

To the Graduate Council:

I am submitting herewith a thesis written by Hyoungh Yon Kim entitled "Genetic characterization of the hypertriglyceridemia and the related traits in obese diabetic TALLYHO/JngJ mice." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Jung Han Kim
Major Professor

We have read this thesis
and recommend its acceptance:

Naima Moustaid-Moussa

Arnold Saxton

Accepted for the Council:

Anne Mayhew

Vice Chancellor and

Dean of Graduate Studies

(Original signatures are on file with official student records.)

**Genetic characterization of the hypertriglyceridemia and the related traits in obese
diabetic TALLYHO/JngJ mice.**

A Thesis
Presented for the
Master of Science Degree
The University of Tennessee, Knoxville

Hyoung Yon Kim
August 2006

DEDICATION

This thesis is dedicated to my parents, Jung-Man Kim and Yonung-Chae Oh, for always loving me and encouraging me.

It is also dedicated to my husband Pyoung Chung Kim for helping and supporting me throughout my studies.

ACKNOWLEDGEMENTS

I would like to start thanking for God for all of his blessings, help and guidance.

I would like to thank my major advisor, Dr. Jung Han Kim for her guidance, effort, patience and support.

I would like to thank Dr. Naima Moustaid-Moussa and Dr. Arnold Saxton for being on my committee and their encouragement and scientific guidance throughout my study.

I would like to thank Pyoung Chung Kim for his encouragement, help and support throughout my study.

I would like to thank my parents and parents in law for their endless love and sacrifices.

Abstract

Type 2 diabetes mellitus (T2DM) is the most common form of human diabetes, accounting for approximately 90% of cases and often coexists with obesity. Elevated triglyceride levels and small dense low density lipoprotein particles and reduced high density lipoprotein cholesterol constitute diabetic dyslipidemia. Diabetic patients often develop hypertriglyceridemia (HTG) at the early stage of the disease, before the onset of overt hyperglycemia (diabetes). It is well-established that genetic factors significantly influence the onset of HTG, yet no susceptibility genes for common forms of HTG have been identified in human populations to date.

TALLYHO/JngJ (TH) mice are a newly established inbred polygenic model for obesity and T2DM. These mice are characterized by insulin resistance, hyperinsulinemia, diabetes (males), obesity, and dyslipidemia. TH male mice rapidly develop HTG at a very young age before the onset of overt diabetes, and this HTG is accompanied by hyperinsulinemia, and precedes glucose intolerance. Accordingly, we hypothesize that the HTG may become a strong risk factor for overt diabetes in these mice and TH allelic differences at the underlying genetic susceptibility loci confer predisposition to HTG in TH mice. In an attempt to test these hypotheses, the aim of this study is to establish the genomic location(s) linked to the HTG in TH mice. In order to achieve this aim, we generated F2 mice from an intercross of F1[C57BL/6J (B6) x TH] mice. The F2 mice were then phenotyped for HTG and related traits including hypercholel^aemia, hyperglycemia, and obesity. F2 mice were also genotyped with simple sequence repeat markers over the entire genome. Statistical analysis to detect significant loci that co-segregate with the traits was conducted using quantitative trait loci (QTLs) mapping.

We found significant QTLs linked to HTG on chromosomes 1 and 8. Chromosome 1 QTL was also associated with hypercholesterolemia. A significant QTL responsible for fat pad weights was detected on chromosome 1 located proximal to the HTG QTL. A QTL on chromosome 4 was significantly linked to hyperglycemia. For all these traits, mice homozygous for TH alleles exhibited higher values than mice heterozygous or homozygous for B6 alleles.

In summary, our genetic study revealed a complex pattern of inheritance and mapped significant QTLs for HTG and related traits in TH mice. Identifying the genes for these QTLs will enhance our understanding of diabetic HTG, and provide new targets to prevent it.

Table of Contents

<u>Chapter</u>	<u>Page</u>
CHAPTER I: INTRODUCTION	1
CHAPTER II: LITERATURE REVIEW	3
1. Obesity and Type 2 Diabetes Mellitus	3
2. Pathogenesis of Type 2 Diabetes Mellitus	5
3. Diabetic Dyslipidemia	8
4. Genetics in Type 2 Diabetes Mellitus and Dyslipidemia.....	13
5. Polygenic Rodent Models of Type 2 Diabetes Mellitus	19
CHAPTER III: EXPERIMENTAL SECTION	24
1. Introduction.....	25
2. Materials and Methods.....	26
3. Results	30
4. Discussion.....	43
CHAPER IV: CONCLUSIONS AND FUTURE DIRECTIONS.....	50
LIST OF REFERENCES	53
VITA.....	68

List of Tables

<u>Table</u>	<u>Page</u>
TABLE 1. LIPID AND LIPOPROTEIN ABNORMALITIES IN TYPE 2 DIABETES.	8
TABLE 2. CHARACTERISTICS OF THE LIPOPROTEINS.	9
TABLE 3. CHARACTERISTICS OF THE MAJOR APOLIPOPROTEINS.	10
TABLE 4. QTLs FOR PLASMA TRIGLYCERIDE LEVELS IN MICE.	16
TABLE 5. QTLs FOR PLASMA CHOLESTEROL LEVELS IN MICE.	17
TABLE 6. QTLs FOR PLASMA INSULIN LEVELS IN MICE.	18
TABLE 7. QTLs FOR PLASMA GLUCOSE LEVELS IN MICE.	18
TABLE 8. QTLs FOR BODY WEIGHT IN MICE.	20
TABLE 9. QTLs FOR FAT WEIGHT IN MICE.	20
TABLE 10. SIMPLE SEQUENCE LENGTH POLYMORPHIC MARKERS USED FOR GENOME SCAN OF F2 MICE FROM AN INTERCROSS OF F1(C57BL/6J x TALLYHO/JngJ) MICE (MALES).	28
TABLE 11. PHENOTYPIC CHARACTERISTICS OF PARENTAL STRAINS AND F1 PROGENY (MALES).	31
TABLE 12. HIGHLY SIGNIFICANT QTLs IN F2 MICE FROM AN INTERCROSS OF F1(B6 x TH) MICE (MALES).	41
TABLE 13. SIGNIFICANT QTLs IN F2 MICE FROM AN INTERCROSS OF F1(B6 x TH) MICE (MALES).	41
TABLE 14. SUGGESTIVE QTLs IN F2 MICE FROM AN INTERCROSS OF F1(B6 x TH) MICE (MALES).	44
TABLE 15. SUMMARY OF SIGNIFICANT QTLs.	47

List of Figures

<u>Figure</u>	<u>Page</u>
FIGURE 1. MODEL OF THE PROGRESSIVE PATHOGENESIS OF TYPE 2 DIABETES MELLITUS.....	6
FIGURE 2. SCHEMATIC REPRESENTATION OF DYSLIPIDEMIA.....	12
FIGURE 3. STEPS IN QUANTITATIVE TRAIT LOCUS MAPPING.....	15
FIGURE 4. HISTOGRAM FOR PLASMA GLUCOSE LEVELS IN F2 MALE MICE (N=182) FROM AN INTERCROSS OF F1(B6 X TH) MICE.	33
FIGURE 5. HISTOGRAM FOR PLASMA TRIGLYCERIDE LEVELS IN F2 MALE MICE (N=182) FROM AN INTERCROSS OF F1(B6 X TH) MICE.	34
FIGURE 6. HISTOGRAM FOR PLASMA TOTAL CHOLESTEROL LEVELS IN F2 MALE MICE (N=182) FROM AN INTERCROSS OF F1(B6 X TH) MICE.....	35
FIGURE 7. HISTOGRAM FOR PLASMA FREE FATTY ACID LEVEL IN F2 MALE MICE (N=182) FROM AN INTERCROSS OF F1(B6 X TH) MICE.	36
FIGURE 8. HISTOGRAM FOR PLASMA INSULIN LEVELS IN F2 MALE MICE (N=182) FROM AN INTERCROSS OF F1(B6 X TH) MICE.	37
FIGURE 9. HISTOGRAM FOR BODY WEIGHT IN F2 MALE MICE (N=182) FROM AN INTERCROSS OF F1(B6 X TH) MICE.	38
FIGURE 10. HISTOGRAM FOR FAT PAD WEIGHT IN F2 MALE MICE (N=182) AT 24 WKS FROM AN INTERCROSS OF F1(B6 X TH) MICE.	39

Chapter I: Introduction

Type 2 diabetes mellitus (T2DM) is a common disease found in 3 to 5% of Western populations (1). T2DM is a chronic and progressive disease characterized by insulin resistance and pancreatic beta cell failure. Three abnormalities cause hyperglycemia in T2DM: impaired insulin secretion, increased hepatic glucose production, and decreased insulin-stimulated uptake of glucose in peripheral tissues (2). Cardiovascular disease (CVD), particularly coronary heart disease (CHD), is a major complication of this disease, and over 50% of T2DM patients die of CHD (1). CHD, sudden death, and myocardial infarction are at least twice as high in patients with T2DM than in non diabetic subjects (3).

T2DM patients are at increased risk for CVD already at diagnosis of diabetes. T2DM is a major cardiovascular risk factor in addition to elevated cholesterol and blood pressure, smoking, low HDL cholesterol and aging (4). A high proportion of patients with T2DM die within 1 year after an acute myocardial infarction, and a considerable number of patients die even before they reach the hospital (5). Hyperglycemia was believed to cause diabetic complications. Poor glycemic control is a strong risk factor for microvascular complications but its significance with respect to CVD is quite limited. Therefore, other risk factors for microangiopathy have to be considered. Dyslipidemia is very common in patients with T2DM and it significantly contributes to the risk of CVD (6).

The development of T2DM is controlled by the interaction of multiple genetic and environmental factors (7). Many candidate genes for insulin resistance and β -cell biology have been studied and a few of them are associated with T2DM (8). Genetic influences have been difficult to elucidate and determination of genes involved has not been easily achieved in humans (9). Analysis of diabetes genes in rodents offers several advantages over the same studies in humans: A large number of animals can be bred, and both environmental influences and genetic heterogeneity can be controlled (10). In addition, the possibility of creating similar genetic lesions in mice of different background can be used to identify important modifiers of disease genes, and new mouse models of diabetes can be created by gene insertions or targeted gene disruptions (11).

TALLYHO/JngJ (TH) mouse strain is a polygenic model for T2DM with obesity. TH mice are characterized by insulin resistance, hyperinsulinemia, hyperglycemia (males), obesity, and dyslipidemia associated with increased triglyceride, free fatty acid and cholesterol levels. In previous studies from our laboratory, we demonstrated that male TH mice rapidly develop hypertriglyceridemia (HTG) at a young age before the onset of overt diabetes. The objective of this study is to identify the underlying genetic susceptibility loci responsible for HTG in TH mice. This study will enhance our understanding of diabetic HTG and provide new therapeutic targets for T2DM and dyslipidemia.

Chapter II: Literature review

1. Obesity and Type 2 Diabetes Mellitus

About of 65% of American adults are either overweight or obese (the Centers for Disease Control and Prevention; CDC) and the number of overweight and obese Americans continues to rise. Between NHANES (The National Health and Nutrition Examination Surveys, a series of surveys conducted periodically by the U.S. Department of Health and Human Services agencies) II (1976-1980) and NHANES 1999-2002, the percentage of obese adults nearly doubled (12).

Obesity increases the risk of T2DM, heart disease, dyslipidemia, arthritis, gallstone formation, and certain cancer, including cancers of the breast, colon, uterus, pancreas, and kidney (13). According to Colditz's study in 1995, obese women are 7 to 13 times more likely than woman of normal weight to develop T2DM (13,14). Even a modest weight gain of 5 to 7 kg (11 to 15 lbs.) increases the risk of diabetes by 50%, while losing as little as 5kg (11 lbs.) reduces the risk by the same amount (14).

As the prevalence of obesity increases, the world faces a epidemic problem of T2DM (3). According to International Diabetes Federation (IDF) the number of diabetic adults between 20 to 79 years at age will increase by around 70% in the next 20 years, from 194 million in 2003 to 330 million in 2025 (<http://www.eatlas.idf.org/>). T2DM causes blindness (15), end-stage renal disease (16), and non traumatic loss of limb (17). Cardiovascular disease (CVD) causes the most serious fatal disease by T2DM, and T2DM patients have two to four times greater cardiovascular morbidity than that of non-diabetic people (18). Diabetic men have 2-3 and diabetic women have 3-5 times the

chance to have CVD compared with the non-diabetic population (19).

The intra-abdominal fat depot or central subcutaneous fat depot is considered a risk factor for reduction in insulin sensitivity (20). Obese people whose fat is stored in visceral adipose depots suffer more adverse metabolic consequences than those with fat stored predominantly in subcutaneous sites. Excess abdominal fat mass is associated with an increased release of non-esterified free fatty acids (FFA) that may trigger a reduction in insulin sensitivity at both the hepatic and the muscular levels (21).

In the liver, this results in an increased glucose output (essentially due to enhanced gluconeogenesis) and increased VLDL production, while in the skeletal muscle, there is a reduction in glucose oxidation and glucose storage as glycogen (22).

Insulin-resistant obese patients suffer from impaired glucose tolerance, dyslipidemia and arterial hypertension, all factors which increase the risk of CVD. Interestingly, ectopic fat accumulation in insulin-sensitive tissues may be associated with insulin resistance independent of overall obesity (23).

T2DM occurs as a late phenomenon in obese subjects and is preceded by years of normal glucose tolerance or impaired glucose tolerance (IGT) as suggested by several cross-sectional studies (24). The progression from IGT to diabetes occurs when the beta cell becomes unable to maintain its previously high rate of insulin secretion in response to glucose (25). During the early years of obesity, subjects are normoglycemic but hyperinsulinemic then progressively become hyperglycemic when hyperinsulinemia is not maintained (26).

2. Pathogenesis of Type 2 Diabetes Mellitus

T2DM develops when the body doesn't make enough insulin or doesn't efficiently use the insulin it makes (27). Insulin resistance is characterized by defective glucose uptake by muscle and adipose tissue (28). The primary abnormality in most cases of T2DM is insulin resistance (29). The initial stage of insulin resistance, pre-diabetic stage, is influenced by genetic factors and environmental factors with changes in insulin levels, and lipids (Figure 1). These changes are followed by cardiovascular complications in genetically susceptible people, before the onset of diabetes (27). The onset of diabetes is characterized by hyperglycemia and hyperinsulinemia, followed by rising fasting plasma glucose or rising peak plasma glucose challenge, levels in response to an oral glucose with hyperglycemia leading to a diagnosis of T2DM.

T2DM is defined as a fasting blood glucose of ≥ 126 mg/dl. "Pre-diabetes" is a condition in which blood glucose levels are higher than normal and precedes diabetes. People with pre-diabetes are at increased risk for developing T2DM and have one of these conditions: impaired fasting glucose (100 ~ 125 mg/dl) and impaired glucose tolerance (fasting glucose less than 126 mg/dl and a glucose level between 140 and 199 mg/dl two hours after taking an oral glucose tolerance test).

Since insulin is produced by pancreatic β -cells, dysfunction of these cell is a critical factor in the pathogenesis of T2DM (30). When insulin action decreases with increased obesity, concentrations of blood glucose as fasting and 2 hours after glucose load will increase slowly (31,32).

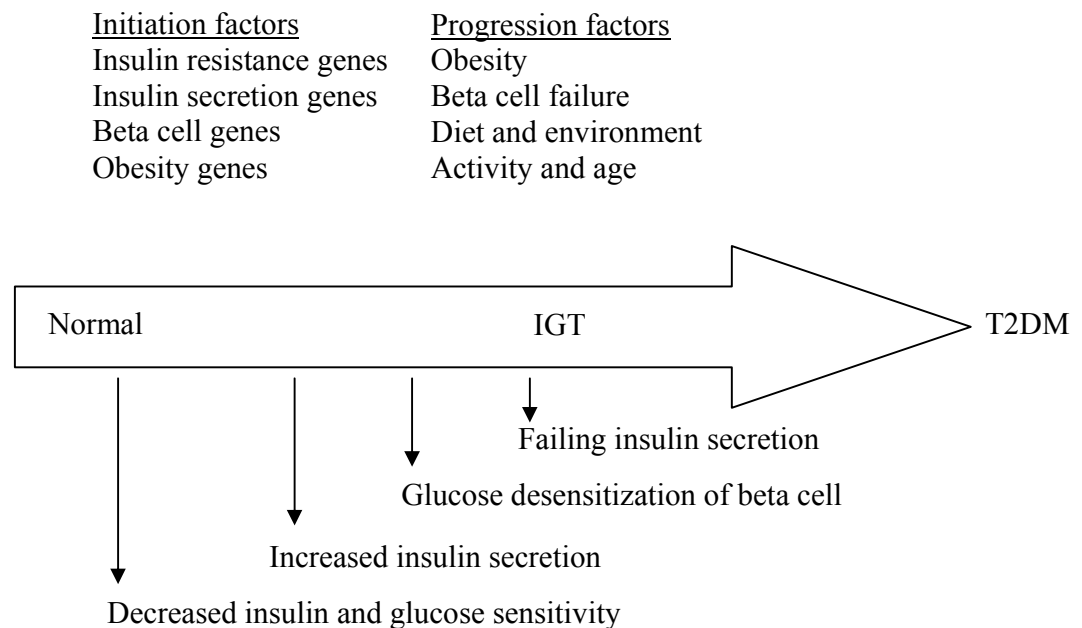


Figure 1. Model of the progressive pathogenesis of type 2 diabetes mellitus. In most individuals there is a slow progression from normal to impaired glucose tolerance (IGT) to diabetes. This depends on interactions between genetic and environmental factors that act on both initiation and progression of the disease (33).

Insulin: Insulin is secreted from β -cells in the pancreas and controls blood glucose. In normal conditions, insulin inhibits glucose production from the liver and stimulates the uptake of glucose into peripheral tissues to use as energy (34). In addition, insulin regulates lipid metabolism. In adipose tissue, insulin stimulates FFA uptake, esterification, and storage into increased TG via lipogenesis. Insulin promotes FFA uptake into the adipose tissue by stimulation of lipoprotein lipase (LPL) which hydrolyzes lipoprotein triglyceride (TG). Also, insulin promotes TG synthesis from glucose. However, insulin inhibits hormone-sensitive lipase (HSL) which is the principal regulator of FFA release from adipose tissue, and prevents lipolysis of TG in adipose tissue.

Insulin signaling: Insulin acts through a subfamily of receptor tyrosine kinases; the insulin receptor (IR) and insulin-like growth factor-I receptor (IGF-IR). They catalyze the phosphorylation of several intercellular substrates, including the insulin receptor substrate (IRS) protein, growth factor receptor bound protein 2-associated protein 1 (GAB-1), src homology 2 domain-containing transforming protein C (Shc), adaptor protein with pleckstrin homology and src (APS), and signal-regulatory proteins (SIRPS) (35,36). The signaling cascades of these two receptors are quite similar. The IR undergoes autophosphorylation and affects signal transduction primarily by activating two pathways, the IRS - phosphoinositide-3 kinase (PI3K) - Akt pathway and the Ras – Raf – Mitogen Activated Protein Kinase (MAPK) pathway. The c-Jun N-terminal kinase (JNK) and p38 MAPK pathways are also activated by insulin. The IGF-IR activates these same pathways in a similar fashion. The IR primarily regulates fuel metabolism and the IGF-IR modulates cell growth and survival (37).

Insulin-sensitive tissues such as liver, fat, and muscle are involved in regulating whole body fuel metabolism. Insulin's effect is controlled by the IRS-PI3K-Akt pathway. In fat and muscle, activation of this pathway leads to translocation of intracellular glucose transporter 4 (GLUT4), to the surface membrane, resulting in enhanced glucose uptake (38). In addition, it has been reported that GLUT4 gene expression in fat and muscle is reduced in T2DM and this leads to reduced uptake of glucose from blood (39). In liver, insulin acts to down regulate glucose production by inhibiting the expression of gluconeogenic genes (40).

3. Diabetic Dyslipidemia

Dyslipidemia is commonly found in patients with T2DM (41). Dyslipidemia precedes onset of T2DM and frequently impaired glucose tolerance (IGT) by years, indicating that disturbance of lipid metabolism may be the primary event in the development of T2DM (42) (Table 1). In recent study, lean insulin resistant offspring of patients with T2DM were shown to have increased intrameyocellular lipid contents, which was attributable to a reduction of mitochondrial phosphorylation (43). Increased liver fat content and resulting non-alcoholic fatty liver or non-alcoholic steatohepatitis are also very commonly found in T2DM subjects with dyslipidemia (43).

