [reference]	1 [reference]	1 [reference]	1 [reference]
Yes	1.71 [0.54-5.44]	0.77 [0.09-6.68]	1.97 [0.60-6.52]	2.86[0.82-10.01]	0.96 [0.11-8.31]	2.15 [0.65-7.11]
Gestational diabetes mellitus						
No	1 [reference]	1 [reference]	1 [reference]	1 [reference]	1 [reference]	1 [reference]
Yes	3.05 [1.61-5.80]	2.94 [1.27-6.82]	2.96 [1.50-5.84]	2.98 [1.39-6.40]	3.32 [1.39-7.94]	3.10 [1.57-6.15]

OFCs: orofacial clefts; CPO: cleft palate only; CLO: cleft lip only; CLP: cleft lip with cleft palate; CL/P: cleft lip with or without cleft palate; CP/L: cleft palate with or without cleft lip.

History of GDM at any pregnancy

History of type 2 diabetes at any time.

Table 4.5 Mean metabolic syndrome-related biomarkers by orofacial cleft case groups and controls: The Utah Cleft 2 Study

Biomarker	Control	Orofacial cleft cases					
		All OFCs	CLO	CL/P	CLP	CPO	CP/L
	n = 353	n = 268	n = 89	n = 208	n = 119	n = 60	n = 179
Systolic BP (mmHg)	109.4±12.2	110.5±12.0	110.4±11.7	109.8±11.8	109.4±11.9	112.8±12.5	110.5±12.2
Unadjusted p-value		0.30	0.53	0.74	0.96	0.05	0.34
Adjusted p-value		0.71	0.58	0.88	0.48	0.23	0.96
	n = 353	n = 268	n = 89	n = 208	n = 119	n = 60	n = 179
Diastolic BP (mmHg)	71.8±9.1	72.5±10.4	72.4±9.8	72.1±10.5	71.8±11.1	73.8±9.8	72.5±10.7
Unadjusted p-value		0.43	0.59	0.79	0.98	0.12	0.49
Adjusted p-value		0.88	0.85	0.64	0.57	0.16	0.81
	n = 352	n = 268	n = 89	n = 208	n = 119	n = 60	n = 179
Waist (cm)	92.3±17.2	94.4±18.0	94.3±17.3	93.9±17.9	93.7±18.4	95.8±18.6	94.4±18.4
Unadjusted p-value		0.14	0.32	0.27	0.44	0.14	0.19
Adjusted p-value		0.06	0.75	0.16	0.09	0.07	0.03
	329	n = 248	n = 82	n = 189	n = 107	n = 59	n = 166
Glucose (mg/dL)	91.0±20.4	93.4±25.7	93.0±30.6	93.0±26.0	93.0±22.0	94.6±24.6	93.6±22.9
Unadjusted p-value		0.22	0.49	0.34	0.39	0.23	0.21
Adjusted p-value		0.07	0.99	0.13	0.04	0.21	0.02
	n = 316	n = 233	n = 78	n = 182	n = 104	n = 51	n = 155
Insulin (µU/mL)	19.7±26.8	21.6±24.4	15.7±15.2	20.4±21.6	24.0±25.0	25.8±32.3	24.6±27.5
Unadjusted p-value		0.40	0.21	0.76	0.16	0.21	0.07
Adjusted p-value		0.01	0.14	0.68	0.10	0.14	0.04
	n = 324	n = 246	n = 83	n = 187	n = 104	n = 59	n = 163
HbA1c (%)	5.42±0.30	5.44±0.53	5.45±0.54	5.43±0.45	5.41±0.36	5.47±0.73	5.43±0.52
Unadjusted p-value		0.66	0.49	0.85	0.74	0.41	0.81
Adjusted p-value		0.58	0.52	0.54	0.69	0.88	0.71

Table 4.5 Mean metabolic syndrome-related biomarkers by orofacial cleft case groups and controls: The Utah Cleft 2 Study (Cont.)

Biomarker	Control	Orofacial cleft cases					
		All OFCs	CLO	CL/P	CLP	CPO	CP/L
	n = 328	n = 247	n = 82	n = 188	n = 106	n = 59	n = 165
Cholesterol (mg/dL)	177.5±33.6	176.3±36.6	173.2±32.2	173.8±34.6	174.2±36.6	184.4±41.4	177.8±38.6
Unadjusted p-value		0.70	0.30	0.23	0.40	0.16	0.91
Adjusted p-value		0.48	0.66	0.27	0.22	0.56	0.51
	n = 329	n = 247	n = 82	n = 188	n = 106	n = 59	n = 165
Triglyceride (mg/dL)	110.3±69.5	118.2±81.3	107.8±54.6	113.4±72.5	117.7±83.7	133.4±103.8	123.3±91.4
Unadjusted p-value		0.21	0.76	0.63	0.37	0.10	0.11
Adjusted p-value		0.03	0.57	0.19	0.19	0.01	0.02
	329	n = 247	n = 82	n = 188	n = 106	n = 59	n = 165
HDL (mg/dL)	55.5±14.4	54.2±14.3	54.1±13.7	54.1±13.9	54.1±14.0	54.6±15.9	54.3±14.7
Unadjusted p-value		0.28	0.40	0.27	0.38	0.66	0.37
Adjusted p-value		0.02	0.40	0.06	0.05	0.08	0.02
	n = 328	n = 244	n = 82	n = 187	n = 105	n = 57	n = 162
LDL (mg/dL)	99.9±28.0	98.6±31.3	97.6±27.9	97.0±30.6	96.6±32.7	103.8±33.10	99.1±32.9
Unadjusted p-value		0.59	0.50	0.27	0.31	0.35	0.78
Adjusted p-value		0.64	0.99	0.45	0.28	0.63	0.53
	n = 328	n = 244	n = 82	n = 187	n = 105	n = 57	n = 162
VLDL (mg/dL)	21.8±13.2	22.7±13.6	21.6±11.0	22.3±13.3	22.8±15.0	24.0±14.4	23.2±14.7
Unadjusted p-value		0.44	0.88	0.70	0.50	0.26	0.28
Adjusted p-value		0.99	0.61	0.93	0.79	0.91	0.79
	n = 328	n = 244	n = 82	n = 187	n = 105	n = 57	n = 162
LDL/HDL ratio	1.92±0.75	1.95±0.86	1.92±0.72	1.93±0.84	1.93±0.93	2.05±0.91	1.97±0.92
Unadjusted p-value		0.60	0.99	0.91	0.88	0.26	0.52
Adjusted p-value		0.88	0.81	0.84	0.91	0.40	0.78

Table 4.5 Mean metabolic syndrome-related biomarkers by orofacial cleft case groups and controls: The Utah Cleft 2 Study (Cont.).

Biomarker	Control	Orofacial cleft cases					
		All OFCs	CLO	CL/P	CLP	CPO	CP/L
	n = 328	n = 247	n = 81	n = 188	n = 107	n = 59	n = 166
Alkaline phosphatase (IU/L)	64.3±18.7	65.8±20.1	64.4±18.9	65.9±20.2	67.1±21.1	65.2±19.7	66.4±20.6
Unadjusted p-value		0.36	0.95	0.35	0.19	0.73	0.25
Adjusted p-value		0.60	0.72	0.50	0.21	0.86	0.37
	n = 328	n = 247	n = 81	n = 188	n = 107	n = 59	n = 166
ALT (SGPT) (IU/L)	18.1±8.9	19.2±10.4	19.5±10.4	19.6±11.2	19.6±11.8	18.1±7.1	19.1±10.4
Unadjusted p-value		0.15	0.20	0.09	0.15	1.00	0.26
Adjusted p-value		0.18	0.30	0.11	0.14	0.96	0.25
	n = 328	n = 247	n = 81	n = 188	n = 107	n = 59	n = 166
AST (SGOT)(IU/L)	18.1±5.1	19.4±13.2	20.3±16.6	19.9±14.8	19.6±13.4	17.9±5.8	19.0±11.3
Unadjusted p-value		0.14	0.25	0.11	0.26	0.74	0.34
Adjusted p-value		0.08	0.05	0.05	0.08	0.94	0.15

OFCs: orofacial clefts; CPO: cleft palate only; CLO: cleft lip only; CLP: cleft lip with cleft palate; CL/P: cleft lip with or without cleft palate; CP/L: cleft palate with or without cleft lip.

BP: blood pressure, WC: waist circumference, HbA1c: Hemoglobin A1c, HDL: high-density lipoprotein cholesterol, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase.

NCEP/ATP III: National Cholesterol Education Program, Adult Treatment Panel III

IDF: International Diabetes Federation

Unadjusted p-value for independent sample t-test

Adjusted p-value form analysis of covariance model including maternal age and smoking (Model)

Table 4.6 Cytokine and adipokine levels of orofacial cleft cases and controls: The Utah Cleft 2 Study

	Control (n = 241)	Orofacial cleft cases					
		All OFCs (n = 182)	CLO (n = 60)	CL/P (n = 138)	CLP (n = 78)	CPO (n = 44)	CP/L (n = 122)
<b>Adiponectin (ng/ml)</b>							
% Undetected	0	0	0	0	0	0	0
Median <sup>1</sup> ±IQR	8921±7379	7689±8220	7360±10204	7893±8412	8522±8223	7230±8120	8273±8083
Mean <sup>2</sup> ±SD	10937±560	10413±644	10792±1185	10489±749	10256±971	10173±1327	10226±767
p-value <sup>a</sup>		0.54	0.91	0.63	0.54	0.60	0.45
p-value <sup>b</sup>		0.70	0.82	0.79	0.61	0.81	0.57
<b>CRP (ng/ml)</b>							
% Undetected	1.2	0	0	0	0	0	0
Median <sup>1</sup> ±IQR	23324 ±123764	33834 ±115461	26365 ±58834	30200 ±73054	35225 ±121045	50447 ±241589	38130 ±146133
Mean <sup>2</sup> ±SD	159345 ±384310	152771 ±427596	79971.1 ±155579.0	147468 ±469983	199388 ±606773	169406 ±255936	188575 ±507720
p-value <sup>a</sup>		0.59	0.47	0.88	0.70	0.11	0.25
p-value <sup>b</sup>		0.67	0.41	0.84	0.72	0.15	0.29
<b>IL-1a (pg/ml)</b>							
% Undetected	21.2	17.6	15.0	17.4	19.2	18.2	18.9
Median <sup>1</sup> ±IQR	3.51±4.50	3.70±4.34	4.19±4.14	3.72±4.40	3.46±4.50	3.28±3.25	3.35±4.27
Mean <sup>2</sup> ±SD	3.69±2.80	3.85±2.63	4.25±2.79	3.93±2.70	3.69±2.63	3.58±2.38	3.65±2.53
p-value <sup>a</sup>		0.99	0.32	0.71	0.74	0.47	0.52
p-value <sup>b</sup>		0.94	0.52	0.88	0.73	0.54	0.58

Table 4.6 Cytokine and adipokine levels of orofacial cleft cases and controls: The Utah Cleft 2 Study (Cont.)

	Control (n = 241)	Orofacial cleft cases					
		All OFCs (n = 182)	CLO (n = 60)	CL/P (n = 138)	CLP (n = 78)	CPO (n = 44)	CP/L (n = 122)
<b>IL-6 (pg/ml)</b>							
% Undetected	11.6	12.6	11.7	13.0	14.1	11.4	13.1
Median <sup>1</sup> ±IQR	3.82±3.15	4.02±3.55	4.38±4.00	4.36±3.56	4.25±3.20	3.10±3.49	3.58±3.38
Mean <sup>2</sup> ±SD	3.87±3.22	3.89±2.55	4.27±2.94	4.04±2.61	3.86±2.32	3.42±2.32	3.70±2.32
p-value <sup>a</sup>		0.93	0.53	0.35	0.40	0.10	0.76
p-value <sup>b</sup>		0.91	0.54	0.34	0.38	0.11	0.83
<b>IL-8 (pg/ml)</b>							
% Undetected	34.9	33.5	35.0	37.7	39.7	20.5	32.8
Median <sup>1</sup> ±IQR	2.85±1.59	3.05±1.78	2.69±1.53	2.71±1.61	2.83±1.72	3.32±2.21	3.12±1.93
Mean <sup>2</sup> ±SD	3.18±1.21	3.45±1.88	3.19±1.33	3.22±1.35	3.24±1.38	4.16±2.89	3.57±2.09
p-value <sup>a</sup>		0.03	0.88	0.40	0.16	<0.001	0.003
p-value <sup>b</sup>		0.02	0.47	0.60	0.16	0.001	0.003
<b>Leptin (ng/ml)</b>							
% Undetected	0.8	0	0	0	0	0	0
Median <sup>1</sup> ±IQR	8.79±15.36	10.14±33.50	8.99±23.68	9.63±31.21	10.78±34.68	11.06±37.48	10.87±35.14
Mean <sup>2</sup> ±SD	18.40±23.16	26.87±39.83	20.14±26.67	26.47±42.91	31.34±51.73	28.13±28.44	30.18±44.64
p-value <sup>a</sup>		0.04	0.83	0.13	0.03	0.01	0.004
p-value <sup>b</sup>		0.06	0.71	0.26	0.05	0.01	0.006
<b>Resistin (ng/ml)</b>							
% Undetected	1.7	1.1	3.3	1.4	0	0	0
Median <sup>1</sup> ±IQR	2924±3284	2686±3283	2686±3298	2671±3239	2516±3150	2817±3368	2689±3274
Mean <sup>2</sup> ±SD	2235±1602	2193±1740	2369±1947	2132±1771	1949±1611	2386±1643	2107±1630
p-value <sup>a</sup>		0.74	0.46	0.54	0.12	0.66	0.35
p-value <sup>b</sup>		0.92	0.45	0.61	0.16	0.47	0.50



Table 4.6 Cytokine and adipokine levels of orofacial cleft cases and controls: The Utah Cleft 2 Study (Cont.)

	Control (n = 241)	Orofacial cleft cases					
		All OFCs (n = 182)	CLO (n = 60)	CL/P (n = 138)	CLP (n = 78)	CPO (n = 44)	CP/L (n = 122)
TNF- $\alpha$ (pg/ml)							
% Undetected	7.1	7.1	5.0	5.8	6.4	11.4	8.2
Median <sup>1</sup> ±IQR	12.07±7.61	12.52±8.36	13.08±6.06	12.68±7.99	12.32±6.36	12.07±9.38	12.32±7.81
Mean <sup>2</sup> ±SD	12.76±11.09	13.09±7.43	14.12±8.05	13.41±7.55	12.87±7.14	12.07±7.04	12.58±7.09
p-value <sup>a</sup>		0.50	0.27	0.43	0.83	0.98	0.86
p-value <sup>b</sup>		0.46	0.31	0.41	0.75	0.96	0.74

OFCS: orofacial clefts; CPO: cleft palate only; CLO: cleft lip only; CLP: cleft lip with cleft palate; CL/P: cleft lip with or without cleft palate; CP/L: cleft palate with or without cleft lip.

