

# Associations Between Metabolic Phenotypes Vary With the Codon 54 Polymorphism of Fatty Acid-binding Protein 2 Gene

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## A B S T R A C T

### OBJECTIVE

The T54 allele of the intestinal fatty acid-binding protein 2 (*FABP2*) gene has been associated with impairment in lipid and carbohydrate metabolism in susceptible populations. We hypothesized that lipid and carbohydrate abnormalities would be present in young, nonobese individuals with the variant allele.

### METHODS

As part of another study, we screened 52 subjects for their *FABP2* genotype. The genotype-phenotype relationships in these young (mean age: 31.7±9.6 years), nonobese (mean body mass index [BMI]: 23.8±3.0 kg/m<sup>2</sup>) individuals were analyzed.

### RESULTS

We found no significant mean differences between the A54 and T54 genotype groups. However, low-density lipoprotein cholesterol (LDL-C) was significantly higher in T54 females vs. A54 females. There were positive correlations between triglycerides (TGs) and BMI ( $r=0.72$ ) and TGs and insulin ( $r=0.67$ ), and a negative correlation between high-density lipoprotein cholesterol (HDL-C) and insulin ( $r=-0.63$ ) in T54 subjects. These correlations were not significant in the

## R É S U M É

### OBJECTIF

L'allèle T54 du gène de la protéine intestinale de la liaison des acides gras (*FABP2*) a été associé à une altération du métabolisme des lipides et des glucides chez les personnes sensibles. Nous avons supposé que les anomalies des lipides et des glucides se manifesteraient chez des personnes jeunes et non obèses présentant la variante du gène.

### MÉTHODES

Dans le cadre d'une autre étude, nous avons déterminé le génotype *FABP2* chez 52 sujets jeunes (âge moyen : 31,7 ± 9,6 ans) et non obèses (indice de masse corporelle [IMC] moyen : 23,8 ± 3,0 kg/m<sup>2</sup>), et avons analysé le lien entre le génotype et le phénotype.

### RÉSULTATS

Nous n'avons pas trouvé de différences moyennes significatives entre les génotypes A54 et T54. Toutefois, le cholestérol des lipoprotéines de basse densité (LDL-C) était significativement plus élevé chez les femmes de génotype T54 que chez celles de génotype A54. Nous avons observé des corrélations positives entre les triglycérides (TG) et l'IMC ( $r = 0,72$ ) et entre les TG et l'insuline ( $r = 0,67$ ) et une corrélation négative entre le cholestérol des lipoprotéines de haute densité (HDL-C) et l'insuline ( $r = -0,63$ ) chez les sujets de génotype T54. Ces corrélations n'étaient pas significatives chez les homozygotes de génotype T54 et les courbes de régression des sujets de génotypes T54 et A54 étaient significativement différentes.

### CONCLUSION

Cette étude démontre que les associations phénotypiques varient selon le génotype *FABP2* dans un groupe de jeunes non obèses même s'il n'y a pas de différences moyennes entre les génotypes *FABP2*.

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A54 homozygotes, and the regression slopes of the T54 and A54 subjects were significantly different.

## CONCLUSION

This study demonstrates that phenotypic associations vary with *FABP2* genotype in a young, nonobese group despite the absence of mean differences between *FABP2* genotypes.

## INTRODUCTION

The intestinal fatty acid-binding protein 2 (*FABP2*) gene encodes the protein intestinal fatty acid-binding protein (IFABP), which is involved in the absorption and cytoplasmic transport of fatty acids in intestinal enterocytes (1). A polymorphism at codon 54 causes a substitution of the amino acid alanine to threonine. Reported frequencies of the threonine (T54) allele of *FABP2* have ranged from 0.14 in aboriginal Canadians (2) to 0.38 in Japanese men (3); in samples of European ancestry, the frequency typically ranges from 0.25 to 0.30 (1,4-6). Thus, despite interethnic differences, the T54 allele is relatively common in most populations.