Dyslipidemia is the raised TG values (≥ 150 mg/dl, fasting), low high density lipoprotein (HDL) cholesterol values (< 40 mg/dl in men, < 50 mg/dl in women, fasting), increased small, dense low density lipoprotein (sd LDL) cholesterol values, and raised apolipoprotein (apo) B values, the structural protein of very low density lipoprotein (VLDL) and LDL (44). Dyslipidemia is an independent risk factor for CVD (45).

Table 1. Lipid and lipoprotein abnormalities in type 2 diabetes.

Diabetes category	Lipids and lipoproteins
Usual levels of glycemia	Increased triglycerides Decreased HDL cholesterol Small and dense LDL particles Increased LDL susceptibility to oxidation
Poor glycemic control	Worsening of hyperglyceridemia
Diabetic nephropathy	Increased triglycerides Decreased HDL cholesterol

HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein.

Hypertriglyceridemia (HTG) is characterized by the over secretion of TG-rich VLDL particles from the liver (46). In abdominal obesity and insulin resistance, FFA release is increased (47). Increased FFA uptake by the liver then stimulates the secretion of TG-rich VLDL in the blood stream. In the peripheral circulation, TG in VLDL is hydrolyzed by LPL and the generated FFA is used by peripheral tissues (48).

Lipoproteins and Apolipoproteins

Lipoproteins are a molecular complex that carries various lipids and proteins in the circulation. The cores of lipoproteins are filled with hydrophobic TG and cholesteryl ester molecules and these are covered with amphipathic (hydrophobic and hydrophilic) phospholipids and proteins (Table 2).

Apolipoproteins are proteins that are located on the surface of the lipoproteins. They are involved in the regulation of plasma lipid and lipoprotein transport (Table 3). Apo A-I is the major structural protein of HDL and it activates lecithin cholesterol-acyl transferase (LCAT), playing a role in reverse cholesterol transport. LCAT esterifies free cholesterol using a fatty acyl moiety derived primarily from phosphatidylcholine within

Table 2. Characteristics of the lipoproteins.

Lipoprotein	Lipid (%)		
	TG	CHOL	PL
Chylomicrons	80-95	2-7	3-9
VLDL	55-80	5-15	10-20
IDL	20-50	20-40	15-25
LDL	5-15	40-50	20-25
HDL	5-10	15-25	20-30

VLDL, Very Low Density Lipoprotein; IDL, Intermediate Density Lipoprotein; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; TG, Triglyceride; CHOL, Cholesterol; PL, Phospholipid.

Table 3. Characteristics of the major apolipoproteins.

Apolipoprotein	Lipoproteins	Metabolic functions
A-I	HDL, chylomicrons	Structural component of HDL;LCAT activator
A-II	HDL, chylomicrons	Unknown
A-IV	HDL, chylomicrons	Unknown; possibly facilitates transfer of apolipoproteins between HDL and chylomicrons
B-48	Chylomicrons	Necessary for assembly and secretion of chylomicrons from the small intestine
B-100	VLDL, IDL, LDL	Necessary for the assembly and secretion of VLDL from the liver; structural protein of VLDL, IDL and LDL; ligand for the LDL receptor
C-I	VLDL, IDL, LDL, chylomicrons	May inhibit hepatic uptake of chylomicrons; VLDL remnants
C-II	VLDL, IDL, LDL, chylomicrons	Activator of lipoprotein lipase
C-III	VLDL, IDL, LDL, chylomicrons	Inhibitor of lipoprotein lipase; inhibits hepatic uptake of chylomicron and VLDL remnants
E	VLDL, IDL, LDL, chylomicrons	Ligand for binding of several lipoproteins to the LDL receptor, LRP, and proteoglycans

HDL, High Density Lipoprotein; LCA,: Lecithin Cholesterol-Acyl Transferase; VLDL, Very Low Density Lipoprotein; IDL, Intermediate Density Lipoprotein; LDL, Low Density Lipoprotein; LRP, LDL-Receptor-related Protein.

HDL particles (49). Apo A-II is the second structural protein of HDL but its function is largely unknown (49). The function of Apo-IV is also unknown and it may serve in apoproteins transfer between HDL and chylomicrons (50). Apo B-48 helps to assemble TG, FFA, and PL with chylomicron in small intestine (51). Apo B-100 is involved in secretion of VLDL, IDL, and LDL. Apo C-II activates LPL (52).

Free fatty acids

Insulin resistance causes fat cells to break down more of their stored TG and release of more FFA into the circulation (53). The primary defect is probably focused in the inability to incorporate the FFAs to TGs by the adipose tissue (44). This results in reduced fatty acid trapping and retention by the adipose tissue. The insulin resistance

also causes reduced retention of FFAs by adipocytes. Both these abnormalities lead to increased flux of FFA to the liver (54) (Figure 2).

Triglycerides

Increased FFA from the periphery to the liver in the insulin resistant state stimulates hepatic TG synthesis by promoting assembly and secretion of TG containing VLDL (28), as well as the apo B production in the liver (55). Also, when insulin resistance occurs, insulin becomes unable to suppress insulin on VLDL secretion (56) (Figure 2).

Small dense LDL cholesterol

In the insulin resistant state, the LDL levels are usually within normal limits or raised slightly. However, the LDL particles have smaller and denser size (sdLDL). It seems that the underlying abnormality causing sdLDL is hypertriglyceridemia, because it has been found that sdLDL is not seen until plasma TG levels exceed 150mg/dl (57). Under these conditions, large TG rich VLDL molecules accumulate, which results in increased TG rich LDL. TG rich LDL is a good substrate for HL that finally generates sdLDL (58) (Figure 2).

HDL cholesterol

Under the increased plasma TG levels, the cholesteryl ester transfer protein mediates TG-cholesteryl ester exchange between LDL and VLDL. Similar lipid TG rich

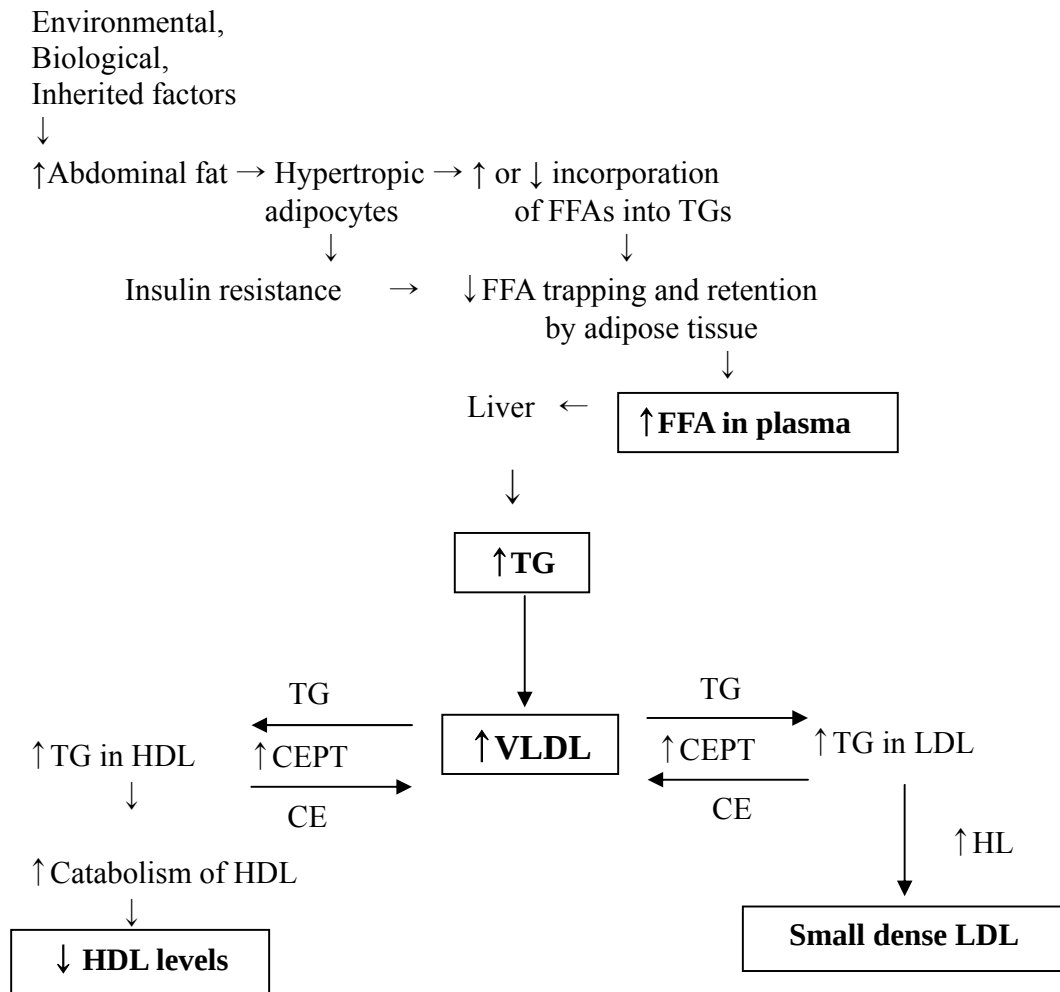


Figure 2. Schematic representation of dyslipidemia (58).

FFA, Free Fatty Acid; TG, Triglyceride; HDL, High Density Lipoprotein; CETP, Cholesteryl Ester Transfer Protein; CE, Cholesterol Ester; VLDL, Very Low Density Lipoprotein; LDL, Low Density Lipoprotein; HL, Hepatic Lipase.

exchange is taking place between LDL and HDL particles, forming TG rich HDL. These but cholesterol depleted HDLs undergo hydrolysis of their TG component and dissociation of their protein component, Apo A (the main protein of HDL) (59) (Figure 2).

4. Genetics in Type 2 Diabetes Mellitus and Dyslipidemia

A. Humans

Genetic factors account for about 50-70% of the causes of T2DM; other factors are environmental (such as high caloric diet and lack of activity) (60). The prevalence of diabetes in offspring of patients with T2DM in both parents is about 60% by the age of 60 years and in one parent is about 38% by the age of 80 years (61,62). In addition, 15-24% of first-degree relatives of T2DM patients showed impaired glucose tolerance or diabetes (62).

In a twin study, the prevalence rate of diabetes in individuals older than 60 years was 35-58% in monozygotic twins and 17-20% in dizygotic twins (63). The impaired glucose tolerance increased in monozygotic twins to 88% (64).

Finding diabetic genes has been complicated because of the complexity and late onset of disease and limitation of family history of diabetes. Until now, it has been found that T2DM is polygenic and is caused by interaction of many genes (65). To identify these genes and their interactions it is necessary to study a very large population (66). Thus far, two loci for obesity associated non-insulin dependent diabetes mellitus (NIDDM), NIDDM1 and NIDDM2, have been found on chromosome 2q in Mexican –

American populations (67) and on chromosome 12q in western Finland population (68), respectively.

B. Animals

Animal models of T2DM are likely to be as complex and heterogeneous as the human condition. (69), However, finding diabetes genes in rodents offer advantages over humans because a large number of animals can be bred for study, and the environmental influence and genetic heterogeneity can be controlled (66).

Quantitative trait loci (QTLs) mapping is a genome-wide inference of the relation between genotype and phenotype. Detecting and mapping QTL method has been used since Thoday's study (70). The purpose of QTL mapping is to localize chromosomal regions that significantly affect the variation of quantitative traits in a population. From this location in the chromosome, scientists attempt to find candidate genes (71). For QTL mapping, it is necessary to cross two differentiated populations and then either backcross to the parents to create backcross population or intercross between brother and sister to create F2 progeny. The backcross or F2 populations are then phenotyped and genotyped using genetic markers (72) (Figure 3). Most frequently used genetic markers include simple sequence length polymorphic (SSLP) markers that consist of a variable number of cytosine-adenine repeats (CA)_n. These repeats are present at ~1,000,000 copies per genome (73).

Using QTL mapping analysis, several loci linked to dyslipidemia have been identified in numerous genetic crosses of rodents.

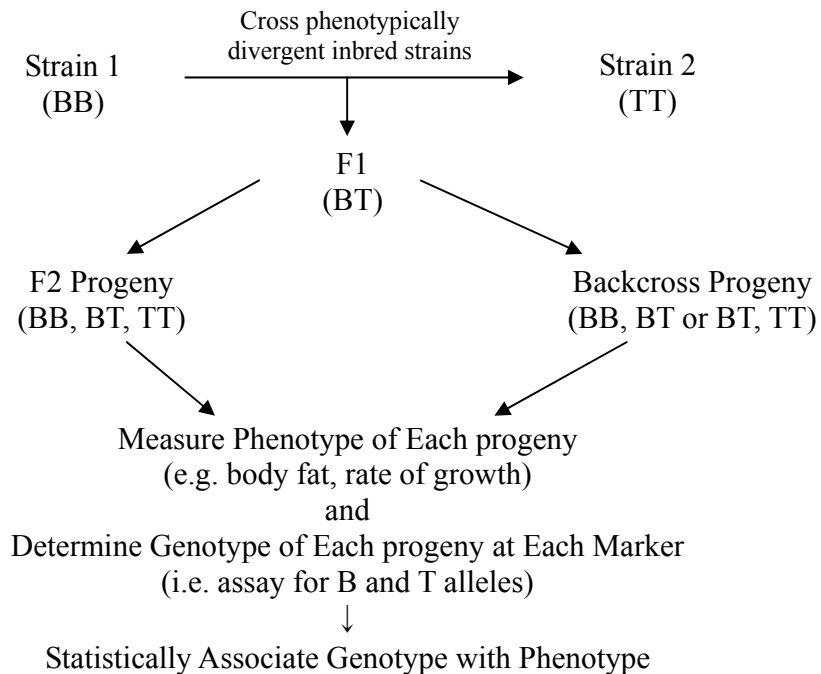


Figure 3. Steps in quantitative trait locus mapping (72).

1) QTLs for plasma triglyceride levels in mice.

In KK/Ta x (BALB/c x KK/Ta)F1 mice, QTLs for serum TG levels were found on chromosomes 8 (51.5 cM) and 4 (59 cM) (74). In the C57BL/6J x 129S1/SvImJ mice, QTLs linked to plasma TG levels were also found on chromosome 18 (42 cM), 9 (66cM) and 4 (58cM) (75). In the LG/J x SM/J mice, QTLs for plasma TG levels were detected on chromosomes 11 (9 cM), 1 (95.8 cM), and 4 (58 cM) (76). Additionally, in the SM/J x A/J recombinant inbred mice, QTLs for plasma TG levels were detected on chromosomes 11 (70 cM), 9 (33 cM), and 8 (30 cM) (77) (Table 4).

Table 4. QTLs for plasma triglyceride levels in mice.

Strains	Chr	cM	Symbol, name	Ref.
KK/Ta x (BALB/c x KK/Ta)F1	8	51.5	Tgl1, triglyceride level 1	(74)
	4	59	Tgls1, triglyceride level suppressor 1	
C57BL/6J x 129S1/SvImJ	18	42	Tgq1, triglyceride QTL 1	(75)
	9	66	Tgq2, triglyceride QTL 2	
	4	58	Tgq3, triglyceride QTL 3	
LG/J x SM/J	11	9	Tgy11, triglyceride level 1	(76)
	1	95.8	Trglyd, triglycerides	
	4	58	Triglq1, triglyceride QTL 1	
SM/J x A/J	11	70	Triglq2, triglyceride QTL 2	(77)
	9	33	Trigq1, triglyceride QTL 1	
	8	30	Trigq2, triglyceride QTL 2	

Chr, Chromosome; cM, Centimorgan; Ref, Reference.

2) QTLs for plasma cholesterol levels in mice

In KK/Ta x (BALB/c x KK/Ta)F1 mice, a QTL for serum total cholesterol levels was found on chromosome 3 (49.2 cM) (74). In the C57BL/6J x C3H/He mice, QTLs linked to plasma total cholesterol were also found on chromosomes 7 (65 cM) and 6 (2.9 cM) (78). In the 129S1/SvImJ x RIIS/J mice, significant QTLs associated with serum cholesterol levels were detected on chromosome 9 (9 and 28 cM) (79). In the DBA/2J x CAST/Ei mice, a QTL on chromosome 9 (5 cM) was linked to plasma total cholesterol levels (80). In the 129S1/SvImJ x CAST/Ei mice, QTLs for plasma cholesterol levels were also found on chromosomes 1 (74 cM), 4 (12 cM) and 17 (54 cM) (81). In the KK x KK-Ay mice, QTLs for plasma cholesterol levels were also found on chromosomes 1 (74 and 100 cM) and 3 (49.7 cM) (82). In the C57BL/6J x RR mice, a QTL for plasma cholesterol levels was also found on chromosome 1 (100 cM) (81) (Table 5).

Table 5. QTLs for plasma cholesterol levels in mice.

Strains	Chr	cM	Symbol, name	Ref.
KK/Ta x (BALB/c x KK/Ta)F1	3	49.2	Tchol1, total cholesterol level 1	(74)
C57BL/6J x C3H/He	7	65	Chol1, cholesterol 1	(78)
	6	2.9	Chol2, cholesterol 2	
129S1/SvImJ x RIIIS/J	9	9	Chol10, cholesterol 10	(79)
	9	28	Chol11, cholesterol 11	
DBA/2J x CAST/Ei	9	5	Chol6, cholesterol 6	(80)
129S1/SvImJ x CAST/Ei	1	74	Chol7, cholesterol 7	(83)
	4	12	Chol8, cholesterol 8	
	17	54	Chol9, cholesterol 9	
KK x KK-Ay	1	81.6	Cq1, cholesterol QTL 1	(82)
	1	100	Cq2, cholesterol QTL 2	
	3	49.7	Cq3, cholesterol QTL 3	
C57BL/6J x RR	1	100	Cq6, cholesterol QTL 6	(81)

Chr, Chromosome; cM, Centimorgan; Ref, Reference.

3) QTLs for plasma insulin levels in mice.

In the DU6i x DBA/2 mice, QTLs associated with plasma insulin were detected on chromosome 10 (38 cM) and 18 (39 cM) (84). In the NOR/Lt x NOD/Jsd mice, QTLs for plasma insulin levels were found on chromosomes 17 (19.5 cM), 9 (55 cM), 3 (19.2 cM), 11 (46 cM), 1 (40 cM) and 6 (73 cM) (85-88) (Table 6).

4) QTLs for plasma glucose levels in mice.

In the C57BL/6 x C3H/He mice, QTLs associated with plasma glucose were detected on chromosomes 2 (87 cM) and 13 (59 cM) (89). In the KK/Ta x (BALB/c x KK/Ta)F1 mice, QTLs for plasma glucose were found on chromosomes 12 (36 cM) and 15 (10.1 cM) (74). In the KK x KK-Ay mice, QTLs for plasma glucose were found on chromosomes 6 (16 cM), 1 (100 cM) and 15 (10.1 cM) (74,90) (Table 7).

Table 6. QTLs for plasma insulin levels in mice.

Strains	Chr	cM	Symbol, name	Ref.
DU6i x DBA/2	10	38	Igf1q1, insulin-like growth factor 1 QTL 1	(84)
	18	39	Igf1q2, insulin-like growth factor 1 QTL 2	
NOR/Lt x	17	19.5	Idd1, insulin dependent diabetes susceptibility 1	(85)
NOD/Jsd	9	55	Idd2, insulin dependent diabetes susceptibility 2	(86)
	3	19.2	Idd3, insulin dependent diabetes susceptibility 3	
	11	46	Idd4, insulin dependent diabetes susceptibility 4	(87)
	1	40	Idd5, insulin dependent diabetes susceptibility 5	
	6	73	Idd6, insulin dependent diabetes susceptibility 6	(88)
Chr, Chromosome; cM, Centimorgan; Ref, Reference; NOD, Nonobese Diabetic mice; NOR, Nonobese Diabetes Resistance mice.				

Table 7. QTLs for plasma glucose levels in mice.