CRP: C-reactive protein, IL-1 $\alpha$ : Interleukin-1 alpha, IL-6: Interleukin-6, IL-8: Interleukin-8, TNF- $\alpha$ : Tumor necrosis factor alpha

<sup>1</sup> median and interquartile range (IQR) from all samples

<sup>2</sup> mean and standard deviation (SD)

<sup>a</sup>Unadjusted p-value for test difference to control among detected group (two-way ANOVA)

<sup>b</sup>Adjusted p-value for test difference to control among detected group with analysis of covariance model including maternal age and smoking (two-way ANOVA).

Table 4.7 Odds ratio analysis of body mass index and biomarkers related to metabolic syndrome and risk of orofacial clefts: The Utah Cleft 2 Study

	Odds ratios (95 percent confidence intervals)					
	All OFCs	CLO	CL/P	CLP	CPO	CP/L
<b>BMI (kg/m<sup>2</sup>)</b>						
Underweight	0.77 [0.22-2.61]	0.63 [0.08-5.18]	1.03 [0.30-3.52]	1.31 [0.33-5.15]	N/A	0.83 [0.21-3.22]
Normal	Reference	Reference	Reference	Reference	Reference	Reference
Overweight	1.09 [0.72-1.63]	0.86 [0.45-1.66]	1.13 [0.73-1.75]	1.31 [0.78-2.20]	0.97 [0.49-1.93]	1.19 [0.76-1.86]
Obese	1.45 [0.99-2.13]	2.01 [1.17-3.45]	1.53 [1.01-2.31]	1.19 [0.71-2.00]	1.24 [0.65-2.35]	1.21 [0.78-1.87]
	p-trend 0.06	p-trend 0.012	p-trend 0.048	p-trend 0.47	p-trend 0.54	p-trend 0.39
<b>Glucose (mg/dl)</b>						
≤ 84	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
85-93	1.13 (0.76-1.69)	0.92 (0.52-1.64)	1.18 (0.77-1.82)	1.47 (0.85-2.54)	0.99 (0.50-1.94)	1.27 (0.80-2.00)
≥ 94	1.16 (0.78-1.75)	0.85 (0.47-1.54)	1.20 (0.77-1.86)	1.58 (0.92-2.74)	1.07 (0.54-2.09)	1.37 (0.86-2.17)
	p-trend 0.46	p-trend 0.59	p-trend 0.42	p-trend 0.10	p-trend 0.86	p-trend 0.19
<b>HbA1c (%)</b>						
≤ 5.30	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
> 5.30 - 5.50	0.73 (0.49-1.08)	0.86 (0.49-1.52)	0.73 (0.47-1.12)	0.63 (0.36-1.08)	0.73 (0.39-1.35)	0.67 (0.43-1.04)
> 5.50	0.87 (0.58-1.32)	0.96 (0.53-1.76)	1.04 (0.67-1.61)	1.09 (0.64-1.85)	0.45 (0.20-0.99)	0.83 (0.52-1.33)
	p-trend 0.41	p-trend 0.85	p-trend 0.98	p-trend 0.90	p-trend 0.04	p-trend 0.33
<b>Insulin (μU/mL)</b>						
≤ 7.80	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
>7.80 -18.10	1.07 (0.70-1.61)	0.91 (0.50-1.64)	1.06 (0.68-1.67)	1.23 (0.70-2.16)	1.07 (0.52-2.23)	1.17 (0.72-1.90)
> 18.10	1.25 (0.83-1.90)	0.87 (0.47-1.61)	1.26 (0.80-1.97)	1.65 (0.95-2.86)	1.24 (0.60-2.56)	1.50 (0.93-2.41)
	p-trend 0.29	p-trend 0.66	p-trend 0.32	p-trend 0.07	p-trend 0.56	p-trend 0.09

Table 4.7 Table 4.7 Odds ratio analysis of body mass index and biomarkers related to metabolic syndrome and risk of orofacial clefts: The Utah Cleft 2 Study (Cont.)

	Odds ratios (95 percent confidence intervals)					
	All OFCs	CLO	CL/P	CLP	CPO	CP/L
<b>Cholesterol (mg/dL)</b>						
≤ 161	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
162-188	1.04 (0.70-1.56)	1.06 (0.59-1.88)	0.99 (0.64-1.53)	0.94 (0.56-1.61)	1.27 (0.61-2.64)	1.04 (0.65-1.65)
≥ 189	1.09 (0.73-1.63)	0.88 (0.48-1.61)	0.92 (0.59-1.43)	0.95 (0.56-1.62)	1.85 (0.93-3.68)	1.21 (0.77-1.90)
	p-trend 0.67	p-trend 0.69	p-trend 0.72	p-trend 0.86	p-trend 0.07	p-trend 0.42
<b>Triglyceride (mg/dL)</b>						
≤ 70	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
71-120	1.07 (0.71-1.60)	1.13 (0.63-2.05)	0.95 (0.61-1.47)	0.82 (0.48-1.41)	1.64 (0.79-3.39)	1.03 (0.65-1.64)
≥ 121	1.20 (0.80-1.79)	1.11 (0.62-2.02)	1.05 (0.68-1.62)	1.00 (0.60-1.69)	1.91 (0.94-3.89)	1.24 (0.79-1.95)
	p-trend 0.38	p-trend 0.72	p-trend 0.84	p-trend 0.99	p-trend 0.08	p-trend 0.36
<b>HDL (mg/dL)</b>						
≤ 48	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
49-61	0.79 (0.53-1.18)	0.71 (0.36-1.41)	0.82 (0.53-1.26)	0.72 (0.42-1.22)	0.96 (0.54-1.71)	0.72 (0.45-1.13)
≥ 62	0.72 (0.48-1.08)	0.80 (0.41-1.55)	0.70 (0.45-1.09)	0.70 (0.41-1.18)	0.71 (0.38-1.30)	0.73 (0.46-1.15)
	p-trend 0.11	p-trend 0.49	p-trend 0.11	p-trend 0.17	p-trend 0.27	p-trend 0.16
<b>LDL (mg/dL)</b>						
≤ 86	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
87-109	0.88 (0.59-1.32)	1.05 (0.59-1.86)	0.84 (0.55-1.29)	0.70 (0.42-1.20)	1.07 (0.52-2.20)	0.80 (0.51-1.27)
≥ 110	0.84 (0.56-1.27)	0.79 (0.43-1.46)	0.72 (0.46-1.12)	0.67 (0.39-1.15)	1.39 (0.69-2.77)	0.87 (0.55-1.37)
	p-trend 0.41	p-trend 0.46	p-trend 0.15	p-trend 0.14	p-trend 0.34	p-trend 0.53

Table 4.7 Odds ratio analysis of body mass index and biomarkers related to metabolic syndrome and risk of orofacial clefts: The Utah Cleft 2 Study (Cont.)

	Odds ratios (95 percent confidence intervals)					
	All OFCs	CLO	CL/P	CLP	CPO	CP/L
VLDL (mg/dL)						
≤ 14	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
15-24	1.10 (0.74-1.65)	1.22 (0.67-2.19)	0.94 (0.61-1.45)	0.76 (0.44-1.31)	1.92 (0.94-3.93)	1.05 (0.66-1.66)
≥ 25	1.19 (0.79-1.78)	1.23 (0.68-2.23)	1.07 (0.70-1.65)	0.97 (0.58-1.64)	1.76 (0.85-3.65)	1.17 (0.74-1.84)
	p-trend 0.41	p-trend 0.49	p-trend 0.77	p-trend 0.87	p-trend 0.14	p-trend 0.52
LDL/HDL ratio						
≤ 1.50	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
> 1.50-2.13	0.78 (0.52-1.18)	0.97 (0.53-1.77)	0.75 (0.48-1.17)	0.62 (0.36-1.07)	0.90 (0.44-1.82)	0.70 (0.44-1.12)
> 2.13	0.99 (0.66-1.48)	1.19 (0.66-2.16)	0.95 (0.61-1.46)	0.80 (0.47-1.36)	1.16 (0.58-2.29)	0.91 (0.58-1.43)
	p-trend 0.97	p-trend 0.56	p-trend 0.80	p-trend 0.39	p-trend 0.67	p-trend 0.67

OFCs: orofacial clefts; CPO: cleft palate only; CLO: cleft lip only; CLP: cleft lip with cleft palate; CL/P: cleft lip with or without cleft palate; CP/L: cleft palate with or without cleft lip.

BMI: body mass index, HbA1c: Hemoglobin A1c, HDL: high-density lipoprotein cholesterol, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase.

BMI: underweight, ≤ 18.5 kg/m<sup>2</sup>; normal, 18.5-24.9 kg/m<sup>2</sup>; overweight, 25.-29.9 kg/m<sup>2</sup>; obese, ≥ 30 kg/m<sup>2</sup>.

Table 4.8 Risk of orofacial clefts associated with NCEP/ATP III and IDF metabolic syndrome criteria and components.

	Odds ratios (95 percent confidence intervals)					
	All OFCs	CLO	CL/P	CLP	CPO	CP/L
MET score (NCEP/ATP III)						
< 3	1 [reference]	1 [reference]	1 [reference]	1 [reference]	1 [reference]	1 [reference]
≥ 3	1.42 [0.92-2.17]	1.06 [0.54-2.05]	1.31 [0.82-2.09]	1.51 [0.88-2.60]	1.78 [0.91-3.49]	1.60 [1.00-2.56]
MET score (IDF)						
WC < 80 cm	1 [reference]	1 [reference]	1 [reference]	1 [reference]	1 [reference]	1 [reference]
WC > 80 cm + 2 more	1.48 [0.98-2.24]	1.16 [0.62-2.17]	1.38 [0.89-2.18]	1.58 [0.93-2.67]	1.78 [0.92-3.42]	1.64 [1.04-2.59]
Systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg	1.51 [0.91-2.53]	1.03 [0.46-2.32]	1.30 [0.73-2.29]	1.50 [0.78-2.89]	2.34 [1.10-4.96]	1.77 [1.02-3.09]
WC > 88 cm <sup>a</sup>	1.09 [0.79-1.50]	1.18 [0.74-1.89]	1.11 [0.79-1.57]	1.06 [0.70-1.61]	1.04 [0.58-1.75]	1.04 [0.73-1.50]
WC > 80 cm <sup>b</sup>	1.13 [0.78-1.64]	1.03 [0.60-1.77]	1.16 [0.77-1.74]	1.27 [0.77-2.10]	1.04 [0.55-1.95]	1.18 [0.77-1.81]
TG ≥ 150 mg/dL	1.05 [0.70-1.58]	0.77 [0.40-1.47]	1.00 [0.64-1.55]	1.18 [0.70-1.97]	1.24 [0.65-2.39]	1.20 [0.77-1.87]
HDL < 50 mg/dL	1.22 [0.87-1.69]	1.16 [0.71-1.90]	1.21 [0.84-1.73]	1.25 [0.81-1.93]	1.23 [0.71-2.15]	1.24 [0.86-1.80]
FPG > 110 mg/dL <sup>a</sup>	1.41 [0.70-2.84]	1.01 [0.33-3.10]	1.30 [0.60-2.81]	1.52 [0.63-3.67]	1.75 [0.62-4.98]	1.61 [0.75-3.42]
FPG > 100 mg/dL <sup>b</sup>	1.20 [0.65-2.23]	0.87 [0.32-2.35]	1.13 [0.57-2.21]	1.32 [0.61-2.87]	1.46 [0.57-3.74]	1.37 [0.70-2.67]

OFCs: orofacial clefts; CPO: cleft palate only; CLO: cleft lip only; CLP: cleft lip with cleft palate; CL/P: cleft lip with or without cleft palate; CP/L: cleft palate with or without cleft lip.

BP: blood pressure, WC: waist circumference, TG: triglyceride, HDL: high-density lipoprotein cholesterol, FPG: fasting plasma glucose

<sup>a</sup>National Cholesterol Education Program, Adult Treatment Panel III

<sup>b</sup>International Diabetes Federation

MET: metabolic syndrome

NCEP/ATP III: National Cholesterol Education Program, Adult Treatment Panel III [raised waist circumference ( $\geq 102$  cm in males,  $\geq 88$  cm in females), raised blood pressure (systolic blood pressure  $\geq 130$  mmHg or diastolic blood pressure  $\geq 85$  mmHg), raised triglyceride ( $\geq 150$  mg/dL), reduced HDL cholesterol ( $<40$  mg/dL in males,  $<50$  mg/dL in females), raised fasting plasma glucose (fasting plasma glucose  $\geq 110$  mg/dL or previous diagnosed type 2 diabetes)]

IDF: International Diabetes Federation [raised waist circumference ( $\geq 94$  cm in males,  $\geq 80$  cm in females), raised blood pressure (systolic blood pressure  $\geq 130$  mmHg or diastolic blood pressure  $\geq 85$  mmHg), raised triglyceride ( $\geq 150$  mg/dL), reduced HDL cholesterol ( $<40$  mg/dL in males,  $<50$  mg/dL in females), raised fasting plasma glucose (fasting plasma glucose  $\geq 100$  mg/dL or previous diagnosed type 2 diabetes)]

WC: Waist circumference

N/A: Not applicable

Table 4.9 Risk of orofacial clefts associated with cytokine and adipokine levels.

Tertile	Odds ratios (95 percent confidence intervals)					
	All OFCs	CLO	CL/P	CLP	CPO	CP/L
<b>Adiponectin (mg/L)</b>						
≤ 6199.51	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
> 6199.52-10907.73	0.73 (0.45-1.17)	0.79 (0.40-1.56)	0.73 (0.44-1.22)	0.68 (0.36-1.30)	0.72 (0.33-1.55)	0.70 (0.41-1.19)
≥ 10907.73	0.80 (0.50-1.27)	0.74 (0.37-1.48)	0.83 (0.50-1.38)	0.90 (0.49-1.66)	0.69 (0.31-1.52)	0.82 (0.49-1.39)
	p-trend 0.34	p-trend 0.39	p-trend 0.48	p-trend 0.76	p-trend 0.35	p-trend 0.47
<b>CRP (mg/L)</b>						
≤ 14061.55	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
> 14061.55-71263.16	1.28 (0.80-2.05)	1.31 (0.68-2.54)	1.28 (0.77-2.11)	1.24 (0.66-2.33)	1.31 (0.55-3.08)	1.26 (0.73-2.18)
> 71263.16	1.17 (0.73-1.88)	0.72 (0.34-1.51)	0.98 (0.58-1.65)	1.21 (0.65-2.27)	1.96 (0.88-4.34)	1.45 (0.85-2.47)
	p-trend 0.51	p-trend 0.43	p-trend 0.96	p-trend 0.55	p-trend 0.09	p-trend 0.18
<b>IL-1a (pg/ml)</b>						
≤ 2.08	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
> 2.08-4.96	1.12 (0.70-1.79)	1.26 (0.61-2.63)	1.02 (0.60-1.71)	0.87 (0.47-1.64)	1.44 (0.68-3.07)	1.06 (0.63-1.80)
> 4.96	1.17 (0.73-1.87)	1.68 (0.84-3.39)	1.27 (0.77-2.11)	1.04 (0.56-1.92)	0.85 (0.36-1.98)	0.97 (0.57-1.67)
	p-trend 0.52	p-trend 0.14	p-trend 0.35	p-trend 0.91	P-trend 0.75	p-trend 0.93
<b>IL-6 (pg/ml)</b>						
≤ 2.78	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
> 2.78-5.01	0.72 (0.45-1.16)	0.77 (0.38-1.56)	0.74 (0.44-1.25)	0.72 (0.38-1.38)	0.66 (0.31-1.42)	0.70 (0.41-1.20)
> 5.01	1.00 (0.63-1.60)	1.10 (0.56-2.18)	1.13 (0.68-1.88)	1.15 (0.62-2.13)	0.67 (0.30-1.48)	0.96 (0.56-1.62)
	p-trend 1.00	p-trend 0.77	p-trend 0.62	p-trend 0.63	p-trend 0.30	p-trend 0.86

Table 4.9 Risk of orofacial clefts associated with cytokine and adipokine levels (Cont.).

Tertile	Odds ratios (95 percent confidence intervals)					
	All OFCs	CLO	CL/P	CLP	CPO	CP/L
<b>IL-8 (pg/ml)</b>						
≤ 2.20	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
> 2.20-3.53	0.93 (0.58-1.49)	1.06 (0.54-2.07)	0.82 (0.49-1.36)	0.65 (0.35-1.24)	1.57 (0.65-3.84)	0.86 (0.50-1.49)
> 3.53	1.21 (0.76-1.93)	0.92 (0.45-1.87)	0.96 (0.58-1.60)	0.99 (0.54-1.81)	2.65 (1.14-6.14)	1.36 (0.81-2.30)
	p-trend 0.43	p-trend 0.83	p-trend 0.85	p-trend 0.93	p-trend 0.018	p-trend 0.24
<b>Leptin (ng/ml)</b>						
≤ 5.95	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
> 5.95-15.44	1.07 (0.67-1.73)	0.81 (0.41-1.61)	0.97 (0.57-1.62)	1.13 (0.59-2.20)	1.55 (0.66-3.65)	1.27 (0.73-2.22)
> 15.44	1.73 (1.07-2.77)	1.07 (0.54-2.12)	1.56 (0.94-2.60)	2.10 (1.12-3.93)	2.46 (1.07-5.63)	2.21 (1.28-3.81)
	p-trend 0.02	p-trend 0.89	p-trend 0.09	p-trend 0.02	p-trend 0.03	p-trend 0.004
<b>Resistin (ng/ml)</b>						
≤ 481.22	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
>481.22- 3328.74	1.20 (0.75-1.92)	1.43 (0.71-2.90)	1.06 (0.64-1.77)	0.86 (0.46-1.59)	1.81 (0.80-4.08)	1.11 (0.65-1.88)
> 3328.74	1.00 (0.62-1.60)	1.24 (0.61-2.51)	0.92 (0.55-1.53)	0.74 (0.40-1.38)	1.36 (0.59-3.14)	0.91 (0.53-1.54)
	p-trend 1.00	p-trend 0.58	p-trend 0.74	p-trend 0.34	p-trend 0.50	p-trend 0.72
<b>TNF-α (pg/ml)</b>						
≤ 9.54	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
> 9.54-14.48	0.82 (0.51-1.31)	0.92 (0.45-1.89)	0.92 (0.55-1.54)	0.92 (0.50-1.70)	0.54 (0.23-1.25)	0.77 (0.45-1.32)
> 14.48	1.09 (0.68-1.74)	1.39 (0.70-2.76)	1.11 (0.66-1.85)	0.92 (0.49-1.74)	1.04 (0.50-2.18)	0.97 (0.57-1.64)
	p-trend 0.72	p-trend 0.34	p-trend 0.69	p-trend 0.80	p-trend 0.93	p-trend 0.89

OFCs: orofacial clefts; CPO: cleft palate only; CLO: cleft lip only; CLP: cleft lip with cleft palate; CL/P: cleft lip with or without cleft palate; CP/L: cleft palate with or without cleft lip.



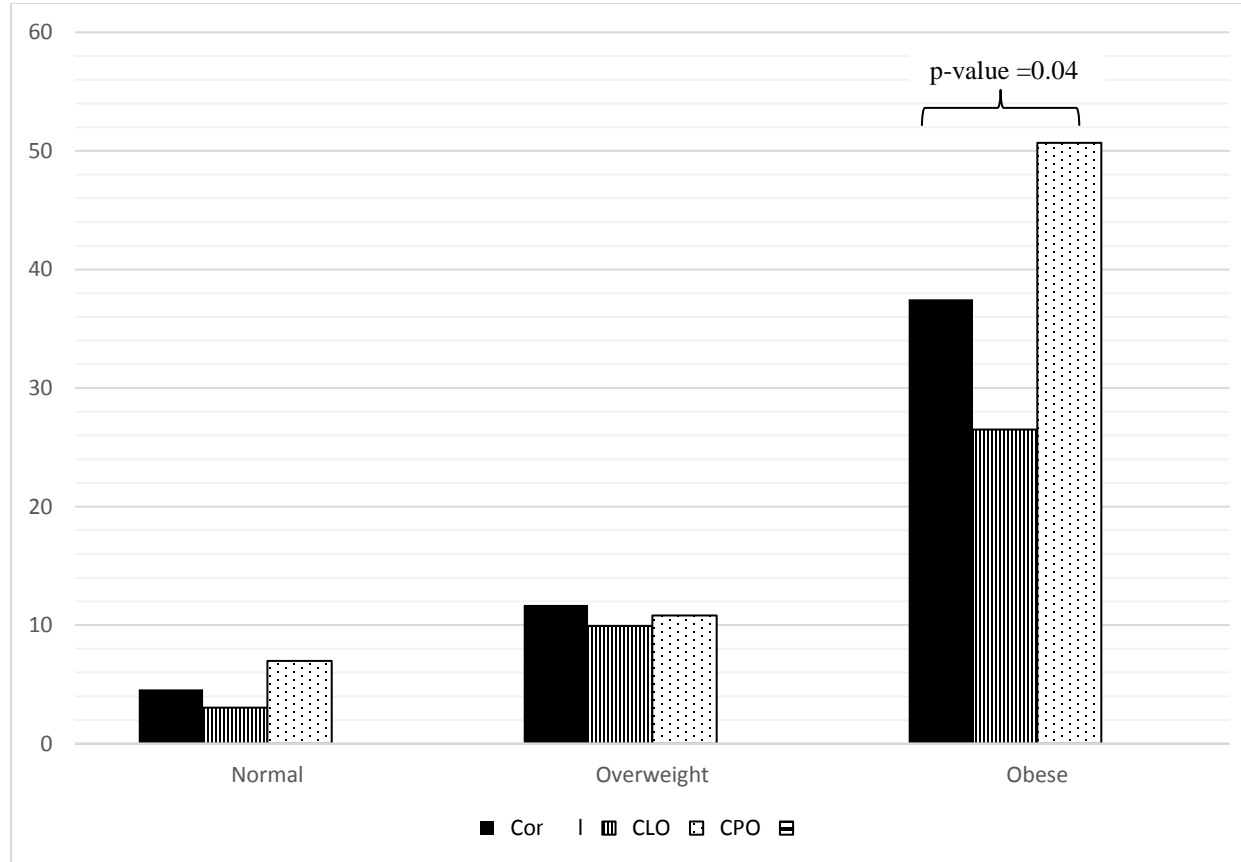


Figure 4.2 Medians of leptin levels divided by maternal body mass index

OFCs: orofacial clefts; CPO: cleft palate only; CLO: cleft lip only; CP/L: cleft palate with or without cleft lip.  
 p-value for test difference to control among detected group with analysis of covariance model including maternal age and smoking (two-way ANOVA)

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## CHAPTER 5

THE ASSOCIATION BETWEEN GENES RELATED TO GESTATIONAL  
DIABETES MELLITUS AND THE RISK OF OROFACIAL CLEFTS**5.1 Abstract**

**Background:** Gestational diabetes mellitus (GDM) has been associated with an increased risk of orofacial clefts (OFCs) and there is some evidence that GDM may be specifically related to palate development rather than lip. Genetic studies have identified many genes associated with non-syndromic OFCs, but none are clearly associated with GDM.

**Objective:** to determine the association between genes related to GDM and the risk of OFCs.

**Methods:** Genetic data from the GENEVA study of OFCs consisted of 892 cleft lip with or without cleft palate (CL/P) and 910 cleft palate with or without cleft lip (CP/L) trios of Asian ancestry and 665 CL/P and 644 CP/L trios of European ancestry. Twenty GDM-related genes were selected for analysis of association with OFCs. Genotypic transmission disequilibrium tests (gTDT) were used to analyze genetic effects and gene-environment (GxE) interactions with periconceptional maternal multivitamin use (PCMV), smoking, and environmental tobacco smoke (ETS).

**Results:** SLC30A8 was associated with CL/P and HNF1B was associated with CP/L in Asian trios. In Europeans, ADRB3 and TNF- $\alpha$  were associated with both CL/P and CP/L; ABCC8 was associated with CL/P only and ADIPOQ and HNF1 were associated with CP/L only. Considering interactions with PCMV, associations were

found for ABCC8 and CDKAL1 in Europeans. Considering interactions with maternal smoking, associations were found for CDKN2A/2B in Asian and for LEP in Europeans. In Asians, FTO, HHEX, and PPARG had associations with OFCs when considering interaction with ETS.

**Conclusion:** Several genes related to GDM were associated with risk of isolated CL/P and CP/L through genotypic effects alone and gene-environment interactions with PCMV, maternal smoking, and ETS. These associations do not point to a single major GDM gene associated with OFCs, but support the hypothesis that GDM may be causally related to OFCs via multiple GDM susceptibility genes and interactions with environmental factors.

## 5.2 Introduction

Orofacial clefts (OFCs) are among the most common congenital birth defects. Globally, approximately 1.43 in 1000 newborns suffer from OFCs (1). The prevalence of OFCs is different among varying ethnic and racial groups (2). National Birth Defects Prevention Network reported the prevalence of OFCs during 2002-2006 of CL/P was 1.33 per 1000 live births, and 0.73 per 1000 live births for CP (3). The prevalence of OFCs in Utah is higher than the overall prevalence of the United States (2.25 per 1000 births) (4).

The etiology of orofacial clefts is not fully understood. The updated evidence suggests that there are the multiple factors for this defect including both genetic and environmental factors (5). Maternal diabetes mellitus, obesity, and underweight have been found as risk factors for OFCs (6-13). Pre-diabetes mellitus and GDM increased risk

of OFCs (6-8). Many case-control studies and a meta-analysis found that maternal obesity increased risk of OFCs. The previous study by Kutbi (11) reported the association between maternal obesity and risk of CL/P and CP/L. In addition, we found that GDM increased risk of cleft palate only (CPO) and CP/L in the previous chapter.

OFCs are classified as either syndromic or non-syndromic. Syndromic OFCs are those occurring with other birth defects, while non-syndromic clefts have no other structural or functional anomalies. The prevalence of syndromic OFCs is around 25 percent of all OFCs; around seventy-five percent of syndromic OFCs can be described by known genetic conditions including Van der Woude syndrome, Bamforth–Lazarus syndrome, Kabuki syndrome, Pierre Robin syndrome, and Treacher Collins syndrome (14). Genetic studies have identified genes related to syndromic OFCs and have provided clear associations between cleft phenotypes and the mutations of genes (15). Genetic studies have also found associations between non-syndromic orofacial clefts and genes related to growth factors, transcription factors, xenobiotic metabolism, immune response, and one-carbon metabolism (16). Genome-wide association studies (GWAS) have identified additional genes associated with non-syndromic orofacial clefts, but none to date are clearly associated with GDM (17-20).

Several environmental factors, including PCMV, smoking, and ETS have been associated with OFCs. These same exposures have been associated with diabetes, therefore it is of interest to explore possible interaction between GDM-related genes and these environmental factors.



There has been no genetic association study focusing on GDM-related genes and OFC risk. Therefore, this study aims to determine the association between genes known to be related to GDM and risk of OFCs through genetic effects and gene-environment interactions.

### **5.3 Subjects and Methods**

#### **5.3.1 Study Design**

The study design is based on trios of children with an isolated orofacial cleft and their mothers and fathers using genome wide association (GWA) data available from the International Genetic Epidemiology study of Oral Clefts, a part of the Gene-Environment Association Studies Initiative (GENEVA) of the National Institutes of Health (NIH) (20). This study is a multi-center, international study of trios from Europe, the U.S., including Utah, China, Taiwan, Singapore, Korea, and the Philippines, which aims to investigate genes associated with oral clefts. Families were recruited from treatment centers or population-based registries. Research protocols for human study were reviewed and approved by institutional review boards of each participating institutions and parents provided informed consent.

Principal Components Analysis (PCA) was used to document ancestral population affiliation. OFC cases were examined by either a clinical geneticist or an experienced clinician to minimize misclassification of the OFCs. All cases with cleft palate with or without cleft lip (CP/L) were analyzed together based on evidence that maternal obesity and diabetes have a specific effect on palate development (11). Trios having CL/P and CP/L were analyzed in this study separately.

### 5.3.2 Maternal exposure assessment

Parents were asked about family history of oral clefts and other birth defects and maternal exposures during the periconceptional period (three months before conception through the first trimester) including smoking, alcohol consumption, environmental tobacco smoke exposure (ETS), and multivitamin use (PCMV).