Strains	Chr	cM	Symbol, name	Ref.
C57BL/6 x C3H/He	2	87	Bglu1, blood glucose level 1	(89)
	13	59	Bglu2, blood glucose level 2	
KK/Ta x (BALB/c x KK/Ta)F1	12	36	Fbgl1, fasting blood glucose 1	(74)
	15	10.1	Fbgl2, fasting blood glucose 2	
KK x KK-Ay	6	16	Fglu, fasting glucose	(90)
	1	100	Fglu2, fasting glucose 2	
	6	2.8	Imgt1, impaired glucose tolerance 1	(74)
Chr, Chromosome; cM, Centimorgan; Ref, Reference.				

5) QTLs for body weight in mice.

In the C57BL/6J x PERA/Ei mice, a QTL associated with body weight was detected on chromosome 8 (16 cM) (91). In the C57BL/6J x FGS/Kist mice, a QTL for body weight was found on chromosome 13 (9 cM) (92). In the DU6i x DBA/2 mice, QTLs for body weight were found on chromosomes 2 (18 cM), 11 (55 cM), 1 (5 cM), and 9 (34 cM) (90), (93). In the C57BL/6J x TallyHo, QTLs for body weight were found on chromosomes 7 (30 cM) and X (16 and 32 cM) (94). In the DU6 x DUKs mice, QTLs for body weight were found on chromosomes 1 (36 and 69 cM), 2 (56 cM), 4 (59 cM), 5 (81 and 82 cM), 6 (35 cM), 7 (28 cM), 11 (14 and 55 cM), 12 (17 cM), 13 (10 and 34 cM), 15 (6 cM), and X (42 cM) (95) (Table 8).

6) QTLs for fat weight in mice

In the NSY x C3H/He mice, a QTL associated with epididymal fat pad weight was detected on chromosome 6 (35 cM) (96). In the C57BL/6J x TallyHo mice, a QTL for mesenteric fat pad weight was found on chromosome 4 (69.8 cM) (97). In the MH x ML mice, QTLs for brown fat pad weight were found on chromosomes 1 (102 cM) and 3 (55 cM) (98) (Table 9).

5. Polygenic Rodent Models of Type 2 Diabetes Mellitus

The Goto Kakizaki (GK) rat

The GK rat is used to understand pathogenesis of nonobese T2DM (99). The GK

Table 8. QTLs for body weight in mice.

Strains	Chr	cM	Symbol, name	Ref.
C57BL/6J x PERA/Ei	8	16	Bdwt, body weight	(91)
C57BL/6J x FGS/Kist	13	9	Bdw1, body weight 1	(92)
DU6i x DBA/2	2	18	Bodw1, body weight 1	(93)
	11	55	Bodw2, body weight 2	
	1	5	Bodw3, body weight 3	
	9	34	Bodw4, body weight 4	
C57BL/6J x TallyHo	7	30	Tabw, TallyHo associated body weight	(97)
	X	18	BW1, body weight QTL 1	(94)
	X	32	BW3, body weight QTL 3	
DU6 x DUKs	11	55	BW4, body weight QTL 4	(95)
	1	36	BW5, body weight QTL 5	
	2	56	BW6, body weight QTL 6	
	4	59	BW7, body weight QTL 7	
	5	82	BW8, body weight QTL 8	
	12	17	BW9, body weight QTL 9	
	13	34	BW10, body weight QTL 10	
	15	6	BW11, body weight QTL 11	
	X	42	BW12, body weight QTL 12	
	5	81	BW13, body weight QTL 13	
	7	28	BW14, body weight QTL 14	
	13	10	BW15, body weight QTL 15	
	11	14	BW16, body weight QTL 16	
	1	69	BW17, body weight QTL 17	
	6	35	BW18, body weight QTL 18	

Chr, Chromosome; cM, Centimorgan; Ref, Reference.

Table 9. QTLs for fat weight in mice.

Strains	Chr	cM	Symbol, name	Ref.
NSY x C3H/He	6	35	Efw, epididymal fat weight	(96)
C57BL/6J x TallyHo	4	69.8	Tafat, TallyHo associated fat	(97)
MH x ML	1	102	Batq1, brown fat QTL 1	(98)
	3	55	Batq2, brown fat QTL 2	

Chr, Chromosome; cM, Centimorgan; Ref, Reference.

rats develop relatively stable hyperglycaemia in adult life. Typically, the fasting blood glucose is only mildly elevated but rises further on challenge with glucose. Both insulin resistance and impaired insulin secretion are present. This rat is usually born with a reduced number of islets (100).

Spontaneously Diabetic Torii (SDT) rat

SDT rats are an inbred strain with spontaneous diabetes without obesity originated from an outbred colony of Sprague-Dawley rats (101). This rat spontaneously develops hyperglycemia and glucosuria after 20 weeks of age with an incidence of 100% at 40 weeks of age in males. Moreover, SDT rats exhibit severe ocular complications such as proliferative retinal degeneration and detachment of retina resembling human diabetic retinopathy (101).

The Otsuka Long-Evans Tokushima fatty (OLETF) rat

The OLETF rats are generated from an outbred colony of Long-Evans rats by selective breeding for glucose intolerance (102). The rats are mildly obese and males are more likely to develop diabetes in adult life than females. Genome wide scans have revealed susceptibility loci for T2DM-related traits on chromosomes 1, 7, 14, and X (103,104). It has also been known that OLETF rats carry a null allele for the cholecystokinin A receptor which may be involved in the regulation of food intake in these rats (105). However, whether this is casually related to the phenotype is still unclear (69).

The KK mouse

The original strain of KK mouse was bred for large body size (106). KK mice exhibit a multigenic syndrome of hyperphagia, moderate obesity, hyperinsulinemia, and hyperglycemia (106,107). Male mice but females develop T2DM after 4 months of age (108). KK/A^y mouse, a congenic strain of the A^y allele at the agouti locus in the KK background, also develops T2DM that is more severely expressed than that in KK. These mice are a suitable model for the study of maturity onset obesity and diabetes in humans (90).

The Nagoya-Shibata-Yasuda (NSY) mouse

NSY mice spontaneously develop diabetes in an age-dependent manner (96). These mice develop diabetes at a very late age with mild obesity and mild insulin resistance. Almost all males develop hyperglycemia, but less than a third of females do (96). This gender difference is a general characteristic of most rodent models of T2DM.

The New Zealand Obese (NZO) mouse

The NZO mice are an inbred strain initially selected for polygenic obesity, characterized by hyperphagia, obesity and insulin resistance (109). These mice have been used to characterize the interactions of obesity and diabetes genes (110).

TALLYHO (TH) mouse

The TH mouse strain was originally derived from a colony of outbred Theiler Original mice which show polyuria and glucosuria (97). The TH mice have been

established by selective breeding based on the hyperglycemia phenotype of the original deviant stock. TH mice exhibit obesity, hyperinsulinemia, hyperlipidemia, and hyperglycemia (male). A genome-wide linkage analysis using backcross progeny from a cross of F1(B6 x TH) and TH mice revealed QTLs linked to body weight on chromosomes 6 and 7 and a QTL affecting hyperglycemia on chromosome 19 (97).

Chapter III: Experimental section

Abstract

Type 2 diabetes mellitus (T2DM) is a chronic disease and closely associated with altered lipid metabolism in humans that often predates the onset of full-blown diabetes. It is well-established that genetic factors significantly influence the development of dyslipidemia, yet no susceptibility genes for the dyslipidemia have been identified in humans. TALLYHO/JngJ (TH) strain of mice is a polygenic model of T2DM with obesity and characterized by insulin resistance, hyperinsulinemia, hyperglycemia, and hyperlipidemia. In a previous study, we have identified that plasma triglyceride (TG) levels are dramatically increased in TH male mouse at 10 wks while plasma insulin levels steadily increased before the onset of diabetes at 30 wks. In order to identify the underlying genetic factors responsible for the hypertriglyceridemia (HTG) in the TH mouse, we intercrossed F1 (C57BL/6J x TH) mice to produce F2 mice and all F2 mice were genotyped using 67 simple sequence length polymorphic markers covering the whole genome. Mice were fasted 4 hours from 7:00 to 11:00 AM, blood was collected at 8, 12, 16, 20, and 24 weeks (wk) of age, and plasma glucose, insulin, free fatty acids, TG and total cholesterol levels were measured. Body weights were also measured at the same time. At the age of 24 wk, mice were killed and five white fat pads (inguinal, epididymal, mesenteric, retroperitoneal, and subscapular) and brown fat pad were dissected and weighed. With the genotype and phenotype data of F2 mice, we conducted QTL analysis using the computer program, Map Manager. In 182 male F2 mice, significant evidence for linkage with plasma TG levels was detected on chromosome 1 at

marker D1Mit113 (93 cM). This QTL was also significantly linked to plasma total cholesterol levels. In addition, a highly significant linkage for the plasma glucose levels was detected at marker D4Mit178 located at chromosome 4 (36 cM). For all these traits, mice homozygous for TH alleles exhibited higher values than mice heterozygous or mice homozygous for B6 alleles. In summary, our genetic study revealed a complex pattern of inheritance for HTG and diabetes and mapped significant QTLs on chromosome 1 for HTG and on chromosome 4 for hyperglycemia in TH mice.

1. Introduction

Type 2 diabetes mellitus (T2DM) is characterized by decreased insulin action and secretion and increased hepatic glucose production (111). T2DM is associated with a variety of risk factors for cardiovascular disease, including elevated triglycerides and small, dense low-density lipoprotein cholesterol levels, and decreased high-density lipoprotein cholesterol levels and obesity. This dyslipidemia is often found before the onset of diabetes in patients (112). Hypertriglyceridemia (HTG) is recognized as a major dyslipidemic feature present in type 2 diabetic patients and as an independent risk factor for coronary artery disease (111).

The importance of the genetic control of lipid metabolism has been demonstrated through epidemiological studies illustrating that 30 to 45% of the phenotypic variance for lipid and lipoprotein phenotypes is accounted by genes whereas environmental factors account for less than 15% of the variance (113). Indeed, many studies have identified the

genomic regions or the candidate genes that affect HTG traits in humans on chromosomes 2, 6, 7, 11, and 15 (114-116), in rat on chromosomes 2, 4, 6, 8, and 17 (117-121), and in mouse on chromosomes 4, 8, 9, 11, 12, and 19 (74,77,122-124) using quantitative trait locus (QTL) mapping.

TALLYHO/JngJ (TH) mice are a newly established inbred polygenic model for T2DM. TH mice are characterized by insulin resistance, hyperinsulinemia, diabetes, obesity and dyslipidemia (increased TG, free fatty acids (FFA) and cholesterol levels) (97). Our longitudinal metabolic study revealed that TH male mice, but not females, rapidly developed HTG at a very young age before the onset of overt diabetes. The HTG was accompanied by hyperinsulinemia, but preceded glucose intolerance (97).

The objective of this study was to identify the underlying genetic susceptibility loci responsible for the HTG in the TH mice. Dissecting the existing genetic variation into loci and mapping them to chromosomal regions are the initial steps for the ultimate identification of genetic factors and their molecular bases. Therefore, the present study will provide valuable information for understanding the pathogenic mechanism of diabetic dyslipidemia and for finding the therapeutic targets.

2. Materials and Methods

Animals: All mice were allowed free access to food and water in a temperature- and humidity-controlled room with a 12:12-h light-dark cycle. Mice were fed a standard rodent chow [4% fat, Harlan Teklad Rodent Diet (W) 8604, Harlan Teklad; Madison, WI].

All animal studies were carried out with the approval of The University of Tennessee Animal Care and Use Committee.

Genetic Crosses: Hypertriglyceridemic male TH mice were mated to normal female C57BL/6J (B6) mice. The resulting F1 hybrid mice were interbred to produce an F2 population in which the genomes of the parental strains are reshuffled by recombination resulting in segregation of HTG and related traits. Since female TH mice do not develop severe HTG only male F2 mice were used for mapping the genetic susceptibility loci for HTG (97).

Phenotype analysis: Blood was collected from the retro-orbital plexus using heparinized microcapillary tubes (no.22-362-566, Fisher Scientific; Pittsburgh, PA) after 4 hour (7:00 - 11:00 A.M.) fasting at 8, 12, 16, 20, and 24 weeks (wk) of age, and plasma was obtained by centrifugation (1,200 g) at 4 °C. The plasma TG, glucose, total cholesterol and FFA levels were analyzed colorimetrically using commercial assay kits (Sigma no. TR0100, Sigma no. 510, Sigma no. 401, Roche no. 1-383-175, respectively) as per manufacturer's specifications and insulin using RIA kits (no. RI-13K, Linco Research; St. Charles, MO). At the age of 24 wk, mice were euthanized by CO₂ asphyxiation and five white fat pads [inguinal, epididymal, mesenteric, retroperitoneal (including perirenal), and subscapular] and brown fat pad were dissected and weighed.

Genotyping: Genomic DNA was prepared from tail tips using proteinase K and two series of salt precipitation steps (125). The DNA was amplified by PCR using 67 simple sequence length polymorphic (SSLP) markers (MapPairs, Invitrogen; Carlsbad, CA) (Table 10), covering the 19 autosomal chromosomes. The thermal cycle consisted of 94°C for 2 min followed by 49 cycles of 94°C (20 s), 50°C (30 s), and 72°C (40 s) and

Table 10. Simple sequence length polymorphic markers used for genome scan of F2 mice from an intercross of F1(C57BL/6J x TALLYHO/JngJ) mice.

Marker	cM	Marker	cM
Chromosome 1		D9Mit212	61.0
D1Mit213	25.7	Chromosome 10	
D1Mit215	47.0	D10Mit80	4.0
D1Mit26	62.1	D10Mit130	31.5
D1Mit113	93.9	D10Mit11	50.0
D1Mit206	95.8	Chromosome 11	
Chromosome 2		D11Mit20	20.0
D2Mit1	1.0	D11Mit86	28.0
D2Mit296	18.0	D11Mit41	49.0
D2Mit56	41.0	D11Mit132	58.0
D2Mit17	69.0	Chromosome 12	
D2Mit22	84.0	D12Mit83	6.0
D2Mit200	107.0	D12Mit34	29.0
Chromosome 3		D12Mit233	52.0
D3Mit304	5.6	Chromosome 13	
D3Mit40	39.7	D13Mit205	5.0
D3Mit106	55.0	D13Mit35	75.0
Chromosome 4		D13Mit26	38.0
D4Mit97	6.3	D13Mit148	59.0
D4Mit178	35.5	Chromosome 14	
D4Mit37	56.5	D14Mit53	12.5
D4Mit312	69.8	D14Mit102	28.3
D4Mit42	81.0	D14Mit107	63.0
D4Mit208	81.5	Chromosome 15	
Chromosome 5		D15Mit174	6.7
D5Mit193	1.0	D15MitNDS2	
D5Mit20	52.0	D15Mit2	46.9
D5Mit101	81.0	Chromosome 16	
Chromosome 6		D16Mit181	4.3
D6Mit93	26.29	D16Mit152	57.0
D6Mit29	36.5	D16Mit4	27.3
D6Mit339	65.5	Chromosome 17	
Chromosome 7		D17Mit133	10.4
D7Mit306	1.7	D17Mit123	56.7
D7Mit231	27.8	D17Mit54	38.5
D7Mit109	66.0	Chromosome 18	
Chromosome 8		D18Mit68	11.0
D8Mit95	8.0	D18Mit47	50.0
D8Mit339	23.0	D18Mit53	27.0
D8Mit242	47.0	Chromosome 19	
Chromosome 9		D19Mit71	54.0
D9Mit323	6.0	D19Mit66	41.0
D9Mit142	31.0	D19Mit30	20.0

cM, centimorgan; The genetic positions of marker loci were based on Mouse Genome Database map locations.

final extension at 72°C (7 min). Amplified products were electrophoretically separated on 3% metaphor (no. 50184, FMC; Rockland ME)/1% agarose (no. 0710-500G, Amresco; Solon, OH) gels in 0.5 x Tris-borate-EDTA buffer, pH 7.4. The DNA was visualized by ethidium bromide (no. E-1510, Sigma, St, Louis, MO) staining (126).

QTL analysis: QTL analysis was performed using the computer program, Map Manager QT (www.mapmanager.org/mmQTX.html). We used two main functions of Map Manager QT for identifying and locating possible quantitative trait loci (<http://www.mapmanager.org/mmQT.html#anchor1452245>). We analyzed genetic markers to identify those which are significantly associated with the trait of interest according to simple regression analysis. We also searched analyzed markers with an interval regression method to map quantitative traits within the intervals defined by the markers (127). In addition, we conducted permutation test to assess the statistical significance of any putative QTL (128). The permutation test estimates an empirical genome-wide probability for given likelihood ratio statistics (LRS) generated by interval mapping (129). Significance was determined by 1,000 permutations to provide LRS that were suggestive, significant or highly significant. The suggestive, significant, and highly significant correspond to the 90th, 95th and 99.9th percentiles (128).

For each QTL, we determined the allelic effect by calculating the phenotype mean for each of the three possible genotypes (homozygous for TH allele, homozygous for B6 allele and heterozygous). We then determined qualitatively which strain contributed the allele that increased phenotypic values and inferred whether that allele caused dominant, additive, or recessive inheritance of the phenotype of interest. A dominant allele was defined as exhibition of a high level by the heterozygous genotype indistinguishable from

the high-phenotype homozygous genotype, whereas a recessive allele was defined as the heterozygous genotype being indistinguishable from the low-phenotype homozygous genotype. An additive allele was defined as an allele that produced an intermediate phenotype between each of the homozygous-low and homozygous-high genotypes (79). Statistical differences in trait values among parental strains and among genotypes in F2 were analyzed by ANOVA with StatView 5.0 (Abacus Concepts, Berkeley, CA). All data are presented as mean $\pm$ SEM.

3. Results

Phenotypic characteristics of parental strains and F1(B6 x TH) mice.

TH mice had heavier body weights and fat pad weights than B6 mice (Table 11). The body weights of F1(B6 x TH) mice were similar to the TH mice, but significantly higher than B6 mice (Table 11). On the other hand, fat pad weights of F1 mice were significantly higher than TH mice (except mesenteric) as well as B6 mice (Table 11).

The mean plasma glucose, TG, total cholesterol, and insulin levels in the TH mice were all significantly higher than B6 or F1 mice (except for plasma insulin levels) (Table 11). The plasma glucose levels of F1 mice were similar to those of B6 mice, suggesting recessive inheritance of this trait. On the other hand, the plasma TG levels of F1 mice were intermediate between the means of the two parental strains (Table 11) semidominance, suggesting that the trait was controlled by both B6 and TH alleles (131). Total plasma cholesterol levels of F1 mice were not significantly different from B6 at 8 wk - 16 wk, but became significantly higher at 20 wk and 24 wk. The plasma insulin

Table 11. Phenotypic characteristics of parental strains and F1 progeny (males).