### 5.3.3 Gene selection and genotyping

Gene selection was based on publications focusing on genes associated with GDM through March 2015. Eighty-three genes were found to have significant associations with GDM. In order to narrow the gene list, GDM candidate genes (Table 5.1) were limited to those with statistically significant associations with GDM and either type 2 diabetes or obesity in at least three candidate gene studies or at least two GWA studies or at least one meta-analysis study. Genomic coordinates of selected genes were obtained from the major human genome assembly released by the Genome Reference Consortium, NCBI36/hg18, via the website of the National Center for Biotechnology Information (NCBI) (21). Case and parent samples were collected as whole blood in EDTA tube for analyzing DNA. DNA samples were previously genotyped using the Illumina Human610 Quad v.1 B BeadChip at the Center for Inherited Disease Research (CIDR) and 99.1% passed quality control standards (20). Genotypes were not called if the quality score was more than 1 HapMap replicate error, more than 1 percent difference in call rate between genders, or more than 4% difference in heterozygote frequency.

### 5.3.4 Statistical analysis

Statistical analyses were performed in the R program 3.1.2 TRIO package (22). Isolated CL/P and CP/L were analyzed separately. Individuals with more than 10 percent missing genotypes were excluded from the study. Four quality control (QC) criteria were used to flag and remove SNPs: 1) high rates of missing genotype calls (>10%), 2) low minor allele frequency (MAF <0.01), 3) high rates of Mendelian errors (> 5%) and 4) significant deviation from Hardy-Weinberg equilibrium ( $p > 10^{-5}$ ). MAFs were calculated using parents only. SNPs within 100kb upstream and downstream of the selected genes were selected and used in this analysis in order include the promoter region (23, 24). Because this study included hypotheses for targeted GDM genes, based on strong evidence from previous studies, a gene-level approach to analysis was employed with Bonferroni adjustment of p-values based on the number of SNPs used in each individual gene region as employed in previous studies (25-28).

The associations between genes related to GDM and risk of OFCs was examined by the genotypic transmission disequilibrium (gTDT) test developed by Schwender et al (29). Europeans and Asians were analyzed separately because of different allelic frequencies. All each SNP, one of the four possible pairs of parental alleles is known to be inherited by the case-child, and the other three untransmitted genotypes are used as pseudo-controls. The gTDT approach can be formulated as a conditional logistic regression model which can be written as:

$$\ln \{P(i^{\text{th}} \text{ case}) / [(1-P(i^{\text{th}} \text{ case}))]\} = \beta_{0i} + \beta_G X_i,$$

where  $X_i$  represents the corresponding risk genotypes under an additive, dominant, or recessive model.  $P(i^{\text{th}} \text{ case})$  is the probability of being the observed case in the case and pseudo-controls set in the  $i^{\text{th}}$  trio. The association between SNPs and OFCs was estimated with odds ratios as  $OR(OFCs) = \exp(\beta_G)$  with 95 percent confidence intervals (CIs) calculated from estimated standard errors of  $\beta_G$ .

The effects of gene-environment (GxE) interactions were examined using the conditional logistic regression model assuming an additive model of inheritance by the following model:

$$\ln \{P(i^{\text{th}} \text{ case}) / [(1-P(i^{\text{th}} \text{ case}))]\} = \beta_{0i} + \beta_G X_i + \beta_E E_i + \beta_{EG} (X_i E_i)$$

where  $E_i$  is environmental factor (exposed or unexposed).  $\beta_{EG}$  represents the coefficient of GxE interaction. Both the gene and GxE interaction terms included in conditional logistic regression model provided an estimate of odds ratio (OR) under the additive model. The estimated OR of being a case with one copy of the risk allele in the unexposed mother was given by  $OR(OFCs|G \text{ no } E) = \exp(\beta_G)$  and among the exposed mothers was  $OR(OFCs|G \text{ and } E) = \exp(\beta_G + \beta_{EG})$  for the odds ratio of being a case with one copy of the risk allele in the presence of maternal exposure. A one-degree of freedom ( $df$ ) likelihood ratio test (LRT) was performed for studying the effect of GxE interaction alone (30) and 2  $df$  LRT examined the inherited effect of genotype while accounting for effects of GxE interaction within the same model (31).

## 5.4 Results

For Asians, 892 CL/P and 910 CP trios, and for Europeans, 665 CL/P and 644 CP/L trios were analyzed (Table 5.2). European mothers had a higher rate of PCMV use than Asians (Tables 5.3 and 5.4) (CL/P: 54.0% vs 14.7%; CP/L: 54.5% vs 16.5). European trios with CL/P and CP/L had high missing rates of ETS (31.4 and 31.7%, respectively). Therefore, GxE interaction with ETS was analyzed only in Asian trios.

### *Test of association considering genotypic effect alone in Asians and Europeans*

In Asian trios, SLC30A8 (rs924388) was associated with CL/P under the dominant model (OR: 1.42, 95% CIs: 1.16-1.74) (Table 5.5). The estimated OR of having a CP/L child carrying the minor allele at rs2158254 in HNF1B gene under the recessive model in Asians was 0.53 (95%CI: 0.37-0.75).

In Europeans, ADRB3 (rs7812866) and TNF- $\alpha$  (rs28470596) were both associated with CL/P and CP/L (Table 5.6). Moreover, both rs2237991 and rs2074315 in ABCC8 showed significant association with CL/P under the dominant model (OR: 0.64, 95%CI: 0.52-0.79; OR: 0.64, 95%CI: 0.52-0.79; respectively). The estimated odds ratios for Europeans for CP/L carrying the minor allele at rs17373877 in ADIPOQ and rs6607292 in HNF1B were 0.55 (95%CI: 0.39-0.78; additive model) and 0.50 (95%CI: 0.35-0.73; recessive model), respectively. There were no significant associations between genes related to GDM and risk of CL/P and CP/L in analyses that combined Asians and Europeans.

*Tests of association jointly considering genotypic effects and GxE interactions in Asians and Europeans: periconceptional maternal multivitamin (PCMV) as exposure.*

ABCC8 (rs4148622) and CDKAL1 (rs12201217) showed evidence of GxPCMV interaction in the 1 *df* LRT in European CL/P and CP/L, respectively (ABCC8: OR (CL/P | G no PCMV): 0.58, 95% CI: 0.42-0.81 and OR (CL/P | G and PCMV): 1.26, 95% CI: 1.01-1.58; CDKAL1: OR (CP/L | G no PCMV): 2.10, 95% CI: 1.45-3.03 and OR (CP/L | G and PCMV): 1.30, 95% CI: 1.04-1.61). After considering GxE interaction effects in the 2 *df* LRT, genotypic effects for FTO (rs836994) in Asian CL/P, ADIPOQ (rs7645316) in European CP/L, and TNF- $\alpha$  (rs28470596) in European CL/P and CP/L became significant (OR (CL/P | G and PCMV): 1.23, 95% CI: 0.88-1.70; (OR (CP/L | G and PCMV): 0.71, 95% CI: 0.57-0.89; OR (CL/P | G and PCMV): 0.49, 95% CI: 0.29-0.82; OR (CP/L | G and PCMV): 0.28, 95% CI: 0.15-0.52; respectively). There was no gene that showed evidence of GxPCMV in Asian CP trios (Table 5.7).

*Test of association jointly considering genotypic effect and GxE interaction in Asians and Europeans: periconceptional maternal smoking (smoke) as exposure.*

CDKN2A/2B (rs1063192) and LEP (rs12538332) showed evidence of gene-environment interactions with maternal smoking in the 1 *df* LRT in Asian CL/P and European CL/P, respectively (CDKN2A/2B: OR (CL/P | G no smoking): 1.16, 95% CI: 0.98-1.37 and OR (CL/P | G and smoking): 0.15, 95% CI: 0.03-0.68; LEP: OR (CL/P | G no smoking): 0.83, 95% CI: 0.68-1.02 and OR (CL/P | G and smoking): 1.52, 95% CI: 1.11-2.13). After considering GxE interaction effects in the 2 *df* LRT, genotypic effects for ABCC8 in European CL/P (rs2237991) and CP/L (rs4148617), and TNF- $\alpha$

(rs28470596) in European CL/P and CP/L became significant (OR (CL/P | G and Smoke): 0.56, 95%CI: 0.40-0.79, OR (CP/L | G and Smoke): 0.52, 95%CI: 0.38-0.73, OR (CL/P | G and smoke): 0.25, 95%CI: 0.12-0.52, and OR (CP/L | G and smoke): 0.29, 95%CI: 0.15-0.60, respectively). There was no gene that showed evidence of GxPCMV in Asian CP/L trios (Table 5.8).

*Test of association jointly considering genotypic effect and GxE interaction in Asians: periconceptional maternal environmental tobacco smoke (ETS) as exposure.*

Four SNPs in FTO (rs3751812, rs8050136, rs9930333, and rs9941349) had protective minor allele associations with CL/P, but only in mothers not exposed to ETS (OR range: 0.59-0.60; Table 5.9). Similar protective associations were found for CP/L for FTO (rs3751812 and rs8050136). Increased risk for CL/P was found for ETS exposed only for five SNPs of HHEX (OR range 1.45-6.00) and found these were also associated with CP/L (OR range 8.50-16.0) though the 95% CI, while not including 1.0, were large. Three SNPs of PPARG were associated with increased risk of CL/P among ETS-exposed mothers (OR range 1.25-1.45).

## 5.5 Discussion

This study found several novel associations between GDM-related genes and OFCs and interaction with maternal environmental factors previously associated with OFCs. The study reported that HNF1B was associated with CP/L in both Asians and Europeans. SLC30A8 had an association with CL/P in Asian trios only. In Europeans, ADRB3 and TNF- $\alpha$  had associations with both CL/P and CP/L. Moreover, ABCC8 and ADIPOQ were associated with CL/P and CP/L, respectively, in Europeans. Five genes

were associated with OFCs when interaction with PCMV was considered -- FTO in Asians and ABCC8, ADIPOQ, CDKAL1, and TNF- $\alpha$  in Europeans. Interactions between genotype and maternal smoking were found for CDKN2A/2B in Asian CL/P, and in Europeans, ABCC8, LEP, and TNF- $\alpha$  were associated with CL/P. In Asian mothers exposed to environmental smoking, FTO and HHEX were associated with an increased risk of CL/P and CP/L, and PPARG was associated with an increased risk of CL/P.

Linkage and association studies have reported over two dozen genes significantly associated with OFCs, but these differ across populations (32). While these studies have revealed the novel genes, it has been difficult to identify the etiologic mechanisms. A family-based study by Meeks et al.(33) focused on genes associated with one-carbon metabolism (OCM), and found that FUT6 (fucosyltransferase 6) and TCN2 (transcobalamin 2) had an association with OFCs in Asian population.

GxE interaction in OFCs have been conducted by many studies but not to date have focused on GDM-related genes. A case-parent trio study by Beaty et al (34) reported genes associated with cleft palate only (CPO) when maternal environmental exposures were considered including BAALC (brain and acute leukemia gene cytoplasmic) with maternal multivitamin use, ZNF236 (Zinc Finger Protein 236) and TBK1(tank-binding kinase 1) with maternal smoking, and MLLT3 (brain and acute leukemia gene cytoplasmic) and SMC2 (structural maintenance of chromosomes 2) with maternal alcohol exposure. Another study by Beaty et al. (25) reported GRID2 (glutamate Ionotropic Receptor Delta Type Subunit 2) and ELAVL2 (ELAV like RNA binding protein 2) had an association with CL/P when interaction with maternal smoking was



considered. A family-based study focusing on a Chinese population by Wu et al (35) showed interactions between IRF6 and both maternal multivitamin use and environmental tobacco smoke in CL/P risk. Another study by Wu et al (36) reported that SLC2A9 (solute carrier family 2 member 9) and WDR1 (WD repeat domain 1) gene increased risk of CP/L if mothers were exposed to environmental tobacco smoke. A family-based study by Wang et al (37) reported a novel gene (FGFR2: fibroblast growth factor receptor 2) was associated with CL/P when maternal multivitamin use and smoking were considered. A study by Meeks (33) found that six genes related to one-carbon metabolism had an association with OFCs if mothers used PCMV (DHFR (dihydrofolate reductase), MMAA (methylmalonic aciduria (cobalamin deficiency) cblA type), MTR (5-methyltetrahydrofolate-homocysteine methyltransferase), and TCN2 in European populations and CBS (cystathionine-beta-synthase) and MTHFD2L (methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2-like) gene in Asian populations).

The association between maternal environmental exposures and risk of orofacial is inconsistent. Maternal use of multivitamin supplements in early pregnancy has been linked to decreased risk of OFCs; in a meta-analysis on overall 25% reduction in birth prevalence of OFCs with multivitamin use was reported (38). However, later data from the National Birth Defects Prevention Study (NBDPS) found no association between maternal use of supplement containing folic acid and risk of CL/P and CPO (39). Maternal smoking has been reported to increase the risk of both CL/P and cleft palate. An international population-based study, including Norway and the United States, reported

that mothers with active smoking increased risk of all types of isolated clefts (OR: 1.27, 95% confidence interval (95% CI): 1.11-1.46 for all OFCs; OR: 1.28, 95% CI: 1.09-1.51 for CL/P, OR: 1.25, 95% CI: 1.01-1.55 for CPO) (40). A study from Brazil by Leite et al. (41), however, presented no association between maternal smoking during the first trimester of gestation and OFCs. In addition, the effect of environmental tobacco smoke from passive exposure on the risk of OFCs still appears to be inconsistent. Case-control studies in China reported that passive smoke exposure of mothers increased the risk of OFCs (42, 43). However, a cohort study in the United States reported that environmental tobacco smoke exposure was not associated with CL/P and CPO (44). Inconsistent association between ETS and risk of OFCs might result from GxE interaction, which requires further study. The statistically significant gene-environment interactions may or may not correspond to biological interaction. Therefore, biological interaction requires further study to confirm the mechanisms involved.

This study found that the presence of the minor allele at rs924388 in SLC30A80 increased risk of CL/P in Asians by 42% under the dominant model. SLC30A8, a member of zinc transporter (ZNT) family, encodes the zinc transporter ZnT8. ZnT proteins transport zinc out of cells when zinc is excess, and sequester cytoplasmic zinc into cell when zinc is replete. Zinc facilitates the formation of dense core granules for insulin crystallization in pancreatic  $\beta$ -cell and has a positive influence on insulin gene transcription (45). Reduced zinc concentration in the secretory granules leads to increased proinsulin to insulin ratio in blood circulation and decreased glucose-induced insulin secretion (45). A cross-sectional study in the Chinese Han population found a strong

interaction between SLC30A8 (rs13266634) variant and plasma zinc concentrations in association with type 2 diabetes (46). Moreover, a cross sectional meta-analysis study also reported the interaction effect between SLC30A8 (rs11558471) variant and total zinc intake on fasting plasma glucose concentration (47). The association between plasma zinc concentration and risk of non-syndromic OFCs was reported in a Philippine population with poor zinc status, but the association was not found in Utah, U.S. population with adequate zinc status (48, 49). However, due to limited data in this study, the interaction between SLC30A8 and plasma zinc levels in association with OFCs cannot be determined. The mechanism explaining the association between SLC30A8 variant and risk of OFCs requires further studies in zinc-poor and zinc-replete populations.

In European trios, the presence of the minor allele of ADRB3 at rs7812866 increased the risk of CL/P and CP/L (44% and 53%, respectively) under the dominant model. ADRB3 is a member of beta-adrenergic receptor family, which regulates energy balance through lipolysis in adipocytes, free fatty acid mobilization from adipose cells and thermogenesis in skeletal muscle (50, 51). The mutation of ADRB3 is associated with decreased resting metabolic rate, obesity, obesity-related diseases (diabetes and hypertension), calorogenic dysfunction, early onset of diabetes mellitus, and increased body weight with aging (51, 52). Therefore, gene related to obesity (Table 5.11) might be associated with CP/L. This hypothesis is supported by a study by Kutbi et al reporting the association between maternal obesity and risk of CP/L and suggesting that obesity has a specific effect on palate formation but not lip formation (11). However, the association between obese genes and risk of CP/L requires further study to confirm the hypothesis.

While ADRB3 has been strongly linked to obesity and diabetes, the mechanisms explaining the association between ADRB3 gene and risk of OFCs require further study.

HNF1B (rs2158254) had an association with CP/L in both European and Asian trios. HNF1B (HNF1 homeobox B), a member of the homeodomain-containing superfamily of transcription factors, is expressed in liver, pancreas, bile ducts, thymus, genital tract, lung, and gut (53). Functions of HNF1B include epithelial differentiation during human organogenesis (54), renal tubulogenesis regulation (55), hepatic insulin sensitivity control (56), and pancreatic endocrine cell generation (57). Moreover, HNF1B gene is also associated with pancreatic  $\beta$  cell dysfunction and insulin resistance (56). Polymorphisms in HNF1B have been strongly associated with diabetes, but the mechanisms explaining the association between HNF1B and risk of OFCs require further study.

ADIPOQ (rs17373877) was associated CP/L in Europeans, and ADIPOQ (rs7645316) was associated with CP in European trios when GxE interaction (PCMV) was considered. ADIPOQ has an influence on adiponectin concentration, which is involved in increased glucose uptake via glucose transporter-4, and increased fatty acid uptake and oxidation (58). Yamauchi reported that adiponectin stimulated phosphorylation of acetyl coenzyme A carboxylase, glucose uptake, lactate production, and fatty acid oxidation through activated 5-prime-AMP-activated protein kinase (59). While ADIPOQ has been strongly linked to obesity and diabetes, the mechanism of ADIPOQ or adiponectin level and risk of OFCs requires further study.

TNF- $\alpha$  (rs28470596) was associated with both CL/P and CP/L in European trios in the gTDT and in the 2 *df* that controlling for gene-environment interactions with

PCMV and smoking. Rs28470596 is located approximately 85 kb upstream of TNF- $\alpha$ , a region with several genes related to immune function in MICB, MHC (major histocompatibility complex) Class I Polypeptide-Related Sequence B, which is expressed in monocytes and normal tissues (60). Polymorphisms of MICB cause autoreactive T-cell stimulation, which relates to relevant differences in immune response against infections, autoimmune diseases, and tumor transformation (61). TNF- $\alpha$  encodes a cell signaling protein produced at inflammatory sites. TNF- $\alpha$  interferes with insulin signaling in adipose, muscle, and liver cells. TNF- $\alpha$  inhibit glucose-induced insulin secretion (62). A cell study by Tsiotra et al. (63) found that TNF- $\alpha$  suppressed both basal and glucose-induced insulin secretion and proinsulin mRNA transcription. TNF- $\alpha$  reduces GLUT4 mRNA levels in adipocytes and myocytes and inhibits insulin-stimulated glucose transport, which induces insulin resistance (64). It is uncertain whether polymorphisms in the TNF- $\alpha$  region are related to the regulation of TNF- $\alpha$  or to effects on other genes in the MHC regions. For example, a study by Rahimov et al of the IRF6-OFC association (65) found that rs642961 (10 kb downstream of IRF6, an IRF6 enhancer), disrupted the binding site of transcription factor AP-2 $\alpha$ , which appeared to be the causal variant associated with cleft lip. The mechanism of rs28470596 on TNF- $\alpha$  and OFCs requires further study

ABCC8 (rs2237991 and rs2074315) showed significant association with CL/P in the dominant model in Europeans. When GxE interactions were considered, ABCC8 was associated with CL/P (rs4148622 for PCMV and rs2237991 for maternal smoking).

ABCC8 influences the K-ATP channel function, which causes increased insulin secretion

by pancreatic  $\beta$ -cells. Elbein et al. reported ABCC8 polymorphism that decreased pancreatic  $\beta$ -cell compensation leading to reduce insulin sensitivity (66).

Interactions with ETS reveal that FTO (rs9930333, rs9941349, rs3751812 and rs8050136), HHEX (rs7078243, rs2497351, rs12784232, rs11187173 and rs1418388) and PPARG (rs7618046, rs3856806 and rs12629751) were associated with CL/P and CP in Asians. FTO shares sequence with iron- and 2-oxoglutarate-dependent oxygenases, and FTO mRNA levels found in the hypothalamus are regulated by feeding and fasting (67). A mouse study by Gao et al (68) found that mice with FTO mutations had postnatal growth retardation (lower body weight, shorter body length, and lower bone mineral density) and decreased insulin-like growth factor 1 (IGF-1) levels. A case-control study in Romanian by Duicu et al found that rs9939609 in FTO was associated with adiponectin and leptin levels. HHEX encodes a transcription factor related to Wnt signaling for cell growth and development. A mouse study found that HHEX knockout mice had impaired forebrain, cardiovascular, thyroid, and liver development (69, 70). PPARG is associated with insulin action, adipocyte differentiation, lipid storage, and fat-specific gene expression (71). PPARG (72) also activates glucose transporter 2 and glucokinase in liver and pancreatic  $\beta$ -cells, which improves glucose homeostasis.

LEP was associated with European CL/P when interaction with maternal smoking was considered. LEP encodes leptin hormone which regulates body weight through leptin receptors. Leptin is involved in food intake inhibition, energy expenditure regulatory, energy and glucose homeostasis, bone formation, immune and inflammatory response, angiogenesis, hematopoiesis, and wound healing (73). Elevated leptin level during

pregnancy result from the production in placenta rather than adipose tissue. Maternal leptin increases the mobilization of maternal fat stores, and regulates placental growth, angiogenesis, and nutrient transfer. (74). A vitro study by Araujo et al (75) reported that increased leptin levels in GDM mothers inhibit placental folic acid transport. The previous chapter (chapter 4) analyzing the association between biomarkers and risk of OFCs reported that mothers having CL/P, CPO, and CP/L offspring had higher leptin levels than control mothers. Many studies have found that leptin concentrations were lower in smokers than in nonsmokers (76-80). The study by Nagayasu et al. (80) also presented that nicotine suppressed leptin gene expression. The study by Larsson and Ahren (81) and Donahue et al. (82) reported that leptin levels were not different between smoker and nonsmoker. However, the study Pertkins and Fonte (83) reported that smoking cessation increased leptin concentrations, and suggested that nicotine in tobacco caused leptinemia by inducing corticosteroid release from adrenal glands. The inconsistent associations between smoking and leptin levels may result from the study designs disregarding the different ethnicities, age, health status, and genetic variation (84). Thus there are many possible mechanism where by leptin may influence OFC risk. The interaction effect between leptin and smoking on risk of OFCs and effect of smoking on LEP gene expression require further study to explore.

Major genes having associations with CL/P and CP/L when considering genotypic effect alone are related to beta-cell dysfunction and insulin resistance (Table 5.11). Five of seven genes showing gene-environment interaction with maternal smoking and ETS are related to beta-cell dysfunction (Table 5.12). A study by Beaty et al (20) reported

MAFB was associated with CL/P based on GWAS data, and had a role in palate formation based on expression study in mice. A mouse study by Banerjee et al (85) found that decreased MAFB expression in maternal beta-cell caused GDM. The hypothesis about the association between genes related to beta-cell dysfunction and insulin resistance and OFCs through genetic effects alone and gene-environment interactions requires further study to confirm. Moreover, the mechanism of gene-environment interaction with maternal smoking and ETS in OFCs, which focuses on genes related to beta-cell function, requires further study.

This study is a multi-center, international family-based study. Family-based designs can control confounding bias from the population stratification which is a critical issue in international multi-center designs. For rare diseases, family-based designs provide better statistical power than case-control designs. Moreover, GxE interaction analysis provided additional genes associated when considering GxE interaction (Table 5.13). However, this study cannot identify the etiological mechanism of significant genes on OFC risk. Biological interaction studies are needed to identify the mechanisms of interaction effects between genes and maternal exposures on lip and palate development.

This study reported the association between novel genes related to GDM and risk of CL/P and CP/L in term of genetic effects or GxE interactions with PCMV, maternal smoking, and ETS. Strong association between GDM and risk of OFCs has been reported in previous studies. Therefore, this study supports the hypothesis that genes related to GDM are associated with OFCs through genotypic effects alone and gene-environment interactions with PCMV, maternal smoking, and ETS. The study does not point to a



single major GDM gene associated with OFCs, but supports the hypothesis that GDM may be causally related to OFCs via multiple GDM susceptibility genes and interactions with environmental factors. Further studies are needed to understand the mechanism through which genes related to diabetes and obesity influence the risk of OFCs.

Table 5.1 Summary of genes associated with gestational diabetes (GDM), diabetes mellitus, and obesity selected for analysis.

Gene ID	Gene name	Mechanism <sup>1</sup>					SNP <sup>2</sup>	References		
		BC	IR	IN	OB	AT		T2DM <sup>3</sup>	GDM <sup>4</sup>	Obesity
ABCC8 (11p15.1)	ATP-binding cassette, sub-family C (CFTR/MRP), member 8	X					rs4148643	(86)	(86)	-
							rs1799854	(86-88)	(86)	-
							rs1799859	(86, 87)	-	-
ADIPOQ (3q27)	Adiponectin, C1Q and collagen domain containing		X	X		X	rs266729	-	(89, 90)	(91)
							rs822396	-	-	(92)
							rs1063537	(93, 94)	-	-
							rs1501299	(95, 96)	-	(91, 92)
							rs2241766	(95, 96)	(97-99)	-
							rs2241767	-	-	(92)
							rs12637534	(100)	-	-
							rs16861194	(93, 94)	-	-
							rs16861209	(100)	-	-
							rs17366568	(100)	-	-
ADRB3 (8p12)	Adrenoceptor beta 3				X		rs4994	(95, 102)	(103)	(104, 105)
							rs72655364	(106)	-	-
							rs72655365	(106)	-	-
CDKAL1 (6p22.3)	CDK5 regulatory subunit associated protein 1-like 1	X					rs2206734	(107)	-	-
							rs2237892	(108)	-	-
							rs4712524	(109)	-	-
							rs7754840	(110-115)	(116, 117)	-
							rs7756992	(110-114)	(116, 118)	-
					rs9295475	(109)	-	-		

Table 5.1 Summary of genes associated with gestational diabetes (GDM), diabetes mellitus, and obesity selected for analysis (Cont.).

Gene ID	Gene name	Mechanism <sup>1</sup>					SNP <sup>2</sup>	References		
		BC	IR	IN	OB	AT		T2DM <sup>3</sup>	GDM <sup>4</sup>	Obesity
CDKAL1 (Cont.)						rs9460546	(109)	-	-	
						rs10946398	(119-122)	-	-	
CDKN2A/2B (9p21)	Cyclin-dependent kinase inhibitor 2A/B	X				rs564398	(120-122)	-	-	
						rs1412829	(123)	-	-	
						rs2383208	-	(124)	-	
						rs10811661	(113-115, 120-122, 125-128)	(116)	-	
FTO (16q12.2)	Fat mass and obesity associated				X	rs965670	-	-	(129)	
						rs1121980	-	-	(129)	
						rs1421085	(130)	-	(130, 131)	
						rs3751812	(132)	-	-	
						rs6499640	(132)	-	-	
						rs6602024	-	-	(129)	
						rs7193144	-	-	(129)	
						rs7907949	-	-	(129)	
						rs8050136	(121, 122, 133)	-	(129)	
						rs965670	-	-	(129)	
						rs9926289	-	-	(129)	
						rs9930506	-	-	(129)	
						rs9939609	(134, 135)	(136)	(129, 130, 137-139)	
rs17817449	(133)	-	-							

Table 5.1 Summary of genes associated with gestational diabetes (GDM), diabetes mellitus, and obesity selected for analysis (Cont.).

Gene ID	Gene name	Mechanism <sup>1</sup>					SNP <sup>2</sup>	References		
		BC	IR	IN	OB	AT		T2DM <sup>3</sup>	GDM <sup>4</sup>	Obesity
GCK (7p15.3- p15.1)	Glucokinase	X					rs1799884	-	(140-142)	-
							rs2244164	(143)	-	-
							rs2268573	(143)	-	-
							rs2284779	(144)	-	-
							rs12534623	(143)	-	-
							rs4604517	(145)		
HHEX (10q23.33)	Haematopoietically expressed homeobox	X					rs7923837	(110, 113, 115, 119, 122, 128, 146)	(116)	-
							rs5015480	(113, 119, 121)	(116)	-
							rs1111875	(110, 113- 115, 119, 122, 127, 128, 146)	(116)	-
HNF1A (12q24.2)	HNF1 homeobox A	X					rs1169288	(147, 148)	(140)	-
							rs2701175	(149)	-	-
							rs7305618	(150)	-	-
							rs7957197	(151)	-	-
							rs21573907	(150)	-	-
HNF1B (17q12)	Hepatocyte nuclear factor 1-beta	X	X				rs10962	(144)	-	-
							rs2688	(88)	-	-
							rs1008284	(143)	-	-

Table 5.1 Summary of genes associated with gestational diabetes (GDM), diabetes mellitus, and obesity selected for analysis (Cont.)