Phenotype	B6	TH	F1	P-value		
				B6 vs TH	B6 vs F1	TH vs F1
Body weight (g)						
8 week	22.1 ±0.4 (14)	30.8±0.5(7)	30.0±0.5(15)	<0.0001	<0.0001	NS
12 week	23.9±0.5(18)	31.8±0.6(16)	33.4±0.6(19)	<0.0001	<0.0001	Ns
16 week	25.4±0.5(18)	34.4±0.8(16)	36.2±0.7(19)	<0.0001	<0.0001	NS
20 week	26.8±0.5(18)	35.9±1.0(16)	38.3±0.7(19)	<0.0001	<0.0001	NS
24 week	27.5±0.4(18)	35.0±1.5(16)	40.0±0.7(19)	<0.0001	<0.0001	0.0029
Plasma glucose (mg/dl)						
8 week	174.6±9.9(9)	292.1±47.6(7)	174.6±11.3(15)	0.0019	NS	<0.0001
12 week	132.1±6.4(14)	252.1±39.6(9)	146.7±8.6(19)	<0.0001	NS	<0.0001
16 week	181.3±13.7(19)	460.5±37.9(16)	166.4±4.2(19)	<0.0001	NS	<0.0001
20 week	177.3±10.4(18)	363.8±35.8(16)	156.1±5.0(19)	<0.0001	NS	<0.0001
24 week	174.6±9.7(18)	387.5±32.6(16)	162.3±8.3(19)	<0.0001	NS	<0.0001
Plasma triglyceride (mg/dl)						
8 week	62.7±12.2(9)	437.6±41.0(7)	153.0±11.8(15)	<0.0001	0.0005	<0.0001
12 week	68.2±7.7(14)	236.6±53.5(9)	186.1±10.7(19)	<0.0001	<0.0001	0.0484
16 week	63.0±2.6(18)	437.9±38.7(16)	168.3±8.2(19)	<0.0001	<0.0001	<0.0001
20 week	70.6±7.4(18)	367.2±31.7(16)	179.8±21.0(19)	<0.0001	<0.0001	<0.0001
24 week	59.7±5.7(18)	367.8±32.8(16)	221.2±18.6(19)	<0.0001	<0.0001	<0.0001
Plasma total cholesterol (mmol/l)						
8 week	2.9±0.2(9)	4.0±0.1(7)	2.3±0.1(15)	<0.0001	NS	<0.0001
12 week	2.6±0.2(14)	2.9±0.1(9)	3.1±0.2(19)	NS	NS	NS
16 week	3.0±0.3(19)	3.6±0.3(16)	2.6±0.1(19)	0.0478	NS	0.0002
20 week	2.1±0.1(18)	4.8±0.3(16)	3.5±0.3(19)	<0.0001	0.0001	<0.0001
24 week	1.9±0.1(18)	4.0±0.4(16)	3.6±0.1(19)	<0.0001	0.0002	NS
Plasma free fatty acid (mmol/l)						
8 week	0.8±0.07(9)	0.8±0.07(7)	0.6±0.03(15)	NS	NS	NS
12 week	0.9±0.09(14)	0.5±0.04(9)	0.7±0.08(19)	0.0014	NS	NS
16 week	0.6±0.07(18)	0.8±0.09(16)	0.5±0.03(19)	NS	NS	NS
20 week	0.6±0.09(18)	0.4±0.06(16)	0.5±0.09(19)	NS	NS	NS
24 week	0.7±0.08(18)	0.8±0.10(16)	0.6±0.06(19)	NS	NS	NS
Plasma insulin (ng/ml)						
8 week	0.07±0.02(9)	1.8±0.41(7)	1.0±0.17(15)	<0.0001	0.0209	0.0446
12 week	0.2±0.07(13)	1.0±0.33(9)	1.1±0.15(19)	0.0112	0.0298	NS
16 week	0.4±0.09(18)	1.0±0.26(15)	1.2±0.13(19)	0.0112	NS	NS
20 week	0.4±0.11(18)	1.6±0.41(16)	2.1±0.26(19)	0.0058	0.0201	NS
24 week	0.4±0.10(10)	2.3±0.56(13)	2.8±0.55(16)	0.0186	0.0390	NS
Fat pad and carcass weights (g, 24wk)						
Ing	0.34±0.02	0.69±0.15	1.40±0.07	0.0190	<0.0001	<0.0001
Epi	0.43±0.03	0.98±0.23	1.86±0.07	0.0048	<0.0001	<0.0001
Mes	0.11±0.01	0.24±0.06	0.11±0.03	0.0399	<0.0001	<0.0001
Retro	0.12±0.06	0.26±0.06	0.64±0.02	0.0294	<0.0001	<0.0001
Subsc	0.14±0.01	0.32±0.09	0.72±0.04	NS	<0.0001	0.0002
TF	1.14±0.06	2.34±0.55	5.17±0.20	0.0217	<0.0001	<0.0001
BF	0.07±0.003	0.22±0.02	0.23±0.01	<0.0001	<0.0001	NS
Carcass	25.52±0.37	30.84±1.05	33.63±0.48	<0.0001	<0.0001	0.0223

Values represent mean ±SEM; NS, not significant. The number of mice used for the tests is provided in the parentheses. The P-values were generated by the Student's t-test. Ing, inginal fat pad; Retro, retroperitoneal (including perirenal) fat pad; Epi, epididymal fat pad; Mes, mesenteric fat pad; Subsc, subscapular fat pad; TF, total fat pad [the sum of inguinal, epididymal, mesenteric, retroperitoneal (including perirenal), and subscapular fat pad weights]; Carcass, body weight without the total fat pad.

levels of F1 mice were significantly higher than those of the B6 mice (except at 16 wk) (Table 11).

No significant differences in plasma free fatty acid levels were found among the parentals and F1 mice (Table 11).

Phenotypic characteristics of F2 intercross mice.

The F2 mice showed a wide distribution pattern of the plasma glucose, TG, and cholesterol levels at all ages determined, indicating polygenic characteristics of the traits (Figures 4-10).

QTLs controlling plasma TG levels.

We found significant linkages for HTG at D1Mit113 on chromosome 1 and at D8Mit242 on chromosome 8, explaining 16.3% and 18.8% phenotypic variance of the trait, respectively (Table 13). Homozygous mice for TH alleles exhibited significantly higher plasma TG levels than mice homozygous for B6 and heterozygous at these loci. Also, the marker D10Mit11 exhibited a highly significant linkage with plasma TG levels at 16 wk and explained 30.6% phenotypic variance of the trait (Table 12). Even though TH is the HTG-susceptible strain, the heterozygotes showed significantly higher plasma TG levels than mice homozygous for the TH allele at this locus (Table 12).

QTLs controlling for plasma glucose levels.

A highly significant linkage for plasma glucose levels (at 20 wk) was found at marker D4Mit178 on chromosome 4, accounting for 23% of the phenotypic variance of

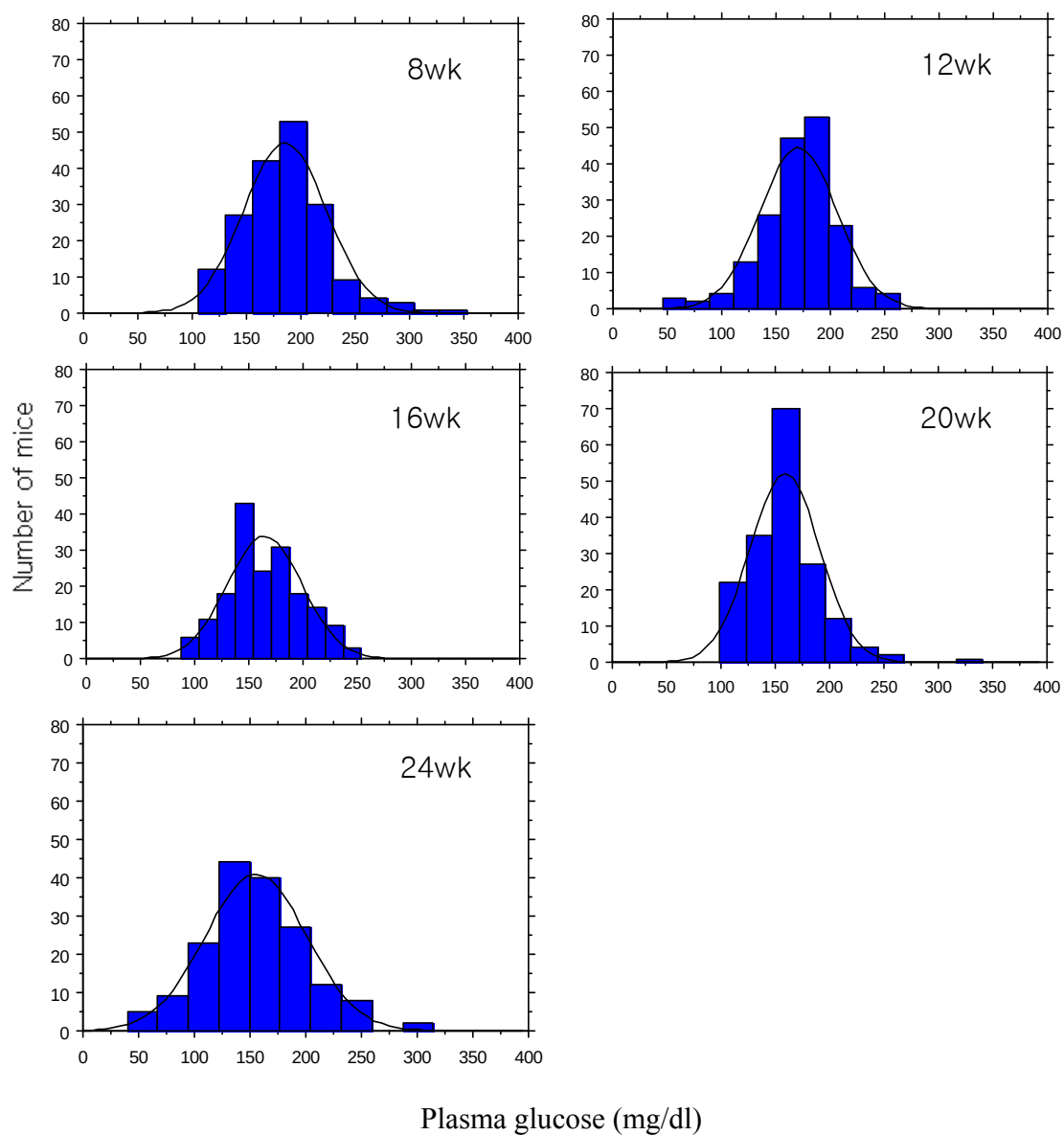


Figure 4. Histogram for plasma glucose levels in F2 male mice (n=182) from an intercross of F1(B6 x TH) mice. Mice were fasted for 4 hours (7:00 – 11:00 A.M.), blood was collected and plasma glucose levels were then measured.

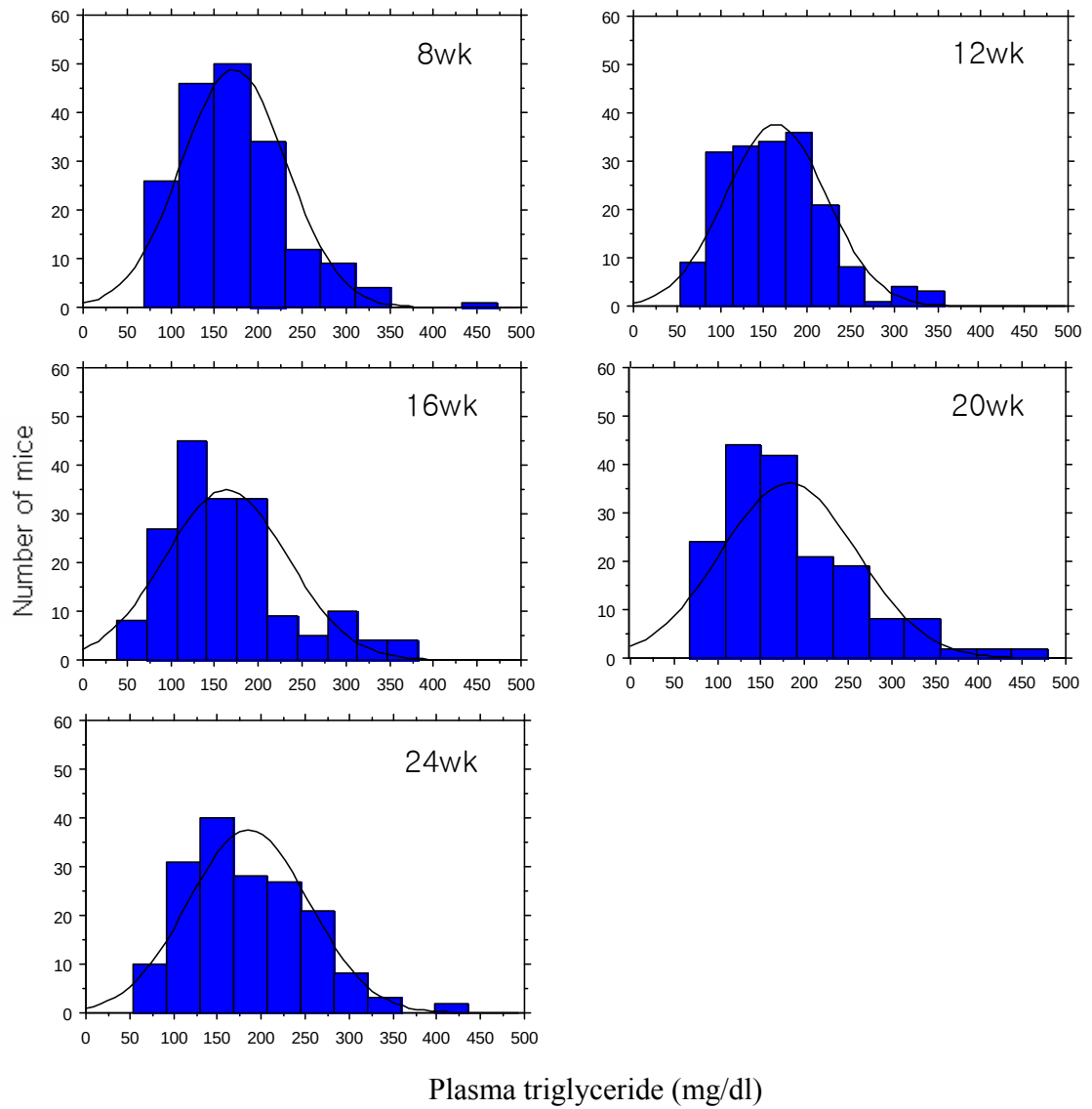


Figure 5. Histogram for plasma triglyceride levels in F2 male mice (n=182) from an intercross of F1(B6 x TH) mice. Mice were fasted for 4 hours (7:00 – 11:00 A.M.), blood was collected and plasma triglyceride levels were then measured.

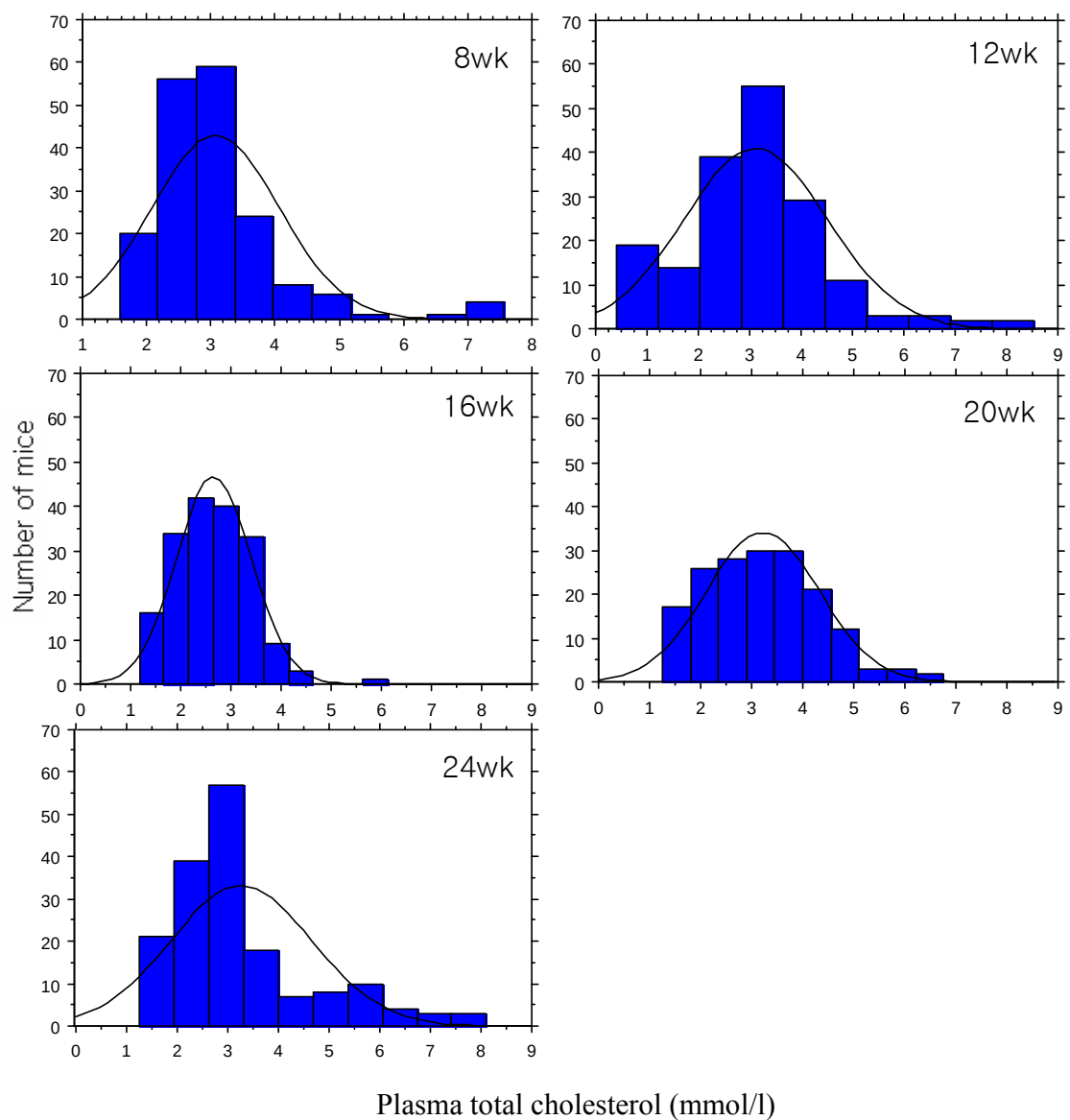


Figure 6. Histogram for plasma total cholesterol levels in F2 male mice (n=182) from an intercross of F1(B6 x TH) mice. Mice were fasted for 4 hours (7:00 – 11:00 A.M.), blood was collected and plasma total cholesterol levels were then measured.

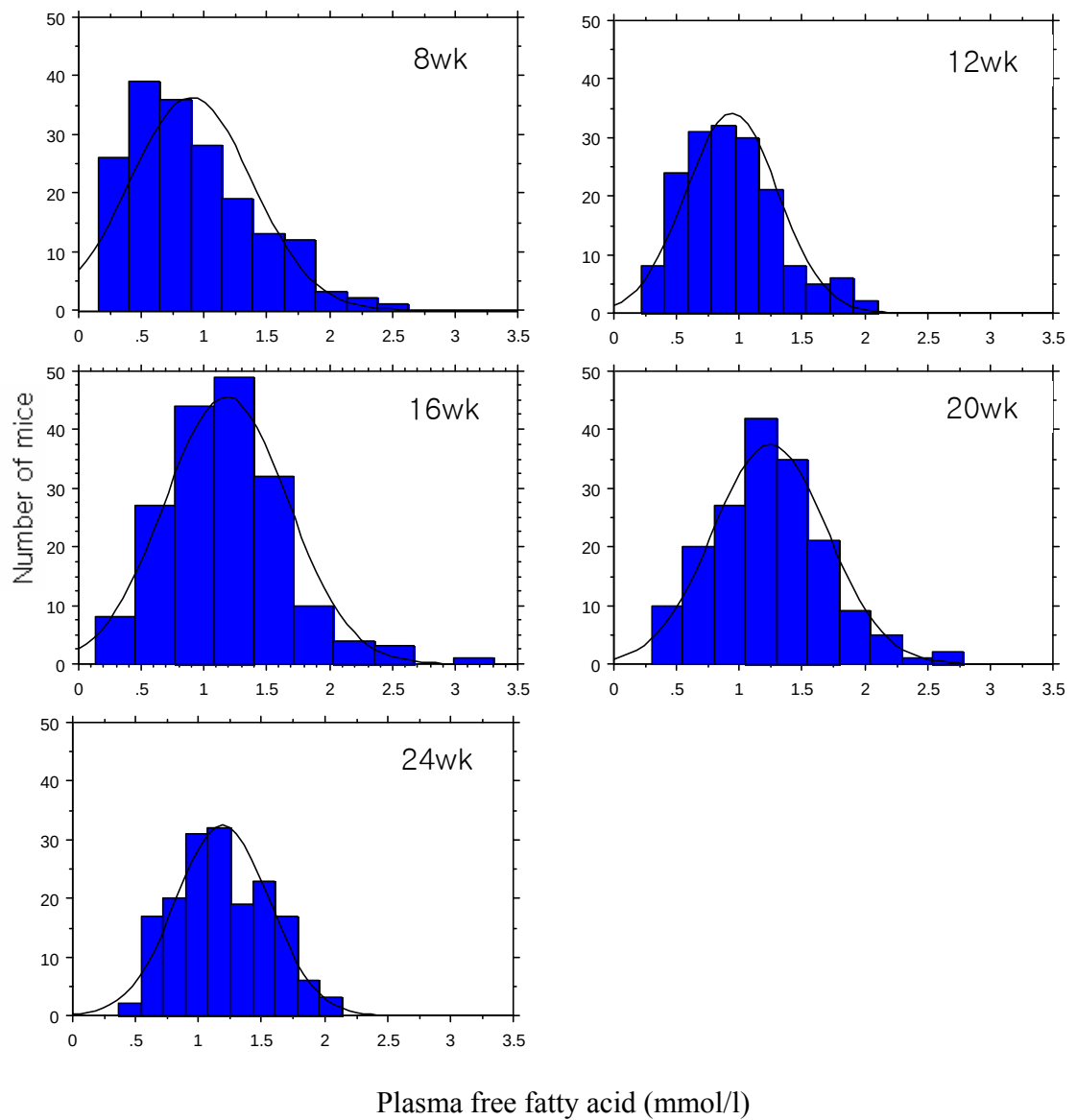


Figure 7. Histogram for plasma free fatty acid level in F2 male mice (n=182) from an intercross of F1(B6 x TH) mice. Mice were fasted for 4 hours (7:00 – 11:00 A.M.), blood was collected and plasma free fatty acid levels were then measured.

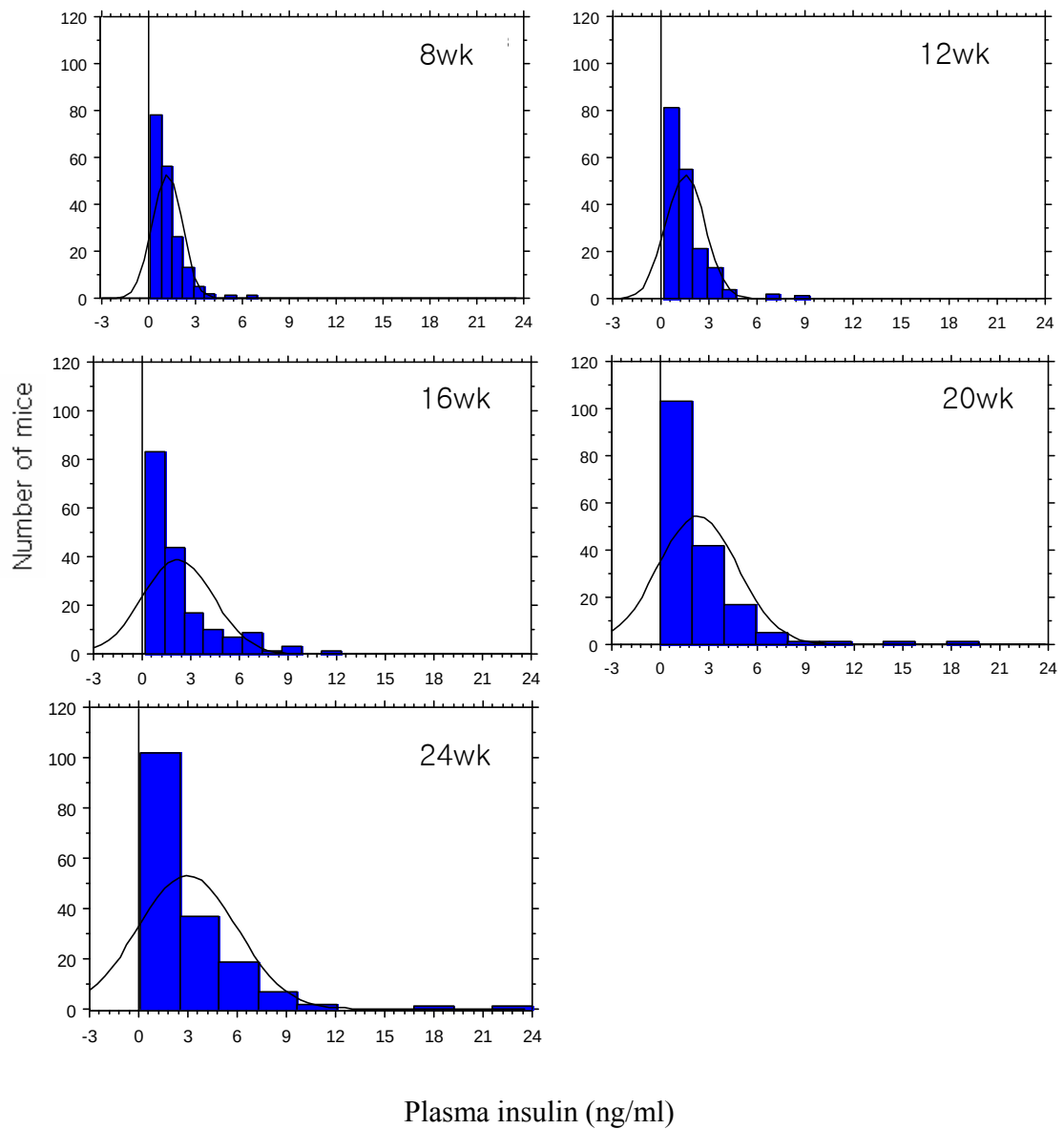


Figure 8. Histogram for plasma insulin levels in F2 male mice (n=182) from an intercross of F1(B6 x TH) mice. Mice were fasted for 4 hours (7:00 – 11:00 A.M.), blood was collected and plasma insulin levels were then measured.

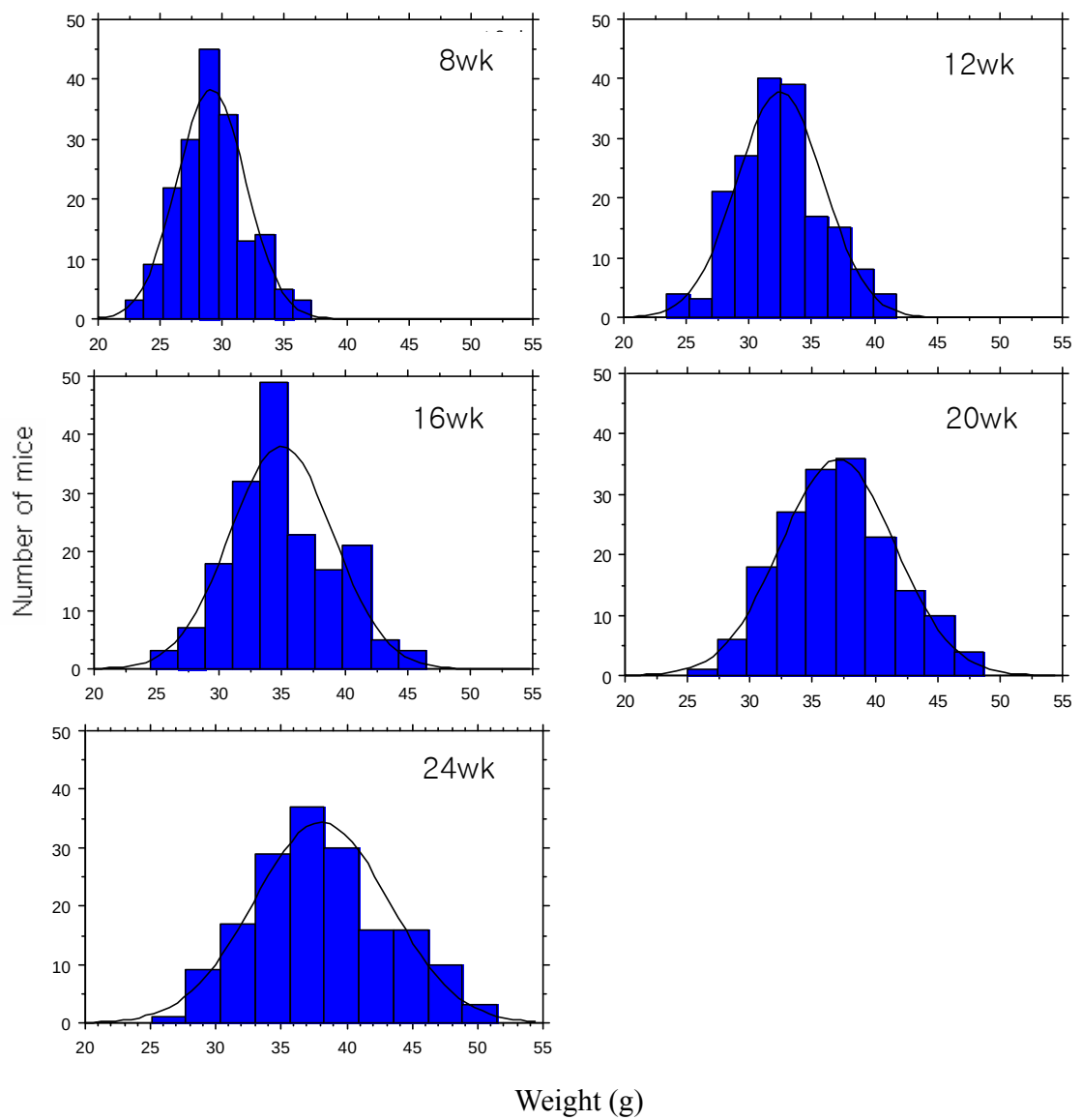


Figure 9. Histogram for body weight in F2 male mice (n=182) from an intercross of F1(B6 x TH) mice.

Figure 10. Histogram for fat pad and carcass weights in F2 male mice (n=182) at 24 wks from an intercross of F1(B6 x TH) mice.

Ing, inginal fat pad; Retro, retroperitoneal (including perirenal) fat pad; Epi, epididymal fat pad; Mes, mesenteric fat pad; Subsc, subscapular fat pad; Total fat pad, the sum of inguinal, epididymal, mesenteric, retroperitoneal (including perirenal), and subscapular fat pad weights; Carcass, body weight without the total fat pad.

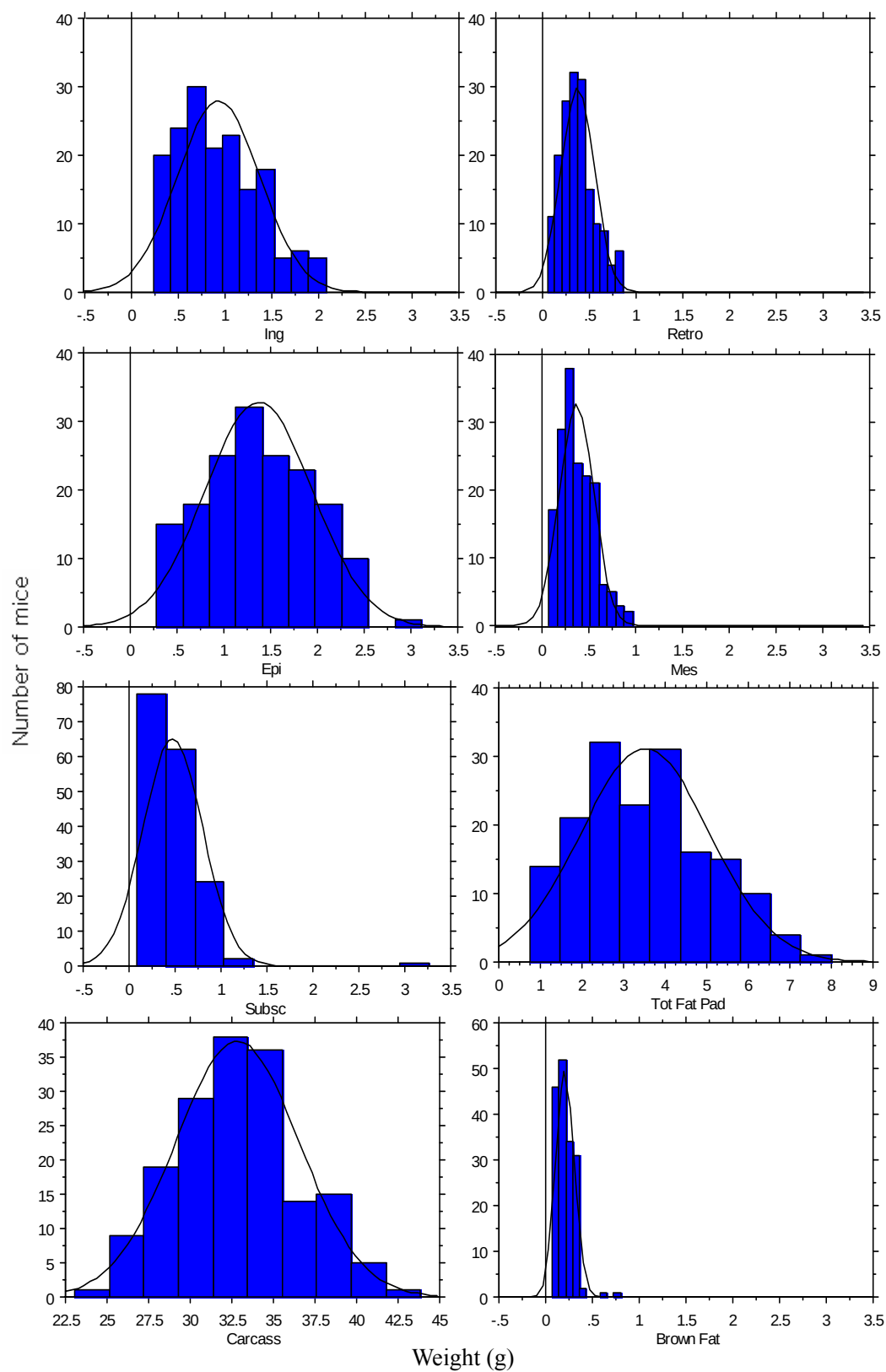


Table 12. Highly significant QTLs in F2 mice from an intercross of F1(B6 x TH) mice (males).

Marker (cM)	Trait	Wk	%	Phenotype values			p value		
				B/B	H (B/T)	T/T	B vs H	B vs T	T vs H
Chr 4									
D4Mit178(35.5)	Glu	20	23	145.0±3.3(36)	157.3±2.7(99)	177.8±7.6(36)	0.0377	<.0001	0.0007
Chr 10									
D10Mit11 (50)	TG	16	30	143.3±6.5(73)	209.6±12.0(43)	141.3±10.3(40)	<.0001	NS	<.0001
Data are presented as mean ± SE; Chr, chromosome; Glu, plasma glucose levels (mg/dl); TG, plasma triglyceride levels (mg/dl); wk, week; %, phenotypic variance explained; B, B6 allele; T, TALLYHO allele; NS, not significant.									

Table 13. Significant QTLs in F2 mice from an intercross of F1(B6 x TH) mice (males).

Marker (cM)	Trait	Wk	%	Phenotype values			p value		
				B/B	H (B/T)	T/T	B vs H	B vs T	T vs H
Chr 1									
D1Mit215 (47)	Ing	24	18	0.7±0.1(45)	1.0±0.1	1.1±0.1(40)	0.0057	<.0001	0.0360
	Epi	24	18.3	1.1±0.1(45)	1.4±0.1(80)	1.7±0.1(40)	0.0143	<.0001	0.0126
	Retro	24	16.6	0.3±0.02(44)	0.4±0.02(80)	0.5±0.03(40)	0.0090	<.0001	0.0428
	TF	24	17.3	2.8±0.2(45)	3.6±0.2(80)	4.2±0.2(40)	0.0026	<.0001	0.0339
	BF	24	17.9	0.17±0.01(44)	0.21±0.01(80)	0.24±0.01(40)	0.0027	<.0001	0.0312
D1Mit26(62.1)	Mes	24	17.6	0.3±0.02(45)	0.4±0.02(80)	0.40±0.03(40)	0.0014	<.0001	NS
D1Mit113 (93.9)	TG	20	16.3	152.8±8.1(44)	182.9±8.4(86)	226.7±15(35)	0.0311	<.0001	0.0040
	Chol	16	22.9	2.4±0.1(47)	2.7±0.1(88)	3.2±0.1(36)	NS	<.0001	0.0002
	Chol	20	19.2	2.8±0.2(44)	3.1±0.1(86)	4.0±0.2(35)	NS	<.0001	0.0001
	Ing	24	15.2	0.8±0.1(43)	0.9±0.1(82)	1.2±0.1(35)	NS	0.0001	0.0052
	BF	24	14.2	0.2±0.01(43)	0.2±0.01(81)	0.3±0.03(35)	NS	0.0004	0.0026
Chr 4									
D4Mit37(56.5)	Glu	24	19.8	129.9±7.3(36)	167.2±4.2(101)	150.9±8.0(31)	<.0001	0.0472	NS
Chr 8									
D8Mit242(47)	TG	24	18.8	156.3±8.1(44)	184.8±7.3(85)	221.0±12.4(37)	0.0207	<.0001	0.0057
Chr 10									
D10Mit11(50)	Chol	16	15.2*	2.6±0.1(73)	3.0±0.1(43)	2.4±0.1(40)	0.0031	NS	0.0002

Data are presented as mean ± SE; Chr, chromosome; WT, weight (g); Chol, plasma cholesterol levels (mmol/l); Glu, plasma glucose levels (mg/dl); TG, plasma triglyceride levels (mg/dl); FFA, plasma free fatty acid levels (mmol/l); INS, plasma insulin levels (ng/ml); Ing, inguinal fat pad (g); Epi, epididymal fat pad (g); Mes, mesenteric fat pad (g); Retro, retroperitoneal (including perirenal) fat pad (g); BF, brown fat pad (g); TF, total fat pad (sum of inguinal, epididymal, mesenteric, retroperitoneal (including perirenal) and subscapular fat pad weights) (g); Carc, carcass weight (Body weight without the total fat pad) (g); wk, week; %, phenotypic variance explained; B, B6 allele; T, TALLYHO allele; NS, not significant.

the trait (Table 12). At this marker, the mean plasma glucose levels in F2 mice homozygous for TH allele was significantly higher than that of mice homozygous for B6 allele and heterozygous. The marker D4Mit37 also showed a linkage for plasma glucose levels at 24wk, accounting for 20% of the phenotypic variance of the trait (Table 13). At this marker, the mean plasma glucose levels in F2 mice homozygous for TH allele was significantly higher than that of mice homozygous for B6, but not that of heterozygous mice (Table 13).

QTLs controlling for plasma total cholesterol levels.

A significant linkage for the plasma total cholesterol levels was detected at the marker D1Mit113 on chromosome 1, accounting for 22.9% and 19.2% of the phenotypic variance of the trait at 16 and 20 wk, respectively (Table 13). Also, the marker D10Mit11 showed a significant linkage for plasma cholesterol levels and accounted for 15.2% of phenotypic variance of the trait at 16 wk. Even though TH is the susceptible strain, the heterozygotes showed significantly higher plasma cholesterol levels than mice homozygous for the TH allele at this locus (Table 13).

QTLs controlling for various fat pads weights.

At 24 wks of age, a significant linkage for fat pad weights was detected at marker D1Mit215 on chromosome 1, accounting for 11-18% of phenotypic variance of the trait (Table 13). At this locus, F2 mice homozygous for TH allele had significantly higher fat pad weights than homozygous mice for B6 allele and heterozygous mice. The marker D1Mit113 also shows a linkage for fat pad weights although the effect was smaller than

at D1Mit215. This QTL explained 9.4-15.2% of phenotypic variance of the trait and mice homozygous for TH allele had significantly higher fat pad weights than homozygous mice for B6 allele and heterozygous mice (Table 13).

4. Discussion

Abnormal lipid metabolism, or dyslipidemia, often precedes the onset of diabetes in T2DM patients. HTG is the most commonly observed form of dyslipidemia and appears to adversely influence insulin action, becoming a risk factor for the development of T2DM or T2DM complications (132).

The TH mouse is a model of T2DM, exhibiting insulin resistance, hyperinsulinemia, hyperglycemia, obesity, and HTG. Multiple QTLs associated with obesity and hyperglycemia in backcross male progeny obtained from a cross between F1(B6xTH) and TH mice were previously identified (97). In this study, QTL analysis was conducted for plasma lipid levels including TG, cholesterol, and FFA in F2 male mice generated from an intercross of F1(B6xTH) mice. Plasma glucose and insulin levels and fat pad and body weights were also included in this analysis. All QTLs detected in this study are summarized in Tables 12-14.

For HTG, two significant QTLs were identified on chromosomes 1 (at marker D1Mit113) and 8 (at marker D8Mit242). QTLs linked to lipid and lipoprotein metabolism have been previously reported in the chromosome 1 region, and these include lipoprotein QTL 3 (Lprq3) found in NZB/BINJ x SM/J cross (133), triglycerides (Trglyd)

Table 14. Suggestive QTLs in F2 mice from an intercross of F1(B6 x TH) mice (males).