Gene ID	Gene name	Mechanism <sup>1</sup>					SNP <sup>2</sup>	References		
		BC	IR	IN	OB	AT		T2DM <sup>3</sup>	GDM <sup>4</sup>	Obesity
HNF1B (Cont.)							rs1016991	(88)	-	-
							rs2285741	(144)	-	-
							rs3110641	(144)	-	-
							rs4430796	(151)	(152)	-
							rs6422978	(144)	-	-
							rs11263755	(144)	-	-
							rs12450628	(143)	-	-
IGF2BP2 (3q27.2)	Insulin-like growth factor 2mRNA binding protein 2	X					rs4376068	(109)	-	-
							rs4402960	(108, 110, 113-115, 128, 153)	(124, 154)	-
							rs6769511	(109)	-	-
							rs7651090	(125)	-	-
IRS1 (2q36)	Insulin receptor substrate- 1		X				rs1801278	(155, 156)	(157-160)	(161)
							rs2943641	(162)	-	-
KCNJ11 (11p15.1)	Potassium channel, inwardly rectifying subfamily J, member 11	X					rs5215	(119, 122)	-	-
							rs5219	(115, 125, 128, 153)	(163)	-
KCNQ1 (11p15.5- p15.4)	Potassium channel, voltage gated KQT-like subfamily Q, member 1	X					rs2074196	(164)	(165)	-
							rs2237892	(150, 164)	(165-167)	-
							rs2237895	-	(166, 168)	-

Table 5.1 Summary of genes associated with gestational diabetes (GDM), diabetes mellitus, and obesity selected for analysis (Cont.)

Gene ID	Gene name	Mechanism <sup>1</sup>					SNP <sup>2</sup>	References		
		BC	IR	IN	OB	AT		T2DM <sup>3</sup>	GDM <sup>4</sup>	Obesity
KCNQ1 (Cont.)							rs2237896	-	(168)	-
							rs231362	(151)		
LEP (7q31.3)	Leptin	X			X		rs2167270	-	(90)	(169, 170)
							rs6966536	-	-	(171)
							rs7799039	-	(172)	-
							rs10954173	(173)	-	-
							rs10954174	-	-	(171)
							rs11761556	(173)	-	-
MTNR1B (11q21-q22)	Melatonin receptor 1B	X					rs1387153	-	(157, 174)	-
							rs10830962	-	(117)	-
							rs10830963	(175-177)	(157, 174, 178)	-
PPARG (3p25)	Peroxisome proliferator- activated receptor gamma		X				rs1801282	(115, 121, 122, 125, 153, 179)	(180)	(181)
							rs3856806	-	(180)	-
SLC30A8 (8q24.11)	Solute carrier family 30 (zinc transporter), member 8	X					rs3802177	(110, 119, 123, 133)	-	-
							rs11558471	(119)	-	-
							rs13266634	(110, 115, 119, 121, 123, 125, 133, 146, 153)	(116)	-

Table 5.1 Summary of genes associated with gestational diabetes (GDM), diabetes mellitus, and obesity selected for analysis (Cont.)

Gene ID	Gene name	Mechanism <sup>1</sup>					SNP <sup>2</sup>	References		
		BC	IR	IN	OB	AT		T2DM <sup>3</sup>	GDM <sup>4</sup>	Obesity
TCF7L2 (10q25.3)	Transcription factor 7-like 2	X					rs4506565	-	(136)	-
							rs6585205	(119)	-	-
							rs7901695	(108)	(182)	-
							rs7903146	(113, 115, 119, 121-123, 126, 146, 153, 183, 184)	(116, 118, 182, 185)	-
							rs10885409	(127)	-	-
							rs114748339	(186)	-	-
							rs12255372	-	(187)	-
TNF- $\alpha$ (6p21.3)	Tumor necrosis factor alpha		X	X			rs361525	(188)	-	-
							rs1800610	(149)	-	-
							rs1800629	(189, 190)	(191, 192)	(193, 194)

<sup>1</sup>Mechanism related to diabetes and metabolic syndrome, BC: beta-cell dysfunction; IR: insulin resistance; IN: inflammation markers (cytokines and adipokines); OB: Obesity; AT: Atherosclerotic processes

<sup>2</sup>SNP, single-nucleotide polymorphism

<sup>3</sup>T2DM, type 2 diabetes mellitus

<sup>4</sup>GDM, gestational diabetes mellitus

Table 5.2 Number of complete trios by recruitment site; GENEVA Orofacial Cleft Consortium

Recruitment Site	Asian Trios		European	
	CL/P	CP/L	CL/P	CP/L
Denmark	-	-	21 (3.2%)	20 (3.1%)
Norway	3 (0.3%)	3 (0.3%)	275 (41.4%)	270 (41.9%)
Iowa, US	-	-	44 (6.6%)	52 (8.1%)
Maryland, US	1 (0.1%)	2 (0.2%)	83 (12.5%)	88 (13.7%)
Pittsburgh, Pennsylvania, US	-	-	87 (13.1%)	73 (11.3%)
Utah, US	-	1 (0.1%)	152 (22.9%)	135 (21.0%)
Philippines	94 (10.5%)	94 (10.3%)	-	-
Singapore	56 (6.3%)	84 (9.2%)	3 (0.4%)	6 (0.9%)
Taiwan	218 (24.4%)	250 (27.5%)	-	-
Weifang, People Republic of China	183 (20.5%)	159 (17.5%)	-	-
Wuhan, People Republic of China	176 (19.7%)	178 (19.6%)	-	-
Chengdu, People Republic of China	106 (11.9%)	101 (11.1%)	-	-
Korea	55 (6.2%)	38 (4.2%)	-	-
<b>Total</b>	<b>892</b>	<b>910</b>	<b>665</b>	<b>644</b>

CL/P: cleft lip with or without cleft palate; CP/L: cleft palate with or without cleft lip



Table 5.3: Environmental maternal exposures in Asian trios by site.

Environmental risk factor exposure by Site	Cleft lip with or without cleft palate (N=892)				Cleft palate with or without cleft lip (N=910)			
	Smoking	PCMV	Drinking	ETS	Smoking	PCMV	Drinking	ETS
Norway								
No	3 (100%)	1 (33.3%)	2 (66.7%)	2 (66.7%)	3 (100%)	1 (33.3%)	2 (66.7%)	2 (66.7%)
Yes	-	1 (33.3%)	1 (33.3%)	1 (33.3%)	-	1 (33.3%)	1 (33.3%)	1 (33.3%)
Missing	-	1 (33.3%)	-	-	-	1 (33.3%)	-	-
Maryland, US								
No	1 (100%)	-	1 (100%)	-	2 (100%)	-	2 (100%)	1 (50.0%)
Yes	-	1 (100%)	-	-	-	2 (100%)	-	-
Missing	-	-	-	1 (100%)	-	-	-	1 (50.0%)
Utah, US								
No	-	-	-	-	1 (100%)	-	1 (100%)	1 (100%)
Yes	-	-	-	-	-	1 (100%)	-	-
Missing	-	-	-	-	-	-	-	-
Philippines								
No	90 (95.7%)	65 (69.1%)	89 (94.7%)	-	90 (95.7%)	65 (69.1%)	89 (94.7%)	-
Yes	4 (4.3%)	29 (30.9%)	5 (5.3%)	-	4 (4.3%)	29 (30.9%)	5 (5.3%)	-
Missing	-	-	-	94 (100%)	-	-	-	94 (100%)
Singapore								
No	51 (91.1%)	29 (51.8%)	54 (96.4%)	42 (75.0%)	78 (92.9%)	44 (52.4%)	78 (92.9%)	67 (79.8%)
Yes	5 (8.9%)	27 (48.2%)	2 (3.6%)	14 (25.0%)	6 (7.1%)	40 (47.6%)	6 (7.1%)	17 (20.2%)
Missing	-	-	-	-	-	-	-	-

Table 5.3: Environmental maternal exposures in Asian trios by site (Cont.).

Environmental risk factor exposure by Site	Cleft lip with or without cleft palate (N=892)				Cleft palate with or without cleft lip (N=910)			
	Smoking	PCMV	Drinking	ETS	Smoking	PCMV	Drinking	ETS
Taiwan								
No	203(93.1%)	173(79.4%)	210(96.3%)	103(47.2%)	233(93.2%)	199(79.6%)	239(95.6%)	120(48.0%)
Yes	15 (6.9%)	44 (20.2%)	8 (3.7%)	113(51.8%)	17 (6.8%)	51 (20.4%)	11 (4.4%)	128(51.2%)
Missing	-	1 (0.5%)	-	2 (0.9%)	-	-	-	2 (0.8%)
Weifang, PRC								
No	182(99.5%)	162(88.5%)	182(99.5%)	116(63.4%)	158(99.4%)	143(89.9%)	157(98.7%)	99 (62.3%)
Yes	1 (0.5%)	11 (6.0%)	-	65 (35.5%)	1 (0.6%)	7 (4.4%)	2 (1.3%)	59 (37.1%)
Missing	-	10 (5.5%)	1 (0.5%)	2 (1.1%)	-	9 (5.7%)	-	1 (0.6%)
Wuhan, PRC								
No	176 (100%)	37 (21.0%)	173(98.3%)	171(97.2%)	178(100%)	38 (21.3%)	175(98.3%)	172(96.6%)
Yes	-	14 (8.0%)	-	3 (1.7%)	-	16 (9.0%)	-	2 (1.1%)
Missing	-	125(71.0%)	3 (1.7%)	2 (1.1%)	-	124(69.7%)	3 (1.7%)	4 (2.2%)
Chengdu, PRC								
No	105(99.1%)	101(95.3%)	103(97.2%)	12 (11.3%)	100(99.0%)	97 (96.0%)	98 (97.0%)	14 (13.9%)
Yes	1 (0.9%)	2 (1.9%)	3 (2.8%)	92 (86.8%)	1 (1.0%)	2 (2.0%)	3 (3.0%)	86 (85.1%)
Missing	-	3 (2.8%)	-	2 (1.1%)	-	2 (2.0%)	-	1 (1.0%)
Korea								
No	55 (100%)	53 (96.4%)	48 (87.3%)	37 (67.3%)	38 (100%)	37 (97.4%)	33 (86.8%)	27 (71.1%)
Yes	-	2 (3.6%)	-	12 (21.8%)	-	1 (2.6%)	-	7 (18.4%)
Missing	-	-	7 (12.7%)	6 (10.9%)	-	-	5 (13.2%)	4 (10.5%)

Smoking: mother smokes in the periconceptional period (3 months prior through 3<sup>rd</sup> month of pregnancy)

PCMV: mother takes multivitamins or prenatal vitamins in the periconceptional period (3 months prior through 3<sup>rd</sup> month of pregnancy)

Drinking: mother has alcohol consumption in the perinatal period (3 months prior through 3<sup>rd</sup> month of pregnancy)

ETS: mother exposed to environmental tobacco smoke during pregnancy or three months before pregnancy

Table 5.4 Environmental maternal exposures in European trios by site.

Site	Cleft lip with or without cleft palate (N=665)				Cleft palate with or without cleft lip (N=644)			
	Smoking	PCMV	Drinking	ETS	Smoking	PCMV	Drinking	ETS
Denmark								
No	14 (66.7%)	9 (42.9%)	15 (71.4%)	-	14 (70%)	8 (40.0%)	14 (70%)	-
Yes	7 (33.3%)	12 (57.1%)	6 (28.6%)	-	6 (30%)	12 (60.0%)	6 (30%)	-
Missing	-	-	-	21 (100%)	-	-	-	20 (100%)
Norway								
No	150(54.5%)	104(37.8%)	130(47.3%)	232(84.4%)	158(58.5%)	108(40.0%)	123(45.6%)	229(84.8%)
Yes	125(45.5%)	87 (31.6%)	145(52.7%)	43 (15.6%)	112(41.5%)	88 (32.6%)	147(54.4%)	41 (15.2%)
Missing	-	84 (30.5%)	-	-	-	74 (27.4%)	-	-
Iowa, US								
No	31 (70.5%)	11 (25.0%)	13 (29.5%)	-	37 (71.2%)	12 (23.1%)	17 (32.7%)	-
Yes	13 (29.5%)	32 (72.7%)	31 (70.5%)	-	15 (28.8%)	39 (75.0%)	35 (67.3%)	-
Missing	-	1 (2.3%)	-	44 (100%)	-	1 (1.9%)	-	52 (100%)
Maryland, US								
No	58 (69.9%)	12 (14.5%)	56 (67.5%)	21 (25.3%)	59 (67.0%)	11 (12.5%)	60 (68.2%)	23 (26.1%)
Yes	23 (27.7%)	66 (79.5%)	25 (30.1%)	6 (7.2%)	27 (30.7%)	73 (83.0%)	25 (28.4%)	7 (8.0%)
Missing	2 (2.4%)	5 (6.0%)	2 (2.4%)	56 (67.5%)	2 (2.3%)	4 (4.5%)	3 (3.4%)	58 (65.9%)
Pittsburgh, Pennsylvania, US								
No	66 (75.9%)	16 (18.4%)	49 (56.3%)	-	53 (72.6%)	14 (19.2%)	41 (56.2%)	-
Yes	21 (24.1%)	70 (80.5%)	37 (42.5%)	-	20 (27.4%)	58 (79.5%)	31 (42.5%)	-
Missing	-	1 (1.1%)	1 (1.1%)	87 (100%)	-	1 (1.4%)	1 (1.4%)	73 (100%)

Table 5.4 Environmental maternal exposures in European trios by site (Cont.)

Site	Cleft lip with or without cleft palate (N=665)				Cleft palate with or without cleft lip (N=644)			
	Smoking	PCMV	Drinking	ETS	Smoking	PCMV	Drinking	ETS
Utah, US								
No	140(92.1%)	63 (41.4%)	132(86.8%)	137(90.1%)	120(88.9%)	57 (42.2%)	119(88.1%)	119(88.1%)
Yes	12 (7.9%)	89 (58.6%)	20 (13.2%)	14 (9.2%)	15 (11.1%)	78 (57.8%)	16 (11.9%)	15 (11.1%)
Missing	-	-	-	1 (0.7%)	-	-	-	1 (0.7%)
Singapore								
No	2 (66.7%)	-	2 (66.7%)	2 (66.7%)	5 (83.3%)	3 (50.0%)	5 (83.3%)	4 (66.7%)
Yes	1 (33.3%)	3 (100%)	1 (33.3%)	1 (33.3%)	1 (16.7%)	3 (50.0%)	1 (16.7%)	2 (33.3%)
Missing	-	-	-	-	-	-	-	-

Smoking: mother smokes in the periconceptional period (3 months prior through 3<sup>rd</sup> month of pregnancy)

PCMV: mother takes multivitamins or prenatal vitamins in the periconceptional period (3 months prior through 3<sup>rd</sup> month of pregnancy)

Drinking: mother has alcohol consumption in the perinatal period (3 months prior through 3<sup>rd</sup> month of pregnancy)

ETS: mother exposed to environmental tobacco smoke during pregnancy or three months before pregnancy

Table 5.5 Conditional logistic regression results from genotypic Transmission Disequilibrium Test (gTDT): risk of orofacial clefts among Asian trios from GENEVA Orofacial Cleft Consortium.

phenotype	Gene ID	Significant SNPs	Allele	MAF (%)	Model fitted	Odds ratios (95% CIs)	Raw p-value <sup>1</sup>	Threshold P-value <sup>2</sup>
CL/P	SLC30A8	rs924388	A/G	17.3	Additive	1.31 [1.10-1.57]	2.7x10 <sup>-3</sup>	7.7x10 <sup>-4</sup>
					Dominant*	1.42 [1.74-1.16]	7.2x10 <sup>-4</sup>	
					Recessive	1.21 [0.77-1.90]	0.41	
CP/L	HNF1B	rs2158254	A/G	27.4	Additive	0.88 [0.76-1.02]	8.9x10 <sup>-2</sup>	8.3x10 <sup>-4</sup>
					Dominant	0.92 [0.77-1.10]	0.38	
					Recessive*	0.53 [0.37-0.75]	4.5x10 <sup>-4</sup>	

CL/P: cleft lip with/without cleft palate; CP/L: cleft palate with or without cleft lip

Allele: minor allele/major allele; MAF: minor allele frequency

Threshold p-value was adjusted for the number of SNPs in each gene as reported in Table 5.10

\* Model achieves significance after Bonferroni correction

<sup>1</sup> p-value significance of the gTDT without multiple testing correction.