Marker (cM)	Trait	Wk	%	Phenotype			p value		
				B/B	H (B/T)	T/T	B vs H	B vs T	T vs H
Chr 1									
D1Mit213 (25.7)	Epi	24	12.2	1.2±0.1(37)	1.3±0.1(87)	1.6±0.1(37)	NS	0.001	0.005
	Mes	24	12	0.3±0.02(37)	0.4±0.02(87)	0.5±0.03(37)	NS	0.0007	0.0184
	BF	24	10.5	0.2±0.01(36)	0.2±0.01(36)	0.2±0.01(37)	NS	0.0015	0.0323
D1Mit215 (47)	Chol	16	10.5	2.5±0.1(50)	2.7±0.1(86)	3.0±0.1(40)	NS	0.0013	0.283
	Subsc	24	11.8	0.3±0.03(45)	0.5±0.05(80)	0.6±0.03(40)	0.0049	0.0016	NS
D1Mit26 (62.1)	Epi	24	10.1	1.2±0.1(42)	1.3±0.1(77)	1.6±0.1(44)	NS	0.0034	0.0091
	BF	24	13.9	0.2±0.01(42)	0.2±0.01(76)	0.2±0.01(44)	0.0053	0.0003	NS
D1Mit113 (93.9)	Glu	16	9.2	166.0±4.7(47)	160.0±3.5(88)	180.2±6.2(36)	NS	NS	0.0027
		20	10.2	153.3±4.0(44)	157.4±3.1(86)	174.0±7.1(36)	NS	0.0040	0.0087
	TG	16	8.7	141.4±8.6(47)	168.7±7.3(88)	185.0±14(36)	0.030	0.0049	NS
		24	11.8	169.5±8.5(44)	180.9±7.1(84)	220.0±14(35)	NS	0.0012	0.0045
		12	14.3	2.5±0.2(46)	3.2±0.1(88)	3.7±0.2(36)	0.0029	0.0001	NS
	WT	16	10.8	33.8±0.6(47)	53.1±0.4(88)	36.7±0.8(36)	NS	0.0011	0.0399
	Epi	24	9.4	1.3±0.1(43)	1.4±0.1(82)	1.6±0.1(35)	NS	0.0035	0.0110
	Mes	24	10.1	0.3±0.02(42)	0.4±0.02(82)	0.5±0.03(35)	NS	0.0017	NS
	Retro	24	10.1	0.3±0.02(42)	0.4±0.02(82)	0.5±0.03(35)	NS	0.0029	0.0076
	TF	24	9.4	3.1±0.2(43)	3.5±0.2(82)	4.3±0.3(35)	NS	0.0007	0.0132
Chr 2									
D2Mit1(1)	BF	24	10.4	0.20±0.01(32)	0.22±0.01(86)	0.17±0.01(46)	NS	NS	0.0015
D2Mit296 (18)	INS	16	11.1	2.7±0.4(33)	1.9±0.2(89)	2.4±0.4(46)	NS	NS	NS
	WT	8	10.2	28.8±0.41(33)	28.7±0.3(90)	30.2±0.5(47)	NS	0.0272	0.0021
D2Mit56(41)	Glu	16	9.6	176.6±5.6(39)	156.7±3.9(81)	166.5±4.2(44)	0.0026	NS	NS
D2Mit200 (107)	Glu	20	10.8	172.2±6.3(36)	153.5±3.4(78)	158.4±3.9(57)	0.0037	0.0408	NS
	BF	24	10.5	0.24±0.02(36)	0.18±0.01(73)	0.21±0.01(55)	0.0018	NS	NS
Chr 4									
D4Mit97(6.3)	TG	20	10.5	162.3±8.8(41)	174.5±7.9(85)	211.1±15.8(36)	NS	0.0045	0.0142
D4Mit178(35.5)	TG	20	17.5	148.9±89(36)	181.4±7.3(98)	219.7±16.3(36)	0.0282	<.0001	0.0099
	Glu	8	12.8	166.6±5.5(38)	190.1±3.5(108)	193.4±7.4(34)	0.001	0.0026	NS
16		10.4	159.2±5.6(37)	161.4±3.2(105)	181.6±6.2(34)	NS	0.0059	0.0028	
20		14.9	143.5±3.7(37)	160.8±3.3(102)	171.9±5.4(32)	0.0039	0.0002	NS	
D4Mit312(69.8)	TG	24	9.3	176.3±11.1(36)	179.2±6.5(101)	219.5±13.9(31)	NS	0.0098	0.0042
		Glu	8	10.9	173.5±4.1(47)	186.3±4.0(95)	201.5±7.4(33)	NS	0.0011
D4Mit208(81.5)	Glu	8	11.6	172.5±4.8(47)	188.2±4.3(91)	202.1±6.0(31)	0.0227	0.0009	NS
Chr 8									
D8Mit339(23)	TG	20	10.4	162.3±8.1(63)	188.8±10.5(58)	202.7±13.3(46)	NS	0.008	NS
D8Mit242(47)	TG	20	13	152.0±6.8(44)	182.7±8.5(84)	212.4±15.8(38)	0.0314	0.0004	0.0471
Chr 10									
D10Mit130(31.5)	Chol	12	10	2.6±0.2(53)	3.4±0.2(75)	3.3±0.2(41)	0.0032	0.0188	NS
D10Mit11 (50)	TG	20	12.6	167.2±7.7(70)	221.5±13.8(41)	164.1±13.2(39)	0.0004	NS	0.001
		20	11.4	2.9±0.1(70)	3.7±0.2(41)	3.2±0.2(39)	0.0005	NS	0.0457
		24	10.1	3.7±0.2(71)	2.9±0.2(41)	3.1±0.2(39)	0.0033	0.0248	NS
	FFA	20	11	1.4±0.1(70)	1.1±0.1(41)	1.1±0.1(39)	0.0019	0.0102	NS
		INS	12	9.6	1.3±0.1(73)	2.1±0.3(43)	1.5±0.2(39)	0.0018	NS
Chr 11									
D11Mit20 (20)	FFA	20	10.7	1.1±0.1(49)	1.3±0.1(71)	1.4±0.1(44)	0.0161	0.0029	NS
	BF	24	10.8	0.2±0.01(45)	0.2±0.01(69)	0.2±0.02(40)	0.0153	0.0018	NS
D11Mit86 (28)	WT	16	9.8	33.5±0.6(49)	35.9±0.5(73)	35.2±0.6(48)	0.0021	0.0386	NS
	WT	20	13.7	35.1±0.6(49)	38.1±0.5(71)	37.6±0.7(45)	0.0004	0.0073	NS
	WT	24	14.8	35.8±0.7(48)	39.4±0.6(69)	38.6±0.8(45)	0.0002	0.0066	NS
	Carc	24	15	31.2±0.5(48)	33.9±0.4(68)	33.3±0.6(45)	0.0002	0.006	NS
	BF	24	13.7	0.17±0.01(48)	0.22±0.01(68)	0.23±0.02(44)	0.0017	0.0009	NS

Table 14. Continued.

Marker (cM)	Trait	Wk	%	Phenotype			p value		
				B/B	H (B/T)	T/T	B vs H	B vs T	T vs H
Chr 12									
D12Mit34	WT	8	9.7	30.1±0.4(48)	28.9±0.3(91)	28.4±0.4(36)	0.0138	0.0039	NS
(29)	WT	12	11.1	33.7±0.5(48)	32.4±0.4(91)	31.3±0.6(36)	0.0321	0.0011	0.0246
Chr 13									
D13Mit148(59)	Chol	24	12.9	2.9±0.2(56)	3.7±0.2(77)	3.0±0.2(30)	0.0012	NS	0.0246
Chr 15									
D15Mit2(46.9)	Chol	16	10.7	2.8±0.1(36)	2.8±0.1(85)	2.4±0.1(49)	NS	0.007	0.0032

Data are presented as mean ± SE; Chr, chromosome; WT, weight (g); Chol, plasma cholesterol levels (mmol/l); Glu, plasma glucose levels (mg/dl); TG, plasma triglyceride levels (mg/dl); FFA, plasma free fatty acid levels (mmol/l); INS, plasma insulin levels (ng/ml); Epi, epididymal fat pad (g); Mes, mesenteric fat pad (g); Retro, retroperitoneal fat pad (g); Subsc, subscapular fat pad (g); BF, brown fat pad (g); TF, total fat pad (sum of inguinal, epididymal, mesenteric, retroperitoneal (including perirenal) and subscapular fat pad weights) (g); Carc, carcass weight (Body weight without the total fat pad); wk. week; %, phenotypic variance explained; B, B6 allele; T, TALLYHO allele ; NS, not significant.

in C57BL/6J x RR cross (81), cholesterol QTL 2 (Cq2) in CAST/Ei x 129S1/SvImJ cross (83), plasma cholesterol 1 (Pcho1) in (AKxD) x (129xSJL129F) cross (134), and fasting glucose 2 (Fglu2) in C57BL/6J x KK-A(y) cross mice (134) (Table 15). Also, a QTL associated with plasma TG levels, Triglyceride level 1 (Tgl1), has been reported on chromosome 8 in BALB/c x KK/Ta cross (74) (Table 15). Whether allelic variants at the TH associated HTG QTLs are responsible for the phenotypes linked to these QTLs remains to be determined.

Apolipoprotein AII (Apoa2) gene isolated near the marker D1Mit113. Apoa2 is a major constituent of human HDL (135). Transgenic mice overexpressing Apoa2 gene exhibit several traits associated with T2DM, including glucose intolerance, insulin resistance, HTG, and obesity (136,137). Furthermore, a number of nucleotide substitutions in Apoa2 cDNA has been reported in several inbred strains (138). Three different Apoa2 alleles, Apoa2a, Apoa2b, and Apoa2C, are known on the basis of amino acid substitutions at four residues (135). Analysis with Apoa2 congenic strains revealed that the Apoa2b allele is unique for increased cholesterol among the three Apoa2 alleles, and that the Ala-to-Val substitution at residue 61 may be crucial as far as cholesterol metabolism is concerned (81). In humans, Apoa2 is located in chromosome 1q23, a region that is associated with combined hyperlipidemia, insulin resistance and T2DM (139). These studies suggest the Apoa2 gene as a logical candidate for the HTG QTL on chromosome 1 that remains to be tested.

For plasma glucose levels, we identified a QTL on chromosome 4, that accounted for 23 % of the phenotypic variance (Table 12). Although there was no evidence of linkage for this QTL to obesity in our study, several QTLs associated with obesity have

Table 15. Summary of significant QTLs.

Marker (cM)	Real peak (cM)	Trait	Overlapping QTL (cM) (ref.)
D1Mit213 (26)	25.76	Epi, Mes, BF	Tgq2 (30) (124)
D1Mit215 (47)	47.12	Chol, Ing, Epi, Retro, Subsc, BF	Bw5 (36) (142) Bwtq1 (54.3) (143)
D1Mit26 (62)	62.61	Epi, Mes, BF	Fatq1 (62) (140) Obwq1 (62) (140) Oppq8 (69.1) (140,144)
D1Mit113 (93)	94.51	Glu, TG, Chol, WT, Ing, Epi, Retro, Subsc, BF	Lprq3 (92) (133)
D1Mit206 (96)	94.53	Glu, TG, Chol, WT, Ing, Epi, Mes,TF, BF	Hdlq20 (96) (145) Trglyd (95.8) (81) Cq2 (100) (82) Pcho1 (100) (79) Fglu2 (100) (134)
D2Mit1 (1)	1	BF	Adip10 (2) (140)
D2Mit296 (18)	18.26	INS, WT	Bodw1 (18) (93)
D2Mit56 (41)	41.13	Glu	Nidd5 (34.5) (141)
D2Mit200 (107)	107	Glu, BF	Bwq5 (81.7) (140,146) Bglu1 (87) (89)
D4Mit97 (6.3)	6.44	TG	Bwq1 (6.3) (140) Triglq1 (6.5) (94)
D4Mit178 (36)	35.76	Glu, TG	Bwq9 (24) (140)
D4Mit37 (57)	57.14	Glu, TG	Mob6, 7(49.6) (141) Bwtq2 (55) (142) Nidds (55) (147) Bw7 (59) (142) Tgq3 (58) (75)
D4Mit312 (70)	70.60	Glu	Afw2 (66) (95,140) Afpq2 (66) (95,140) Tafat (69.8) (97) Adip12 (70) (140) Dob1 (70) (140,148)
D4Mit208 (82)	81.5	Glu	
D8Mit339 (23)	23.35	TG	Trigg2 (30) (124)
D8Mit242 (47)	47	TG	Obq16 (48) (75) Tgl1 (51.5) (74)
D10Mit130 (32)	31.92	Chol	Scfpq1 (33) (140,149)
D10Mit11 (50)	50	TG, Chol, FFA, INS	Igf1sl2 (51) (150) Adip15 (52) (140)
D11Mit20 (20)	20.12	FFA, BF	Bw16 (14) (151)
D11Mit86 (28)	28.25	WT, Carc, BF	Bwtq5 (27.8) (142)

Table 15. Continued.

Marker (cM)	Real peak (cM)	Trait	Overlapping QTL (cM) (ref.)
D12Mit34 (29)	29.18	WT	Afpq10 (18) (84,140) Fob2 (19) (140,152) Afw10 (21) (84,140) Adip16 (26) (140)
D13Mit148 (59)	59.91	Chol	Tanidd2 (60) (97)
D15Mit2 (47)	46.9	Chol	Bwtq12 (34) (140)

cM, centimorgan; ref, reference; Tgq2, triglyceride QTL 2; Bw5, body weight QTL 5; Bwtq1, body weight QTL 1; Fatq1, percent fat, subcutaneous and gonadal, QTL 1; Obwq1, obesity and body weight QTL 1; Obq8, obesity QTL 8; Lprq3, lipoprotein QTL 3; Hdlq20, HDL QTL 20; Trglyd, triglycerides; Cq2, cholesterol QTL 2; Pcho1, plasma cholesterol 1; Fglu2, fasting glucose 2; Adip10, adiposity 10; Bwtq8, body weight QTL 8; Bodw1, body weight 1; Nidd5, non-insulin-dependent diabetes mellitus 5; Bwq5, body weight, QTL 5; Bglu1, blood glucose level 1; Bwq1, body weight QTL 1; Trigql1, triglyceride QTL 1; Bwq9, body weight, QTL 9; Mob6, multigenic obesity 6; Bwtq2, body weight QTL 2; Nidds, non-insulin-dependent diabetes mellitus in SJL; Bw7, body weight QTL 7; Tgq3, triglyceride QTL 3; Afw2, abdominal fat weight QTL 2; Afpq2, abdominal fat percent QTL 2; Tafat, TallyHo associated fat; Adip12, adiposity 12; Dob1, dietary obesity 1; Trigq2, triglyceride QTL 2; Obq16, obesity QTL 16; Tgl1, triglyceride level 1; Scfpq1, subcutaneous fat pad percentage QTL 1; Igflsl2, IGF-1 serum levels 2; Adip15, adiposity 12; Bw16, body weight QTL 16; Bwtq5, body weight QTL 5; Afpq10, abdominal fat percent QTL 10; Fob2, F-line obesity QTL 2; Afw10, abdominal fat weight QTL 10; Adip16, adiposity 16; Tanidd2, TallyHo associated non-insulin dependent diabetes mellitus 2; Bwtq12, body weight QTL 12; Chol, Plasma Cholesterol; Glu, Plasma Glucose; TG, Plasma Triglyceride; INS, Plasma Insulin; FFA, Plasma Free Fatty Acid; Ing, Inguinal fat pad; Epi, Epididymal fat pad; Mes, Mesenteric fat pad; Retro, Retroperitoneal fat pad; BF, Brown fat pad; TF, Total fat pad [sum of inguinal, epididymal, mesenteric, retroperitoneal (including perirenal) and subscapular fat pad weights]; Subsc, Subscapular fat pad; Carc, Carcass weight (Body weight without the total fat pad) ; WT, Body weight.

been detected in this region. These include body weight QTL 9 (Bwq9, 24 cM) found in SM/J x NZB/BINJ cross (140), multigenic obesity 6 (Mob6, 49.6 cM) in SM/J x A/J cross (141), body weight QTL 2 (Bwtq2, 55 cM) in A/a x A^{vy}/A cross (142), and body weight 7 (Bw7, 59 cM) in A/a x A^{vy}/A cross (142). Also, QTLs associated with diabetes and lipid metabolism including non-insulin-dependent diabetes (Nidds, 55 cM) and triglyceride QTL 3 (Tgq3, 58 cM) have been detected in NZO x SJL cross and B6 x 129/SvImJ cross, respectively.

A major QTL linked to fat pad weights was detected on chromosome 1, proximal to the HTG QTL (Table 13). Several QTLs associated with obesity have been previously reported in this region, including body weight QTL 5 (Bw5, 36 cM) in A/a x A^{vy}/A cross (142), obesity QTL 16 (Obq16, 48 cM) in C57BL/6J x 129S1/SvImJ cross (75), body weight QTL 1 (Bwtq1, 54.3 cM) in B6 x DBA/2J cross (143), percent fat, subcutaneous and gonadal, QTL 1 (Fatq1, 62 cM) in SM/J x NZB/BINJ cross, obesity and body weight QTL 1 (Obwq1) in SM/J x NZB/BINJ cross, and obesity QTL 8 (Obq8) in SM/J x NZB/BINJ cross (140). A QTL linked to plasma TG levels, triglyceride level 1 (Tgl1, 51.5 cM), was also reported in this region in BALB/c x KK/Ta cross (74).

Previously reported QTLs that overlap with significant and suggestive QTLs detected in our study are summarized in Table 15.

In conclusion, this study reveals complex inheritance associated with diabetes, obesity and dyslipidemia and identifies significant QTLs contributing to the development of these metabolic abnormalities in TH mice.

Chapter IV: Conclusions and Future Directions

Diabetes is a disorder characterized by alterations in carbohydrate and lipid metabolism. HTG is the dominant feature of dyslipidemia observed in T2DM patients. It is well-recognized that genetic factors significantly influence the development of HTG, yet no susceptibility genes for common forms of HTG have been identified in humans to date. In the present study, we have found significant QTLs contributing to HTG, obesity and diabetes in TH mice.

Our ultimate goal includes identifying the molecular bases of these QTLs. One plausible approach by which this goal can be accomplished is conducting expression QTL (eQTL) analysis using the F2 progeny. The diversity in gene expression is one of the mechanisms for diversity among individuals, such as susceptibility to disease. A differential gene expression could be due to a *cis*-acting polymorphism at the gene itself (153,154), or due to a polymorphism of a gene upstream. Therefore, analysis of determinants of differential gene expression is important not only to study the architecture of transcriptional regulation, but also to identify causative genes for various diseases. However, it is often difficult to determine whether differential gene expression is a cause or consequence, in other words, whether it is due to a *cis*-acting or *trans*-acting polymorphism (155). A new approach to this issue is to treat mRNA expression levels as quantitative traits, and to map QTLs that control the expression levels *in vivo*. Using cDNA microarray technology, several studies performed comprehensive analysis of mRNA expression and QTLs controlling the expression in budding yeast (156), eucalyptus (157), mice (158), rats (159), and humans (158,160-162). The studies

indicated that this approach, eQTL analysis, is effective for identification of the cause of the expression differences *in vivo*, and can be applied to various genes.

Quantitative gene expression profiling of liver, adipose, tissue, skeletal muscle, and pancreas can be carried out in the F2 mice from this study by linkage QTL mapping analysis, treating the levels of gene expression as quantitative traits. To search for candidate gene(s) QTLs, linked to the levels of gene expression can then be examined for overlap with QTLs linked to HTG found in this study. If gene(s) showing transcriptional variation maps to the QTL region for HTG, this gene(s) becomes a strong candidate gene based on the idea that the susceptibility gene(s) responsible for the HTG acts in *cis*. Otherwise, candidates will be searched among the transcriptional regulatory genes.

We can also narrow the genomic intervals (fine mapping) of the QTLs and pursue positional cloning to identify the actual allelic variants (molecular basis). The first step for this experiment is the development of congenic strains in which the individual QTL is separated as new inbred strain on a common genetic background such as B6 strain (163). In this simpler system, we can apply conventional genetic mapping and positional cloning methods for identification of each QTL. This strategy has proven to be highly successful in fine mapping the type 1 diabetes susceptibility genes including *Idd3*, and interleukin-2 is strongly demonstrated as a candidate gene for *Idd3* within the interval (164). Also, using congenic strain, Clee et al. have recently identified *Sorcs 1* gene for the QTL of type 2 diabetes mellitus-2 locus (T2dm2) affecting fasting insulin levels in a leptin-deficient obese F2 mice derived from B6 and BTBR T+tf/J (BTBR) mice (165).

Once genes responsible for HTG and diabetes are identified in mice, they can

serve as crucial candidates for humans. Therefore, the present study is important to establish a framework of mapping QTLs for candidate gene(s) and pathway(s) for the susceptibility to the HTG and diabetes in TH mice. This information can contribute to identifying targets for lipid lowering therapy and ultimately to manage and prevent diabetes and CHD in T2DM patients.

List of References

1. Haffner, S. M., Lehto, S., Ronnemaa, T., Pyorala, K. & Laakso, M. (1998) Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med.* 339: 229-234.
2. Noble, J., Baerlocher, M. O. & Silverberg, J. (2005) Management of type 2 diabetes mellitus. Role of thiazolidinediones. *Can Fam Physician.* 51: 683-687.
3. Laakso, M. & Lehto, S. (1997) Epidemiology of macrovascular disease in diabetes. *Diabet Rev.* 5: 294-315.
4. Grundy, S. M., Benjamin, I. J., Burke, G. L., Chait, A., Eckel, R. H., Howard, B. V., Mitch, W., Smith, S. C. & Sowers, J. R. (1999) Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. *Circulation.* 100: 1134-1146.
5. Miettinen, H., Lehto, S., Salomaa, V., Mahonen, M., Niemela, M., Haffner, S. M., Pyorala, K. & Tuomilehto, J. (1998) Impact of diabetes on mortality after the first myocardial infarction. The FINMONICA Myocardial Infarction Register Study Group. *Diabetes Care.* 21: 69-75.
6. Taskinen, M. R. (1995) Insulin resistance and lipoprotein metabolism. *Curr Opin Lipidol.* 6: 153-160.
7. Permutt, M. A., Wasson, J. & Cox, N. (2005) Genetic epidemiology of diabetes. *J Clin Invest.* 115: 1431-1439.
8. Parikh, H. & Groop, L. (2004) Candidate genes for type 2 diabetes. *Rev Endocr Meta Disord.* 5: 151-176.
9. Wolford, J. K. & Vozarova, D. C. (2004) Genetic basis of type 2 diabetes mellitus: implications for therapy. *Treat Endocrinol.* 3: 257-267.
10. Luna, M. T. (2005) Genes and type 2 diabetes mellitus. *Arch Med Res.* 36: 210-222.
11. Leiter, E. H. (2002) Mice with targeted gene disruptions or gene insertions for diabetes research: problems, pitfalls, and potential solutions. *Diabetologia.* 45: 296 –308.
12. Flegal, K. M., Graubard, B. I., Williamson, D. F. & Gail, M. H. (2005) Excess deaths associated with underweight, overweight, and obesity. *JAMA.* 292: 1861-1867.
13. Mokdad, A. (2003) Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA.* 289: 76-79.
14. Colditz, G. (1995) Weight gain as a risk factor for clinical diabetes mellitus in women. *Am Intern Med.* 122: 481-486.

15. Williamson, D. F., Vinicor, F. & Browman, B. A. (2004) Primary prevention of type 2 diabetes mellitus by lifestyle intervention: implications for health policy. *Ann Intern Med.* 40: 951-957.
16. System., U. R. D. (2005) USRDS 2004 Annual Data Report: Atlas of End-Stage Renal Disease in the United States. Available at: http://www.usrds.org/atlas_2004.htm.
17. Ulbrecht, J. S., Cavanagh, P. R. & Caputo, G. M. (2004) Foot problems in diabetes: an overview. *Clin Infect Dis.* 39: S73-S82.
18. Zimmet, P., Alberti, K. G. & Shaw, J. (2001) Global and societal implications of the diabetes epidemic. *Nature.* 414: 782-787.
19. Kannel, W. B. & McGee, D. L. (1979) Diabetes and cardiovascular risk factors: the Framingham study. *Circulation.* 59: 8-13.
20. Williams, C. M. (2004) Lipid metabolism in women. *Proc Nutr Soc.* 63: 153-160.
21. Carey, D. G., Jenkins, A. B., Campbell, L. V., Freund, J. & Chrisholm, D. J. (1996) Abdominal fat and insulin resistance in normal and overweight women: Direct measurements reveal a strong relationship in subjects at both low and high risk of NIDDM. *Diabetes.* 45: 633-638.
22. Staehr, P., Hother-Nielsen, O. & Beck-Nielsen, H. (2004) The role of the liver in type 2 diabetes. *Reviews in Endocrine & Metabolic Disorders.* 5: 105-110.
23. Ravussin, E. & Smith, S. R. (2002) Increased fat intake, impaired fat oxidation, and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus. *Ann N Y Acad Sci.* 967: 363-378.
24. Montague, C. T. & O'Rahilly, S. (2000) The perils of portliness. Causes and consequences of visceral adiposity. *Diabetes.* 49: 883-888.
25. Scheen, A. J. (2000) From obesity to diabetes: why, when and who? *Acta Clin Belg.* 55: 9-15.
26. Scheen, A. J. (2003) Pathophysiology of type 2 diabetes. *Acta Clin Belg.* 58: 335-341.
27. Corella, D. & Ordovas, J. M. (2004) The metabolism syndrome: a crossroad for genotype-phenotype associations in atherosclerosis. *Curr Atheroscler Rep.* 6: 186-196.
28. Gorter, P. M., Olijhoek, J. K., van der Graaf, Y., Algra, A., Rabelink, T. J., Visseren, F. L. & Group, S. S. (2004) Prevalence of the metabolic syndrome in patients with coronary heart disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic

aneurysm. *Atherosclerosis*. 173: 363-369.

29. Szoke, E. & Gerich, J. E. (2005) Role of impaired insulin secretion and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *Compr Ther*. 31: 106-112.

30. Weyer, C., Bogardus, C., Mott, D. M. & Pratley, R. E. (1999) The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest*. 104: 787-794.

31. Bergman, R. N. (1989) Toward physiological understanding of glucose tolerance. Minimal-model approach. *Diabetes*. 38:1512-1527.

32. Stumvoll, M., Goldstein, B. J. & van Haeften, T. W. (2005) Type 2 diabetes: principles of pathogenesis and therapy. *Lancet*. 365: 1333-1346.

33. Kahn, S. E. (2003) The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia*. 46: 3-19.

34. Alberti, K.G. & Zimmet, P.Z. (1999) Definition, diagnosis and classification of diabetes mellitus and its complications. Report of WHO consultation, part 1: diagnosis and classification of diabetes mellitus. *Diabet Med*. 15: 539-53.

35. LeRoith, D. (1995) Molecular and cellular aspects of the insulin-like growth factor I receptor. *Endocrine Reviews*. 16: 143-163.

36. Ullrich, A., Gray, A., Tam, A. W., Yanf-Feng, T., Tsubokawa, M. & Collins, C. (1986) Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. *EMBO Journal*. 5: 2503-2512.

37. LeRoith, D. & Gavrilova, O. (2006) Mouse models created to study the pathophysiology of type 2 diabetes. *The International Journal of Biochemistry & Cell Biology*. 38: 904-912.

38. Chang, L., Chiang, S. H. & Saltiel, A. R. (2003) Insulin signaling and the regulation of glucose transport. *Mol Med*. 10: 65-71.

39. Dohm, G. L., Elton, C. W., Friedman, J. E., Pilch, P. F., Pories, W. J., Atkinson, S. M. & Caro, J. F. (1991) Decreased expression of glucose transporter in muscle from insulin-resistant patients. *Am J Physiol*. 260: E459-E463.

40. Barthel, A. & Schmoll, D. (2003) Novel concepts in insulin regulation of hepatic gluconeogenesis. *Am J Physiol Endocrinol Metab*. 285: E685-E692.

41. Harris, M. I., Flegal, K. M. & Cowie, C. C. (1998) Prevalence of diabetes, impaired

fasting glucose, and impaired glucose tolerance in U.S. adults. The Third National Health and Nutrition Examination Survey, 1988-1994. *Diabetes Care*. 21: 518-524.

42. Shafir, E. & Raz, I. (2003) Diabetes mellitus or lipidus? *Diabetologia*. 46: 433-440.

43. Neuschwander-Tetri, E. A., Brunt, E. M., Wehmeier, K. R., Oliver, D. & Bacon, B. R. (2003) Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. *Hepatology*. 38: 1008-1017.

44. Ginsberg, H. N. & Huanf, L. S. (2000) The insulin resistance syndrome: impact on lipoprotein metabolism and atherothrombosis. *J Cardiovasc Risk*. 7: 325-331.

45. Genest, J. G. (2000) Dyslipidemia and coronary artery disease. *Can J Cardiol*. 16: 3-4A.

46. Ginsberg, H. N. (1996) Diabetic dyslipidemia: basic mechanisms underlying the common hypertriglyceridemia and low HDL cholesterol levels. *Diabetes*. 45: S27-S30.

47. Arner, P. (2002) Insulin resistance in type 2 diabetes: role of fatty acids. *Diabetes Metab Res Rev*. 18: S5-S9.

48. Carr, M. C. & Brunzell, J. D. (2004) Abdominal obesity and dyslipidemia in the metabolic syndrome: importance of type 2 diabetes and familial combined hyperlipidemia in coronary artery disease risk. *J Clin Endocrinol Metab*. 89: 2601-2607.

49. Jones, R. J., Owens, D., Brennan, C., Collins, P. B., Johnson, A. H. & Tomkin, G. H. (1996) Increased esterification of cholesterol and transfer of cholesteryl ester to apo B-containing lipoproteins in Type 2 diabetes: relationship to serum lipoproteins A-I and A-II. *Atherosclerosis*. 26: 151-157.

50. Verges, B. (1995) Apolipoprotein A-IV in diabetes mellitus. *Diabete Metab*. 21: 99-105.

51. Ginsberg, H. N., Zhang, Y. L. & Hernandez-Ono, A. (2005) Regulation of plasma triglycerides in insulin resistance and diabetes. *Arch Med Res*. 36: 232-240.

52. Murray, R. K., Granner, D. K., Mayes, P. A. & Rodwell, V. W. (2003) *Harper's illustrated biochemistry*, Twenty-sixth edition ed. The McGraw-Hill companies.

53. Unger, R. H. (1995) Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes*. 44: 863-870.

54. Shephard, J. M. (2005) Does statin monotherapy address the multiple lipid abnormalities in type 2 diabetes? *Atherosclerosis*. 6: 15-19.

55. Lewis, G. F., Uffelman, K. D., Szeto, L. W., Weller, B. & Steiner, G. (1995) Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. *J Clin Invest.* 95.
56. Mero, N., Syvanne, M. & Taskinen, M. R. (1998) Postprandial lipid metabolism in diabetes. *Atherosclerosis.* 141: S53-55.
57. Packard, C. J. (2003) Triacylglycerol-rich lipoproteins and the generation of small, dense low-density lipoprotein. *Biochem Soc Trans.* 31: 1066-1069.
58. Kolovou, G. D., Anagnostopoulou, K. K. & Cokkinos, D. V. (2005) Pathophysiology of dyslipidaemia in the metabolic syndrome. *Postgrad Med J.* 81: 358-366.
59. Rader, D. J. (2003) Regulation of reverse cholesterol transport and clinical implications. *Am J Cardiol.* 18: 42J-49J.
60. Smith, C., Falconer, D. S. & Duncan, L. J. (1972) A statistical and genetical study of diabetes. II. Heritability of liability. *Ann Hum Genet.* 35: 281-299.
61. Tattersal, R. B. & Fajans, S. S. (1975) Prevalence of diabetes and glucose intolerance in 199 offspring of thirty-seven conjugal diabetic parents. *Diabetes.* 24: 452-462.
62. Pierce, M., Keen, H. & Bradley, C. (1995) Risk of diabetes in offspring of parents with non-insulin-dependent diabetes. *Diabet Med.* 12: 6-13.
63. Kaprio, J., Tuomilehto, J. & Koskenvuo, M. (1992) Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes melitus in a population-based cohort of twins in Finland. *Diabetologia.* 35: 1060-1067.
64. Henkin, L., Bergman, R. N. & Bowden, D. W. (2003) Genetic epidemiology of insulin resistance and visceral adiposity. The IRAS Family Study design and methods. *Ann Epidemiol.* 13: 211-217.
65. Almind, K., Kulkarni, R. N., Lannon, S. M. & Kahn, C. R. (2003) Identification of interactive loci linked to insulin and leptin in mice with genetic insulin resistance. *Diabetes.* 52: 1535-1543.
66. Almind, K., Kulkarni, R.N., Lannon, S.M. & Kahn, C.R. (2003) Identification of interactive loci linked to insulin and leptin in mice with genetic insulin resistance. *Diabetes.* 52: 1535-1543.
67. Hanis, C. L., Boerwinkle, E., Chakraborty, T., Ellsworth, D. L., Spielman, R. S., Gogolin-Ewens, K. J., Shephard, J. M., Williams, S. R. & Risch, N. et al. (1996) A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. *Nat Genet.* 13: 161-166.

68. Mahtani, M. M., Widen, E., Lehto, M., Thomas, J., McCarthy, M., Brayer, J., Bryant, B., Chan, G., Daly, M., Forsblom, C., Kannien, T., Kirby, A., Kruglyak, L., Munnelly, K., Parkkonen, M., Reeve-Daly, M. P., Weaver, A., Brettin, T., Duyk, G., Lander, G. & Groop, L.C. (1996) Mapping of a gene for type 2 diabetes associated with as insulin secretion defect by a genome scan in Finnish families. *Nat Genet.* 14: 90-94.
69. Rees, D. A. & Alcolado, J. C. (2005) Animal models of diabetes mellitus. *Diabetic Medicine.* 22: 359-370.
70. Thoday, J. M. (1961) Location of polygenes. *Nature.* 191: 368-370.
71. Zeng, S. M., Yankowitz, J., Widness, J. A. & Strauss, R. G. (2003) Sequence-based polymorphisms in members of the apoptosis Bcl-2 gene family and their association with hematocrit level. *J Gend Specif Med.* 6: 36-42.
72. Fislser, J. S. & Warden, C. H. (1997) Mapping of mouse obesity genes: A generic approach to a complex trait. *J Nutr.* 127: 1901S-1916S.
73. Dietrich, W., Katz, H., Lincoln, S.E., Shin, H.S., Friedman, J., Dracopoli, N. C. & Lander, E. S. (1992) A genetic map of the mouse suitable for typing intraspecific crosses. *Genetics.* 131: 423-447.
74. Shike, T., Hirose, S., Kobayashi, M., Funabiki, K., Shirai, T. & Tomino, Y. (2001) Susceptibility and negative epistatic loci contributing to type 2 diabetes and related phenotype in a KK/Ta mouse model. *Diabetes.* 50: 1943-1948.
75. Ishimori, N., Li, R., Kelmenson, P. M., Korstanje, R., Walsh, K. A., Churchill, G. A., Forsman-Semb, K. & Paigen, B. (2004) Quantitative trait loci that determine plasma lipids and obesity in C57BL/6J and 129SI/SvImJ inbred mice. *J Lipid Res.* 45: 1624-1632.
76. Cheverud, J.M., Fhrich, T.H., Hrbek, T., Kenney, J. P. Pletscher, L.S. & Semenkovich, C.F. (2004) Quantitative trait loci for obesity- and diabetes-related traits and their dietary responses to high-fat feeding in LGXSM recombinant inbred mouse strains. *Diabetes.* 53: 3328-3336.
77. Anunciado, R. V., Ohno, T., Mori, M., Ishikawa, A., Tanaka, S., Horio, F., Nishimura, M. & Namikawa, T. (2000) Distribution of body weight, blood insulin and lipid levels in the SMXA recombinant inbred strains and the QTL analysis. *Exp Anim.* 49: 217-224.
78. Warden, C. H., Fislser, J. S., Pace, M. J., Svenson, K. L. & Lusis, A. J. (1993) Coincidence of genetic loci for plasma cholesterol levels and obesity in a multifactorial mouse model. *J Clin Invest.* 92: 773-779.

79. Lyons, M. A., Korstanje, R., Li, R., Walsh, K. A., Churchill, G. A., Carey, M. C. & Paigen, B. (2004) Genetic contributors to lipoprotein cholesterol levels in an intercross of 129S1/SvImJ and RIIS/J inbred mice. *Physiol Genomics*. 17: 114-121.
80. Lyons, M. A., Wittenburg, H., Li, R., Walsh, K. A., Churchill, G. A., Carey, M. C. & Paigen, B. (2003) Quantitative trait loci that determine lipoprotein cholesterol levels in DBA/2J and CAST/Ei inbred mice. *J lipid Res*. 44: 953-967.
81. Suto, J., Takahashi, Y. & Sekikawa, K. (2004) Quantitative trait locus analysis of plasma cholesterol and triglyceride levels in C57BL/6J x RR F2 mice. *Biochemical Genetics*. 42: 347-363.
82. Suto, J., Matsuura, S., Yamanaka, H. & Sekikawa, K. (1999) Quantitative trait loci that regulate plasma lipid concentration in hereditary obese KK and KK-Ay mice. *Biochim Biophys Acta*. 1453: 385-395.
83. Lyons, M. A., Wittenburg, H., Li, R., Walsh, K. A., Korstanje, R., Churchill, G. A., Carey, M. C. & Paigen, B. (2004) Quantitative trait loci that determine lipoprotein cholesterol levels in an intercross of 129S1/SvImJ and CAST/Ei inbred mice. *Physiol Genomics*. 17: 60-68.
84. Brockmann, G. A., Kratzsch, J., Haley, C. S., Renne, U., Schwerin, M. & Karle, S. (2000) Single QTL effects, epistasis, and pleiotropy account for two-thirds of the phenotypic F(2) variance of growth and obesity in DU6i x DBA/2 mice. *Genome Res*. 10: 1941-1957.
85. Prochazka, M., Le, P. H., Serreze, D., Coleman, D. & Leiter, E. H. (1996) Genetic control of insulin-dependent diabetes in non-obese diabetic (NOD) mice. *Mouse News Lett*. 75: 32.
86. Laloraya, M., Davoodi-Semiromi, A., Kumar, G. P., McDuffie, M. & She, J. X. (2006) Impaired Crkl expression contributes to the defective DNA binding of Stat5b in nonobese diabetic mice. *Diabetes*. 55: 734-741.
87. Mayo, S., Kohler, W., Kumar, V. & Quinn, A. (2006) Insulin-dependent diabetes loci Idd5 and Idd9 increase sensitivity to experimental autoimmune encephalomyelitis. *Clin Immunol*. 118: 219-228.
88. Guler, M. L., Ligons, D. L., Wang, Y., Bianco, M., Broman, K. W. & Rose, N. R. (2005) Two autoimmune diabetes Loci influencing T cell apoptosis control susceptibility to experimental autoimmune myocarditis. *J Immunol*. 174: 2167-2173.
89. Kayo, T., Fukita, H., Nozaki, J. & Koizumi, A. (2000) Identification of two chromosomal loci determining glucose intolerance in a C57BL/6 mouse strain. *Comp Med*. 50: 296-302.