<sup>2</sup> p-value significance of the gTDT after gene-level Bonferroni correction, calculated as 0.05 divided by the number of SNPs in each gene.

Table 5.6 Conditional logistic regression results from genotypic Transmission Disequilibrium Test (gTDT): risk of orofacial clefts among European trios from GENEVA Orofacial Cleft Consortium.

phenotype	Gene ID	Significant SNPs	Allele	MAF (%)	Model fitted	Odds ratios (95% CIs)	Raw p-value <sup>1</sup>	Threshold P-value <sup>2</sup>
CL/P	ABCC8	rs2237991	C/T	27.9	Additive*	0.72 [0.60-0.86]	$2.5 \times 10^{-4}$	$3.1 \times 10^{-4}$
					Dominant*	0.64 [0.52-0.79]	$3.6 \times 10^{-5}$	
					Recessive	0.90 [0.62-1.30]	0.58	
		rs2074315	G/T	27.2	Additive	0.73 [0.61-0.87]	$4.1 \times 10^{-4}$	
					Dominant*	0.64 [0.52-0.79]	$4.3 \times 10^{-5}$	
					Recessive	0.93 [0.65-1.35]	0.71	
CL/P	ADRB3	rs7812866	C/A	47.3	Additive	1.07 [0.92-1.24]	0.41	$5.6 \times 10^{-3}$
					Dominant*	1.44 [1.13-1.83]	$3.5 \times 10^{-3}$	
					Recessive	0.81 [0.64-1.03]	$8.4 \times 10^{-2}$	
CL/P	TNF- $\alpha$	rs28470596	T/C	5.0	Additive*	0.36 [0.24-0.55]	$9.6 \times 10^{-7}$	$2.4 \times 10^{-4}$
					Dominant*	0.36 [0.24-0.55]	$1.3 \times 10^{-6}$	
					Recessive	N/A	N/A	
CP/L	ADIPOQ	rs17373877	A/G	6.1	Additive*	0.55 [0.39-0.78]	$6.7 \times 10^{-4}$	$8.5 \times 10^{-4}$
					Dominant	0.56 [0.40-0.80]	$1.4 \times 10^{-3}$	
					Recessive	N/A	N/A	
CP/L	ADRB3	rs7812866	C/A	46.8	Additive	1.11 [0.94-1.30]	0.21	$5.6 \times 10^{-3}$
					Dominant*	1.53 [1.19-1.97]	$9.2 \times 10^{-4}$	
					Recessive	0.83 [0.65-1.06]	0.14	
CP/L	HNF1B	rs6607292	A/G	35.5	Additive	0.82 [0.69-0.98]	$2.6 \times 10^{-2}$	$7.1 \times 10^{-4}$
					Dominant	0.96 [0.76-1.20]	0.71	
					Recessive*	0.50 [0.35-0.73]	$3.6 \times 10^{-4}$	

Table 5.6 Conditional logistic regression results from genotypic Transmission Disequilibrium Test (gTDT): risk of orofacial clefts among European trios from GENEVA Orofacial Cleft Consortium (Cont.).

phenotype	Gene ID	Significant SNPs	Allele	MAF (%)	Model fitted	Odds ratios (95% CIs)	Raw p-value <sup>1</sup>	Threshold P-value <sup>2</sup>
CP/L	TNF- $\alpha$	rs28470596	T/C	4.7	Additive*	0.27 [0.17-0.42]	8.75x10 <sup>-9</sup>	2.4x10 <sup>-4</sup>
					Dominant*	0.27 [0.17-0.42]	1.1x10 <sup>-8</sup>	
					Recessive	N/A	N/A	

CL/P: cleft lip with/without cleft palate; CP/L: cleft palate with or without cleft lip

Allele: minor allele/major allele; MAF: minor allele frequency

Threshold p-value was adjusted for the number of SNPs in each gene as reported in Table 5.10

\* Model achieves significance after Bonferroni correction

<sup>1</sup> p-value significance of the gTDT without multiple testing correction.

<sup>2</sup> p-value significance of the gTDT after gene-level Bonferroni correction, calculated as 0.05 divided by the number of SNPs in each gene.

Table 5.7 Estimated odds ratio (OR) (case|G no E) and OR (case|G and E) from conditional logistic regression using cases and 3 pseudo-controls in both Asian and European trios from GENEVA Orofacial Cleft Consortium for 21 genes related to gestational diabetes mellitus considering G-E interaction between each SNP and mother taking multivitamins or prenatal vitamins in the perinatal period (PCMV)

Ancestry	phenotype	Gene ID	Significant SNPs	Allele	MAF (%)	OR (case G no PCMV)	OR (case G and PCMV)	LRT 1 df P-value	LRT 2 df P-value
Asian	CL/P	FTO**	rs836994	A/G	50.1	1.36 [1.16-1.60]	1.23 [0.88-1.70]	0.58	3.0x10 <sup>-4</sup>
European	CL/P	ABCC8*	rs4148622	T/C	26.8	0.58 [0.42-0.81]	1.26 [1.01-1.58]	1.0x10 <sup>-4</sup>	5.0x10 <sup>-4</sup>
	CL/P	TNF- $\alpha$ **	rs28470596	T/C	5.0	0.22 [0.10-0.50]	0.49 [0.29-0.82]	0.09	3.6x10 <sup>-6</sup>
	CP/L	ADIPOQ**	rs7645316	C/T	33.5	0.70 [0.52-0.93]	0.71 [0.57-0.89]	0.90	6.8x10 <sup>-4</sup>
	CP/L	CDKAL1*	rs12201217	T/C	37.8	2.10 [1.45-3.03]	1.30 [1.04-1.61]	6.5x10 <sup>-5</sup>	3.5x10 <sup>-4</sup>
	CP/L	TNF- $\alpha$ **	rs28470596	T/C	4.7	0.25 [0.12-0.54]	0.28 [0.15-0.52]	0.81	2.53x10 <sup>-8</sup>

CL/P: cleft lip with/without cleft palate; CP/L: cleft palate with or without cleft lip

Allele: minor allele/major allele; MAF: minor allele frequency

<sup>1</sup> Raw p-value for 1 degree of freedom likelihood ratio test examining the exclusive effect of gene-environment interaction.

<sup>2</sup> Raw p-value for 2 degree of freedom likelihood ratio test examining the effect of genotype after considering effect of gene-environment interaction.

\* only 1 degree of freedom likelihood ratio test achieved significance after Bonferroni correction (Table 5.10)

\*\* only 2 degree of freedom likelihood ratio test achieved significance after Bonferroni correction (Table 5.10)



Table 5.8 Estimated odds ratio (OR) (case|G no E) and OR (case|G and E) from conditional logistic regression using cases and 3 pseudo-controls in both Asian and European trios from GENEVA Oralfacial Cleft Consortium for 21 genes related to gestational diabetes mellitus considering G-E interaction between each SNP and maternal smoking in the perinatal period.

Ancestry	phenotype	Gene ID	Significant SNPs	Allele	MAF (%)	OR (case G no smoke)	OR (case G and smoke)	LRT 1 df P-value <sup>1</sup>	LRT 2 df P-value <sup>2</sup>
Asian	CL/P	CDKN2A/2B*	rs1063192	C/T	19.5	1.16 [0.98-1.37]	0.15 [0.03-0.68]	1.2x10 <sup>-3</sup>	2.4x10 <sup>-3</sup>
European	CL/P	ABCC8**	rs2237991	C/T	27.9	0.79 [0.64-0.98]	0.56 [0.40-0.79]	0.09	2.7x10 <sup>-4</sup>
	CL/P	LEP*	rs12538332	C/A	29.7	0.83 [0.68-1.02]	1.52 [1.11-2.13]	1.3x10 <sup>-4</sup>	5.6x10 <sup>-3</sup>
	CL/P	TNF-α**	rs28470596	T/C	5.0	0.45 [0.28-0.74]	0.25 [0.12-0.52]	0.18	7.5x10 <sup>-7</sup>
	CP/L	ABCC8**	rs4148617	T/C	24.5	0.89 [0.71-1.12]	0.52 [0.38-0.73]	9.2x10 <sup>-3</sup>	2.6x10 <sup>-4</sup>
	CP/L	TNF-α**	rs28470596	T/C	4.7	0.25 [0.14-0.46]	0.29 [0.15-0.60]	0.76	2.2x10 <sup>-9</sup>

CL/P: cleft lip with/without cleft palate; CP/L: cleft palate with or without cleft lip

Allele: minor allele/major allele; MAF: minor allele frequency

<sup>1</sup> Raw p-value for 1 degree of freedom likelihood ratio test examining the exclusive effect of gene-environment interaction.

<sup>2</sup> Raw p-value for 2 degree of freedom likelihood ratio test examining the effect of genotype after considering effect of gene-environment interaction.

\* only 1 degree of freedom likelihood ratio test achieved significance after Bonferroni correction (Table 5.10)

\*\* only 2 degree of freedom likelihood ratio test achieved significance after Bonferroni correction (Table 5.10)

Table 5.9 Estimated odds ratio (OR) (case|G no E) and OR (case|G and E) from conditional logistic regression using cases and 3 pseudo-controls in both Asian and European trios from GENEVA Oral Cleft Consortium for 21 genes related to gestational diabetes mellitus considering G-E interaction between each SNP and maternal exposure to environmental tobacco smoke in the perinatal period (ETS).

Ancestry	phenotype	Gene ID	Significant SNPs	Allele	MAF (%)	OR (case G no ETS)	OR (case G and ETS)	LRT 1 df P-value <sup>1</sup>	LRT 2 df P-value <sup>2</sup>
Asian	CL/P	FTO	rs9930333***	G/T	4.4	0.62 [0.49-0.78]	1.17 [0.86-1.59]	1.2x10 <sup>-3</sup>	2.5x10 <sup>-4</sup>
			rs9941349***	T/C	16.9	0.62 [0.49-0.79]	1.16 [0.85-1.59]	1.6x10 <sup>-3</sup>	2.8x10 <sup>-4</sup>
			rs3751812***	T/G	12.4	0.59 [0.45-0.78]	1.35 [0.94-1.93]	3.5x10 <sup>-3</sup>	3.0x10 <sup>-4</sup>
			rs8050136***	A/C	12.4	0.60 [0.45-0.79]	1.37 [0.95-1.96]	3.0x10 <sup>-3</sup>	3.3x10 <sup>-4</sup>
CL/P	HHEX	rs7078243*	A/C	17.5	0.72 [0.56-0.92]	1.45 [1.08-1.94]	3.1x10 <sup>-4</sup>	1.3x10 <sup>-3</sup>	
		rs2497351*	A/G	3.1	0.78 [0.44-1.38]	6.00 [1.77-20.37]	7.1x10 <sup>-4</sup>	1.1x10 <sup>-2</sup>	
		rs12784232*	A/G	2.9	0.81 [0.46-1.43]	6.00 [1.77-20.37]	9.3x10 <sup>-4</sup>	1.3x10 <sup>-2</sup>	
		rs11187173*	A/G	2.9	0.81 [0.46-1.43]	6.00 [1.77-20.37]	9.3x10 <sup>-4</sup>	1.3x10 <sup>-2</sup>	
		rs1418388*	A/G	2.9	0.81 [0.46-1.43]	6.00 [1.77-20.37]	9.3x10 <sup>-4</sup>	1.3x10 <sup>-2</sup>	
		rs7618046*	C/T	45.8	0.87 [0.73-1.04]	1.50 [1.18-1.90]	3.5x10 <sup>-4</sup>	1.2x10 <sup>-3</sup>	
		rs3856806*	T/C	22.6	0.86 [0.69-1.07]	1.60 [1.21-2.12]	5.6x10 <sup>-4</sup>	1.5x10 <sup>-3</sup>	
CL/P	PPARG	rs12629751*	T/C	28.8	0.82 [0.67-1.00]	1.45 [1.12-1.88]	6.0x10 <sup>-4</sup>	2.7x10 <sup>-3</sup>	
		rs3751812***	T/G	12.3	0.58 [0.44-0.76]	1.25 [0.87-1.81]	9.2x10 <sup>-4</sup>	1.9x10 <sup>-4</sup>	
		rs8050136***	A/C	12.3	0.59 [0.45-0.78]	1.27 [0.88-1.84]	9.4x10 <sup>-4</sup>	3.0x10 <sup>-4</sup>	
CP/L	FTO	rs12784232***	A/G	2.5	1.21 [0.66-2.22]	16.0 [2.12-120.65]	1.5x10 <sup>-3</sup>	2.8x10 <sup>-4</sup>	
		rs11187173***	A/G	2.5	1.15 [0.63-2.09]	16.0 [2.12-120.65]	1.1x10 <sup>-3</sup>	3.1x10 <sup>-4</sup>	
		rs1418388***	A/G	2.8	1.15 [0.63-2.09]	16.0 [2.12-120.65]	1.1x10 <sup>-3</sup>	3.1x10 <sup>-4</sup>	
		rs2497351**	A/G	2.7	1.21 [0.66-2.22]	8.50 [1.96-36.79]	4.9x10 <sup>-3</sup>	7.4x10 <sup>-4</sup>	

CL/P: cleft lip with/without cleft palate; CP/L: cleft palate with or without cleft lip

Allele: minor allele/major allele; MAF: minor allele frequency

<sup>1</sup> Raw p-value for 1 degree of freedom likelihood ratio test examining the exclusive effect of gene-environment interaction.