90. Suto, J., Matsuura, S., Imamura, K., Yamanaka, H. & Sekikawa, K. (1998) Genetics of obesity in KK mouse and effects of Ay allele on quantitative regulation. *Mamm Genome*. 9: 506-510.
91. Seidemann, S. B., De Luca, C., Leibel, R. L., Breslow, J. L., Tall, A. R. & Welch, C. L. (2005) Quantitative trait locus mapping of genetic modifiers of metabolic syndrome and atherosclerosis in low-density lipoprotein receptor-deficient mice: identification of a locus for metabolic syndrome and increased atherosclerosis on chromosome 4. *Arterioscler Thromb Vasc Biol*. 25: 204-210.
92. Kim, E. H., Lee, C. H., Hyun, B. H., Suh, J. G., Oh, Y. S., Namikawa, T. & Ishikawa, A. (2005) Quantitative trait Loci for glomerulosclerosis, kidney weight and body weight in the focal glomerulosclerosis mouse model. *Exp Anim*. 54: 319-325.
93. Carlborg, O., Brockmann, G. A. & Haley, C. S. (2005) Simultaneous mapping of epistatic QTL in DU6i x DBA/2 mice. *Mamm Genome*. 16: 481-494.
94. Brown, A. C., Olver, W. I., Donnelly, C. J., May, M. E., Naggert, J. K., Shaffer, D., J. & Roopenian, D. C. (2005) Searching QTL by gene expression: analysis of diabetes. *BMC Genetics*. 6: 1-9.
95. Brockmann, G. A., Haley, C. S., Renne, U., Knott, S. A. & Schwerin, M. (1998) Quantitative trait loci affecting body weight and fatness from a mouse line selected for extreme high growth. *Genetics*. 150: 369-381.
96. Ueda, H., Ikegami, H. & Kawaguchi, Y. (1999) Genetic analysis of late-onset type 2 diabetes in a mouse model of human complex trait. *Diabetes*. 48: 1168-1174.
97. Kim, J. H., Sen, S. & Avery, C. S. (2001) Genetic analysis of a new mouse model for non-insulin-dependent diabetes. *Genomics*. 47: 273-286.
98. Moody, D. E., Pomp, D., Nielsen, M. K. & Van Vleck, L. D. (1999) Identification of quantitative trait loci influencing traits related to energy balance in selection and inbred lines of mice. *Genetics*. 152: 699-711.
99. Goto, Y., Suzuki, K., Ono, T., Sasaki, M. & Toyota, T. (1988) Development of diabetes in the non-obese NIDDM rat (GK rat). *Adv Exp Med Biol*. 246: 29-31.
100. Mrialles, F. & Portha, B. (2001) Early development of beta-cells is impaired in the GK rat model of type 2 diabetes. *Diabetes*. 50: S84-S88.
101. Shinohara, M., Masuyama, T., Shoda, T., Takahashi, T., Katsuda, Y., Komeda, K., Kuroki, M., Kakehashi, A. & Kanazawa, Y. (2000) A new spontaneously diabetic non-obese Torii rat strain with severe ocular complications. *Int J Exp Diabetes Res*. 1: 89-100.

102. Kawano, K., Hirashima, T., Mori, S., Saitoh, Y., Kurosumi, M. & Natori, T. (1992) Spontaneous long-term hyperglycemic rat with diabetic complications. *Diabetes*. 41: 1422-1428.
103. Kose, H., Moralejo, D. H., Ogino, T., Mizuno, A., Yamada, T. & Matsumoto, K. (2002) Examination of OLETF-derived non-insulin-dependent diabetes mellitus QTL by construction of a series of congenic rats. *Mamm Genome*. 13: 558-562.
104. Moralejo, D. H., Wei, S., Wei, K., Yamada, T. & Matsumoto, K. (1998) X-linked locus is responsible for non-insulin-dependent diabetes in the OLETF rat. *J Vet Med Sci*. 60: 373-375.
105. Moralejo, D. H., Ogino, T., Zhu, M., Toide, K., Wei, S. & Wei, K. (1998) A major quantitative trait locus co-localizing with cholecystokinin type A receptor gene influences poor pancreatic proliferation in a spontaneously diabetogenic rat. *Mamm Genome*. 9: 794-798.
106. Nakamura, M. & Yamada, K. (1967) Studies on a diabetic (KK) strain of the mouse. *Diabetologia*. 3: 121-221.
107. Ikeda, H. (1994) KK mouse. *Diabetes Res Clin Pract*. 24: S313-S316.
108. Reddi, A. S. & Camerini-Davalos, R. A. (1988) Hereditary diabetes in the KK mouse: an overview. *Adv Exp Med Biol*. 246: 7-15.
109. Orlepp, J. R., Kluge, R. & Giessen, K. (2000) A Metabolic Syndrome of Hypertension, Hyperinsulinaemia, and Hypercholesterolemia in the New Zealand Obese (NZO) Mouse. *Eur J Clin Invest*. 30: 195-202.
110. Leiter, E. H., Reifsnyder, P. C., Flurkey, K., Partke, H. J., Junger, E. & Herberg, L. (1998) NIDDM genes in mice, deleterious synergism by both parental genomes contributes to diabetogenic thresholds. *Diabetes*. 47: 1287-1295.
111. Seda, O., Kazdova, L., Krenova, D. & Kren, V. (2002) Rosiglitazone improves insulin resistance, lipid profile and promotes adiposity in a genetic model of metabolic syndrome X. *Folia Biol (Praha)*. 48: 237-241.
112. Mykkanen, L., Kuusisto, J., Pyorala, K. & Laakso, M. (1993) Cardiovascular disease risk factors as predictors of type 2 (non-insulin-dependent) diabetes mellitus in elderly subjects. *Diabetologia*. 36: 553-559.
113. Mitchell, B. D., Kammerer, C. M., Blangero, J., Mahaney, M. C., Rainwater, D. L., Dyke, B., Hixson, J. E., Henkel, R. D., Sharp, R. M., Comuzzie, A.G., VandeBerg, J. L., Stern, M. P. & MacClure, J. W. (1996) Genetic and environmental contributions to

cardiovascular risk factors in Mexican Americans. The San Antonio Family Heart Study. *Circulation*. 94: 2159-2170.

114. Bosse, Y., Chagnon, Y. C., Despres, J. P., Rice, T., Bouchard, C., Perusse, L. & Vohl, M. C. (2004) Genome-wide linkage scan reveals multiple susceptibility loci influencing lipid and lipoprotein levels in the Quebec Family Study. *J lipid Res*. 45: 419-426.

115. Naoumova, R. P., Bonney, S. A., Eichenbaum-Voline, S., Patel, H. N., Jones, B., Jones, E. L., Amey, J., Colilla, S., Neuwirth, C. K., Allotery, R., Seed, M., Betteridge, D.J., Galton, D.J., Cox, N. J. Bell, G. I., Scott, J. & Shoulders, C. C. (2003) Confirmed locus on chromosome 11p and candidate loci on 6q and 8p for the triglyceride and cholesterol traits of combined hyperlipidemia. *Arterioscler Thromb Vasc Biol*. 1: 2070-2077.

116. Austin, M. A., Edwards, K. L., Monks, S. A., Koprowicz, K. M., Brunzell, J. D., Motulsky, A. G., Mahaney, M. C. & Hixson, J. E. (2003) Genome-wide scan for quantitative trait loci influencing LDL size and plasma triglyceride in familial hypertriglyceridemia. *J Lipid Res*. 44: 2161-2168.

117. Ueno, T., Tremblay, J., Kunes, J., Zicha, J., Dobesova, Z., Pausova, Z., Deng, A. Y., Sun, Y. L., Jacob, H. J. & Hamet, P. (2004) Rat model of familial combined hyperlipidemia as a result of comparative mapping. *Physiol Genomics*. 12: 38-47.

118. Seda, O., Liska, F., Krenova, D., Kazdova, L., Sedova, L., Zima, T., Peng, J., Tremblay, J., Kren, V. & Hamet, P. (2003) Differential linkage of triglyceride and glucose levels on rat chromosome 4 in two segregating rat populations. *Folia Biol*. 49: 223-226.

119. Kloting, I., Kovacs, P. & van den Brandt, J. (2001) Quantitative trait loci for body weight, blood pressure, blood glucose, and serum lipids: linkage analysis with wild rats (*Rattus norvegicus*). *Biochem Biophys Res Commun*. 29: 1126-1133

120. Yamasaki, Y., Watanabe, T. K., Okuno, S., Ono, T., Oga, K., Mizoguchi-Miyakita, A., Goto, Y., Shinomiya, H., Momota, H., Miyao, H., Hayashi, T., Asai, T., Suzuki, M., Harada, Y., Hishigaki, H. Wakitani, S., Takagi, T. & Nakamura, Y. (2000) Quantitative trait loci for lipid metabolism in the study of OLETF x (OLETF x Fischer 344) backcross rats. *Clin Exp Pharmacol Physiol*. 27: 881-886.

121. Okuno, S., Watanabe, T. K., Ono, T., Yamasaki, Y., Goto, Y., Miyao, H., Asai, T., Kanemoto, N., Oga, K. Mizoguchi-Miyakita, A., Takagi, T., Takahashi, E., Nakamera, Y. & Tanigami, A. (1999) Genetic determinants of plasma triglyceride levels in (OLETF x BN) x OLETF backcross rats. *Genomics*. 15: 350-355.

122. Suto, J., Matsuura, S., Yamanaka, H. & Sekikawa, K. (1999) Quantitative trait loci that regulate plasma lipid concentration in hereditary obese KK and KK-Ay mice. *Biochim Biophys Acta*. 1453: 385-395.

123. Anunciado, R. V., Nichimura, M., Mori, M., Ishikawa, A., Tanaka, S., Horio, F., Ohno, T. & Namikawa, T. (2003) Quantitative trait locus analysis of serum insulin, triglyceride, total cholesterol and phospholipid levels in the (AM/J X A/J)F2 mice. *Exp Anim.* 52: 37-42.
124. Suto, J. & Sekikawa, K. (2003) Quantitative trait locus analysis of plasma cholesterol and triglyceride levels in KK x RR F2 mice. *Biochem Genet.* 41: 325-341.
125. Buffone, G. J. & Darlington, G. J. (1985) Isolation of DNA from biological specimens without extraction with phenol. *Clin Chem.* 31: 164-165.
126. Saiki, R. K., Gelfand, D. M., Stoffel, S., Sharf, S., Higuchi, R., Horn, G., Mullis, K. B. & Ehrlich, H. A. (1985) Primer-detected enzymatic amplification of DNA with a thermostable DNA polymerase. *Science.* 239: 487-491.
127. Haley, C. S. & Knott, S. A. (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity.* 69: 315-324.
128. Churchill, G. A. & Doerge, R. W. (1994) Empirical threshold values for quantitative trait mapping. *Genetics.* 138: 963-971.
129. Manly, K. F. & Olson, J. M. (1999) Overview of QTL mapping software and introduction to Map Manager QT. *Mamm Genome.* 10: 327-334.
130. Lander, E. & Kruglyak, L. (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet.* 11: 241-247.
131. Silver, L. M. (1995) *Mouse genetics.* Oxford University Press, Inc., New York.
132. Jonkers, I. J., Smelt, A. H. & van der Laarse, A. (2001) Hypertriglyceridemia: associated risks and effect of drug treatment. *Am J Cardiovasc Drugs.* 112: 75-79.
133. Purcell-Huynh, D. A., Weinreb, A., Castellani, L. W., Mehrabian, M. & Doolittle, M. H. (1995) Genetic factors in lipoprotein metabolism. *J Clin Invest.* 96: 1845-1858.
134. Suto, J. & Sekikawa, K. (2002) A quantitative trait locus that accounts for glucose intolerance maps to chromosome 8 in hereditary obese KK-A(y) mice. *Int J Obes.* 26: 1517-1519.
135. Fullerton, S. M., Clark, A. G., Weiss, K. M., Taylor, S. L., Stengård, J. H., Salomaa, V., Boerwinkle, E. & Nickerson, D. A. (2002) Sequence polymorphism at the human apolipoprotein AII gene (APOA2): unexpected deficit of variation in an African-American sample. *Human Genetics.* 111: 75-87.

136. Castellani, L. W., Gargalovic, P., Febbraio, M., Charugundla, S., Jien, M. L. & Lusis, A. J. (2004) Mechanisms mediating insulin resistance in transgenic mice overexpressing mouse apolipoprotein A-II. *J lipid Res.* 45: 2377-2387.
137. Castellani, L. W., Goto, A. M. & Lusis, A. J. (2001) Studies with apolipoprotein A-II transgenic mice indicate a role for HDLs in adipoprotein A-II transgenic mice indicate a role for HDLs in adiposity and insulin resistance. *Diabetes.* 50: 643-651.
138. Doolittle, M. H., Leboeuf, R. C., Warden, C. H., Bee, L. M. & Lusis, A. J. (1990) A polymorphism affecting apolipoprotein A-II translational efficiency determines high density lipoprotein size and composition. *J Biol Chem.* 265: 16380-16388.
139. Martin-Campos, J. M., Escola-Gil, J. C., Ribas, V. & Blanco-Vaca, F. (2004) Apolipoprotein A-II, genetic variation on chromosome 1q21-q24, and disease susceptibility. *Curr Opin Lipidol.* 15: 247-253.
140. Stylianou, I. M., Korstanje, R., Li, R., Sheehan, S., Paigen, B. & Churchill, G. A. (2006) Quantitative trait locus analysis for obesity reveals multiple networks of interacting loci. *Mamm Genome.* 17: 22-36.
141. Kobayashi, M. F., Kawai, T., Kumazawa, M., Ikegami, H., Nishimura, M., Ohno, T. & Horio, F. (2006) Major quantitative trait locus on chromosome 2 for glucose tolerance in diabetic SMXA-5 mouse established from non-diabetic SM/J and A/J strains. *Diabetologia.* 49: 486-495.
142. Kaput, J., Klein, K. G., Reyes, E., J., Kibbe, W. A., Cooney, C. A., Jovanovic, B., Visek, W. & Wolff, G. L. (2004) Identification of genes contributing to the obese yellow Avy phenotype: caloric restriction, genotype, diet x genotype interactions. *Physiol Genomics.* 18: 316-324.
143. Morris, K. H., Ishikawa, A. & Keightley, P. D. (1999) Quantitative trait loci for growth traits in C57BL/6J x DBA/sJ mice. *Mamm Genome.* 10: 225-228.
144. Taylor, B. A., Wnek, C., Schroeder, D. & Phillips, S. J. (2001) Multiple obesity QTLs identified in an intercross between the NZO (New Zealand obese) and the SM (small) mouse strains. *Mamm Genome.* 12: 95-103.
145. Korstanje, R., Li, R., Howard, T., Kelmenson, P., Marshall, J., Paigen, B. & Churchill, G. A. (2004) Influence of sex and diet on quantitative trait loci for HDL cholesterol levels in an SM/J by NZB/BINJ intercross population. *J Lipid Res.* 45: 881-888.
146. Reed, D. R., Li, X., McDaniel, A. H., Lu, K., Li, S., Tordoff, M. G., Price, R. A. & Bachmanov, A. A. (2003) Loci on chromosomes 2, 4, 9, and 16 for body weight, body length, and adiposity identified in a genome scan of an F2 intercross between the 129P3/J

and C57BL/6ByJ mouse strains. *Mamm Genome*. 14: 302-313.

147. Plum, L., Giesen, K., Kluge, R., Junger, E., Linnartz, K., Schurmann, A., Becker, W. & Joot, H. G. (2002) Characterisation of the mouse diabetes susceptibility locus *Nidd/SJL*: islet cell destruction, interaction with the obesity QTL *Nob1*, and effect of dietary fat. *Diabetologia*. 45: 823-830.

148. West, D. B., Waguespack, J., York, B., Goudey-Lefevre, J. & Price, R. A. (1994) Genetics of dietary obesity in AKR/J x SWR/J mice: segregation of the trait and identification of a linked locus on chromosome 4. *Mamm Genome*. 5: 546-552.

149. Rocha, J. L., Eisen, E. J., Van Vleck, L. D. & Pomp, D. (2004) A large-sample QTL study in mice: II. Body composition. *Mamm Genome*. 15: 100-113.

150. Rosen, C. J., Churchill, G. A., Donahue, L. R., Shultz, K. L., Burgess, J. K., Powel, D. R. & Beamer, W. G. (2000) Mapping quantitative trait loci for serum insulin-like growth factor-1 levels in mice. *Bone*. 27: 521-528.

151. Brockmann, G. A., Kratzsch, J., Haley, C. S., Renne, U., Schwerin, M. & Karle, S. (2000) Single QTL effects, epistasis, and pleiotropy account for two-third of the phenotypic F(2) variance of growth and obesity in DU6I x DBA/2 mice. *Genome Res*. 10: 1941-1957.

152. Horvat, S., Bunger, L., Falconer, V. M., Mackay, P., Law, A., Bulfield, G. & Keightley, P. D. (2000) Mapping of obesity QTLs in a cross between mouse lines divergently selected on fat content. *Mamm Genome*. 11: 2-7.

153. Baier, L. J., Permana, P. A., Yang, X., Pratley, R. E., Hanson, R. L., Shen, G. Q., Mott, D., Knowler, W. C., Cox, N. J., Horikawa, Y., Oda, N., Bell, G.I. & Bogardus, C. (2000) A calpain-10 gene polymorphism is associated with reduced muscle mRNA levels and insulin resistance. *J Clin Invest*. 106: R69-R73.

154. Kuramoto, T., Nomoto, T., Sugimura, T. & Ushijima, T. (2001) Linkage mapping of the rat interleukin 1 receptor antagonist gene on chromosome 3. *Exp Anim*. 50: 355-357.

155. Yamashita, S., Wakazono, K., Nomoto, T., Tsujino, Y., Kuramoto, T. & Ushijima, T. (2005) Expression quantitative trait loci analysis of 13 genes in the rat prostate. *Genetics*. 171: 1231-1238.

156. Brem, R. B., Yvert, G., Clinton, R. & Kruglyak, L. (2002) Genetic dissection of transcriptional regulation in budding yeast. *Science*. 296: 752-755.

157. Kirst, M., Basten, C. J., Myburg, A. A., Zeng, Z. B. & Sederoff, R. R. (2005) Genetic architecture of transcript-level variation in differentiating xylem of a eucalyptus hybrid. *Genetics*. 169: 2295-2303.

158. Schadt, E. E., Monks, S. A., Drake, T. A., Lusis, A. J., Che, N., Colinayo, V., Ruff, T. G., Milligan, S. B., Lamb, J. R., Cavet, G., Linsley, P.S. Mao, M., Stoughton, R. B. & Friend, S. H. (2003) Genetics of gene expression surveyed in maize, mouse and man. *Nature*. 20: 297-302.
159. Hubner, N., Wallace, C. A., Zimdahl, H., Petretto, E., Schulz, H., Maciver, F., Mueller, M., Hummel, O., Monti, J. Zidek, V., Musilova, A., Kren, V., Causton, H., Game, L., Born, G., Schmidt, S., Muller, Q. Cook, S.A., Kurtz, T. W., J., Pravenec, M. & Aitman, T. J. (2005) Integrated transcriptional profiling and linkage analysis for identification of genes underlying disease. *Nat Genet*. 37: 243-253.
160. Cheung, V. G., Jen, K. Y., Weber, T., Morley, M., Devlin, J. L., Ewens, K. G. & Spielman, R. S. (2003) Genetics of quantitative variation in human gene expression. *Cold Spring Harb Symp Quant Biol*. 68: 403-407.
161. Monks, S. A., Leonardson, A., Zhu, H., Cundiff, P., Pietrusiak, P., Edwards, S., Phillips, J. W., Sachs, A. & Schadt, E. E. (2004) Genetic inheritance of gene expression in human cell lines. *Am J Hum Genet*. 75: 1094-1105.
162. Morley, M., Molony, C. M., Weber, T. M., Devlin, J. L., Ewens, K. G., Spielman, R. S. & Cheung, V. G. (2004) Genetic analysis of genome-wide variation in human gene expression. *Nature*. 12: 743-747.
163. Rogner, U. C. & Avner, P. (2003) Congenic mice: cutting tools for complex immune disorders. *Nat Rev Immunol*. 3: 243-252.
164. Podolin, P. L., Denny, P., Lord, C. J., Hill, N. J., Todd, J. A., Peterson, L. B., Wicker, L. S. & Lyons, P. A. (1997) Congenic mapping of the insulin-dependent diabetes (Idd) gene, Idd10, localizes two genes mediating the Idd10 effect and eliminates the candidate *Fcgr1*. *J Immunol*. 159: 1835-1843.
165. Clee, S. M., Yandell, B. S., Schueler, K. M., Rabaglia, M. E., Richards, O. C., Raines, S. M., Kabara, E. A., Klass, D. M., Mui, E. T., Stapleton, D. S., Gray-Keller, M. P., Young, M. B., Stoehr, J. P., Lan, H., Boronenkov, I., Rasee, P.W., Flowers, M. T. & Attie, A. D. (2006) Positional cloning of *Sorcs1*, a type 2 diabetes quantitative trait locus. *Nat Genet*. 38: 688-693.

VITA

Hyoungh Yon Kim was born in Chonju, South Korea. She grew up in Chonju just before moving to USA. She went to Wonkwang University from 1995 to 1999 and finished a Bachelor of Science degree in Food and Nutrition. In 1999, she went to Chonbuk National University and earned a Master of Science degree in Food and Nutrition in 2001. She worked as research assistance in the department of Pharmacology in Wonkwang University from 2002-2003. In 2003, she moved to Knoxville, Tennessee in USA to study. She has earned a Master of Science degree in Nutrition Science.