<sup>2</sup> Raw p-value for 2 degree of freedom likelihood ratio test examining the effect of genotype after considering effect of gene-environment interaction.

\* only 1 degree of freedom likelihood ratio test achieved significance after Bonferroni correction (Table 5.10)

\*\* only 2 degree of freedom likelihood ratio test achieved significance after Bonferroni correction (Table 5.10)

\*\*\* both 1 and 2 degree of freedom likelihood ratio test achieved significance after Bonferroni correction (Table 5.10)

Table 5.10 Threshold p-value was adjusted for the number of SNPs in each gene as reported.

Gene	Asian trios		European trios	
	No. SNP	Threshold P-value	No. SNP	Threshold P-value
ABCC8	152	$3.3 \times 10^{-4}$	159	$3.1 \times 10^{-4}$
ADIPOQ	56	$8.9 \times 10^{-4}$	59	$8.5 \times 10^{-4}$
ADRB3	10	$5.0 \times 10^{-3}$	9	$5.6 \times 10^{-3}$
CDKAL1	252	$2.0 \times 10^{-4}$	280	$1.8 \times 10^{-4}$
CDKN2A/2B	43	$1.2 \times 10^{-3}$	44	$1.1 \times 10^{-3}$
GCK	45	$1.1 \times 10^{-3}$	45	$1.1 \times 10^{-3}$
FTO	124	$4.0 \times 10^{-4}$	128	$3.9 \times 10^{-4}$
HHEX	34	$1.5 \times 10^{-3}$	38	$1.3 \times 10^{-3}$
HNF1A	38	$1.3 \times 10^{-3}$	43	$1.2 \times 10^{-3}$
HNF1B	60	$8.3 \times 10^{-4}$	70	$7.1 \times 10^{-4}$
IGF2BP2	43	$1.2 \times 10^{-3}$	47	$1.1 \times 10^{-3}$
IRS1	38	$1.3 \times 10^{-3}$	44	$1.1 \times 10^{-3}$
KCNJ11	105	$4.8 \times 10^{-4}$	108	$4.6 \times 10^{-4}$
KCNQ1	179	$2.8 \times 10^{-4}$	184	$2.7 \times 10^{-4}$
LEP	33	$1.5 \times 10^{-3}$	38	$1.3 \times 10^{-3}$
MTNR1B	44	$1.1 \times 10^{-3}$	47	$1.1 \times 10^{-3}$
PPARG	52	$9.6 \times 10^{-4}$	59	$8.5 \times 10^{-4}$
SLC30A8	65	$7.7 \times 10^{-4}$	66	$7.6 \times 10^{-4}$
TCF7L2	59	$8.5 \times 10^{-4}$	70	$7.1 \times 10^{-4}$
TNF- $\alpha$	188	$2.7 \times 10^{-4}$	210	$2.4 \times 10^{-4}$

Table 5.11 Summary genes showing significant association with orofacial clefts with the genotypic Transmission Disequilibrium (gTDT) test.

Gene	Mechanism <sup>1</sup>					SNP	Asian MAF	European MAF	Asians		Europeans	
	BC	IR	IN	OB	AT				CL/P	CP/L	CL/P	CP/L
ABCC8	X					rs2237991	26.1	27.9			Additive	
						rs2074315	25.3	27.2			Dominant	
ADIPOQ		X	X		X	rs17373877	9.9	6.1				Additive
ADRB3				X		rs7812866	29.1	46.8			Dominant	Dominant
HNF1B	X	X				rs2158254	27.4	45.4		Recessive		
						rs6607292	46.7	35.5				Recessive
SLC30A8	X					rs924388	17.3	9.6	Additive			
TNF- $\alpha$		X	X			rs28470596	12.9	4.7			Additive	Additive
											Dominant	Dominant

CL/P: cleft lip with/without cleft palate; CP/L: cleft palate with or without cleft lip

<sup>1</sup>Mechanism based on gene function; BC: Beta-cells dysfunction; IR: Insulin resistance; IN: Inflammatory markers (cytokines and adipokines); OB: Obesity; AT: Atherosclerotic process

Table 5.12 Summary genes showing significant gene-environment interaction (GxE) at 1df LRT

Gene	Mechanism <sup>1</sup>					Asians						Europeans				
						PCMV		Smoke		ETS		PCMV		Smoke		
	BC	IR	IN	OB	AT	CL/P	CP/L	CL/P	CP/L	CL/P	CP/L	CL/P	CP/L	CL/P	CP/L	
ABCC8*	X												X			
CDKAL1	X													X		
CDKN2A/2B	X							X								
FTO				X		X				X	X					
HHEX	X									X	X					
LEP	X			X											X	
PPARG		X								X						

CL/P: cleft lip with/without cleft palate; CP/L: cleft palate with or without cleft lip

<sup>1</sup>Mechanism related to diabetes and metabolic syndrome, BC: Beta-cells dysfunction; IR: Insulin resistance; IN: Inflammatory markers (cytokines and adipokines); OB: Obesity; AT: Atherosclerotic process

\* significant genetic association (genotypic transmission disequilibrium; gTDT) among European trios

GxE, PCMV: Periconceptional multivitamin, Smoke: maternal smoking, ETS: Environment Tobacco Smoke.

Table 5.13 Summary genes showing significant when considering gene-environment interaction (GxE) at 2 *df* LRT.

Gene	Mechanism <sup>1</sup>					Asians						Europeans				
						PCMV		Smoke		ETS		PCMV		Smoke		
	BC	IR	IN	OB	AT	CL/P	CP/L	CL/P	CP/L	CL/P	CP/L	CL/P	CP/L	CL/P	CP/L	
ABCC8*	X														X	X
ADIPOQ*	X													X		
FTO				X		X				X	X					
HHEX	X									X	X					
PPARG		X								X						
TNF- $\alpha$ *		X	X										X	X	X	X

CL/P: cleft lip with/without cleft palate; CP/L: cleft palate with or without cleft lip

<sup>1</sup>Mechanism related to diabetes and metabolic syndrome, BC: Beta-cells dysfunction; IR: Insulin resistance; IN: Inflammatory markers (cytokines and adipokines); OB: Obesity; AT: Atherosclerotic process

\* significant genetic association (genotypic transmission disequilibrium; gTDT) among European trios  
GxE, PCMV: Periconceptual multivitamin, Smoke: maternal smoking, ETS: Environment Tobacco Smoke.

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## CHAPTER 6

## CONCLUSION

**6.1 Summary**

Epidemiological research on orofacial clefts (OFCs) has determined the etiology of OFCs to be multifactorial including genetic factors, environmental factors, and combination of these factors. Environmental factors including maternal smoking, maternal alcohol consumption, maternal nutritional status, certain medicines and supplement intakes, eating behavior during pregnancy and prior to pregnancy, and maternal medical conditions (diabetes and obesity) have been reported to be associated with the incidence of OFCs (1-5). Insulin resistance and obesity are considered as risk factors of metabolic diseases. Several biomarkers including HDL, triglyceride, liver function tests (ALT: alanine aminotransferase and GGT: gamma-glutamyltransferase) (6-8), cytokine (CRP: C-reactive protein, IL-6: interleukin-6, TNF-  $\alpha$ : tumor necrosis factor alpha, and leptin) (9-12), and adipokines (adiponectin) (10-12) are associated with metabolic syndrome. Genetic studies have identified several genes associated with OFCs most of which have unknown etiologic mechanism. Genome-wide association studies have reported the significant genetic effects alone and with gene-environment interaction effect on the risk of OFCs but these effects differ across populations (13).

The results from this dissertation support the association between maternal obesity and diabetes and risk cleft palate. Moreover, underweight mothers had a decreased risk of cleft lip only (CLO) and an increased risk of cleft palate only (CPO). The protective effect of underweight mothers on CLO risk is inconsistent with previous

studies (14). Maternal body mass index (BMI) and maternal diabetes might have effects on facial formation in a variety of pathways. It is difficult to identify the independent effects of maternal obesity and diabetes on OFCs because the relationship between obesity and diabetes is complex. Obesity increases the risk of inadequate folate status (15-18); BMI may have an adverse effect on cellular uptake and tissue distribution of folate. Obesity leads to insulin resistance because of the impairment of insulin sensitivity in sites of glucose disposal, which can develop to type 2 diabetes mellitus and GDM (19). Elevated blood glucose and insulin stimulates the production of ketone bodies, branched chain amino acid, inflammatory markers, and advanced glycation end products. These products alter expression levels of specific genes and increase the variation of gene expression levels (20-23). Inadequate folate status and adverse metabolic production may disrupt normal embryonic development.

The results suggest that gestational diabetes, maternal obesity, and metabolic syndrome are associated with increased risk of cleft palate. Mothers having cleft palate with or without cleft lip (CP/L) offspring had an increased risk of developing metabolic syndrome based on both NCEP/ATP III and IDF definitions. When compared with control mothers, mothers having cleft palate offspring had a higher insulin, IL-8, and leptin levels. Insulin resistance associated with insulin and IL-8 levels is a risk factors of developing GDM (24). Leptin levels are significant higher in pregnancies with GDM compared with normal pregnancies (25-27). Therefore, insulin, IL-8, and leptin levels may be links among GDM, metabolic syndrome, and OFCs. Having OFCs offspring might be a risk factor of developing metabolic syndrome among mothers later in life.

Insulin, IL-8 and leptin might be performed as biomarkers for predicting OFC occurrence.

The strong association between maternal diabetes and risk of OFCs leads to the genetic association study related to these metabolic conditions. The study of genetic effects alone found that two genes (SLC30A8 and HNF1B) were associated with Asian OFCs and five genes (ABCC8, ADIPOQ, ADRB3, HNF1 and TNF- $\alpha$ ) were associated with European OFCs. Two genes were associated with OFCs when interaction with perinatal period (PCMV) was considered-- ABCC8 and CDKAL1 in Europeans. Interactions between genotype and maternal smoking were found for CDKN2A/2B in Asians, and for LEP in Europeans. When considering gene-environment interaction with environmental tobacco smoke (ETS), FTO, HHEX, and PPARG increased risk of OFCs in Asians. Our result suggests that genes related to GDM have an effect on risk of non-syndromic OFCs through either genetic effect alone or gene-environment interaction effects with maternal periconceptional multivitamin use, smoking, and environment tobacco smoke. Therefore, the etiology of non-syndromic OFCs is multifactorial between genes and environment.

## 6.2 Future Direction

This dissertation examines role of maternal diabetes mellitus in orofacial clefts through studies of medical histories, biomarkers, and genes. Further study is required to confirm the result and transcend the limitations of this project.

The completeness of the data from registries is a limitation in this dissertation. We found under-reporting of maternal medical conditions and a large amount of missing data

of potential confounders (smoking and alcohol consumption). Training programs might help registry recorders give priority to complete data. Moreover, the small number of participants with diabetes results in wide confidence intervals and small mediation effects of maternal diabetes on the association between maternal overweight and obesity and risk of OFCs. Therefore, further studies require larger sample sizes in order to confirm the association and mediation effect.

This dissertation found associations between maternal biomarkers of metabolic syndrome and orofacial clefts. We found that insulin, IL-8, and leptin levels were higher in mother having cleft palate offspring than control mother. Therefore, a prospective study that regularly monitors insulin, IL-8 and leptin levels before the conception period until the next pregnancy may be in order to determine predictability for OFC occurrence. Moreover, the association between cytokines and adipokines and risk of OFCs requires further study with larger sample sizes and different ethnic groups in order to confirm and compare the associations.

Genetic association have found that many SNPs in same gene were associated with OFCs; therefore, haplotype analysis and gene-gene interactions may find more regions or genes which have small effects individually but show strong statistical evidence of linkage and association when combined. This dissertation analyzed gene-environment interaction associations between genes related to diabetes and obesity and maternal environmental factors (maternal multivitamin use, smoking, and environmental tobacco smoke). Additional environmental data for gene-environment interaction analysis

is required in further studies such as maternal BMI and biomarkers related to metabolic syndrome and diabetes mellitus.

Observational studies provide evidence of association between exposures and diseases, but often cannot identify the etiological mechanisms of disease from exposure. Therefore, strong associations between maternal diabetes and obesity on risk of OFCs found through maternal medical conditions, maternal biomarkers and genetic associations require further research to explore the etiological mechanisms. Further studies will be required to understand how maternal diabetes and obesity has an effect on OFCs occurrence. Moreover, biological interaction studies needs to identify the mechanisms of interaction effects between genes and maternal exposures on fetal development.

### **6.3 Public Health Significance**

The association between maternal diabetes and obesity and risk of OFCs may lead to increased awareness among people and health care providers. Effective interventions are needed for promoting healthy body weight and metabolic status in reproductive age women in order to reduce the risk of OFCs. Moreover, early screening for GDM risk is needed for the periconceptional period and in the first month of gestation in order to allow early interventions for controlling hyperglycemia, hyperinsulinemia and other associated metabolic abnormalities to prevent OFCs and other congenital malformations.

Mothers having an OFC child developed metabolic syndrome and had abnormal biomarkers later in life may and it may be proposed that having OFC child is a risk indicator for subsequent maternal metabolic syndrome. These mothers may receive regularly metabolic syndrome monitoring and intervention to prevent developing



metabolic syndrome and related diseases. On the contrary, metabolic syndrome score, insulin, IL-8, and leptin levels may be focus as indicator for OFC prediction. Moreover, the association of biomarkers before or during periconceptional period and risk of OFCs requires further study.

The association between genetic effects alone and gene-environment interaction effects and risk of OFCs might be difficult to apply in public health because the biological function of detected variants is still difficult to interpret and people may carry the effects of one or more variants in several genes. Strategies for dealing with multiple causal genes and applying the strategy effectively are required in genetic studies of OFCs. These associations do not point to a single major GDM gene associated with OFCs, but support the hypothesis that GDM may be causally related to OFCs via multiple GDM susceptibility genes and interactions with environmental factors. However, the finding in this dissertation confirms that etiology of OFCs is multifactorial including genetics and environment.

Individuals with OFCs face both physical and mental health problems, which require multi-specialty team care. OFC prevention and prediction are important to public health. This dissertation reported that maternal diabetes mellitus, maternal pre-pregnancy weight and genes related to GDM had associations with the risk of OFCs. Mothers having an OFC child had an increased risk of developing metabolic abnormalities later in life. Potential risk factors were reported in this dissertation that can be applied for OFC prevention. This dissertation also reported potential biomarkers for predicting OFCs.

Moreover, mothers having an OFC child require regular monitoring for metabolic abnormalities later in life.

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