In vitro studies have indicated that the variant gene product has a greater affinity for long-chain fatty acids (1). Similarly, in an organ culture model the T54 allele was associated with increased secretion of newly esterified triglycerides (TGs) and elevated chylomicron output (7). In humans, the T54 allele has been associated with increased body mass index (BMI) in aboriginal Canadians (2), greater intra-abdominal fat thickness in Japanese men (8), higher cholesterol and TG levels in discordant sibling pairs (5), increased fat oxidation and insulin resistance in Pima Indians (1), and increased postprandial lipemia in a small group of Finns (9).

We hypothesized that if the only mechanism responsible for the lipid and carbohydrate abnormalities observed in groups with the T54 variant is increased intestinal absorption, these abnormalities should also be observed in a young, nonobese population. As part of the screening process for another study, we collected cross sectional data for 52 subjects, including *FABP2* genotype and various parameters of lipid and carbohydrate metabolism, from which we investigated the genotype-phenotype relationships.

## METHODS

### Study subjects and design

Fifty-two adult volunteers were recruited (24 males and 28 females) from the greater Toronto area in Ontario, Canada. Subjects were excluded if they had a self-reported BMI >30 or if they had any previously diagnosed endocrine or gastrointestinal disorder. The Ethics Committee of the University of Toronto, Toronto, Ontario, Canada, approved the study, and all subjects gave informed written consent.

Subjects reported to the Clinical Nutrition and Risk Factor Modification Centre at St. Michael's Hospital for a fasting blood sample (at least 12 hours after their last meal) and height and weight measurements. Blood samples were

used to determine total cholesterol (TC), TGs, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), free fatty acids (FFAs), fasting plasma glucose (FPG), HbA<sub>1c</sub>, insulin, leptin, and *FABP2* and *apolipoprotein E (APOE)* genotypes.

### Biochemical and genetic analyses

FPG was analyzed using a Vitros Analyzer 950 (Johnson & Johnson Clinical Diagnostics, Rochester, New York, United States [US]), and fasting HbA<sub>1c</sub> was determined using a Diamet high-performance liquid chromatograph (HPLC) (Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ontario, Canada). Frozen plasma samples were analyzed for insulin and leptin by radioimmunoassay (RIA) (Pharmacia AB, Uppsala, Sweden, and Linco Research, St. Charles, Missouri, US, respectively), and enzymatically for FFAs (Wako Chemical Co., Osaka, Japan).

Plasma TC, TGs and HDL-C were determined on fresh samples at the J. Alick Little Lipid Laboratory, St. Michael's Hospital, Toronto, Ontario, Canada, certified by the National Heart, Lung, and Blood Institute—Centers for Disease Control Lipid Standardization Program (Lipid Research Clinics [LRC] Program, 1982). The Technicon RA 1000 and Technicon enzymatic reagents were used to determine TC (Technicon method SM4-0139G86) and TGs (Technicon method SM4-0173G90 with TG blank reagent number T01-2013-01) (Technicon-Miles, Mississauga, Ontario, Canada) (10). HDL-C was measured as the cholesterol in the supernatant after precipitation of the non-HDL-C using dextran sulfate magnesium chloride (11). LDL-C was calculated as  $TC - (HDL-C + [TGs/2.2])$ .

Established procedures were used to extract leukocyte DNA and determine the genotypes of *FABP2* codon 54 (1) and *APOE* (12), with known genotypic controls run as standards for each electrophoresis.

The homeostasis model assessment (HOMA) was used as a validated method to derive beta cell function ( $\% \text{ beta cell function} = 20 \times \text{insulin} / [\text{FPG} - 3.5]$ ) and insulin resistance ( $\text{insulin} / 22.5e^{-\ln \text{FPG}}$ ) (13) from FPG and insulin measurements. In 4 subjects, the calculations for beta cell function could not be used because the denominator was  $\leq 0$ .

### Statistical analysis

SAS 6.12 (Cary, North Carolina, US) was used for all statistical comparisons. Univariate analysis was performed for all quantitative variables to assess normality. Log transformations were performed for TGs, LDL-C, FFAs, insulin, insulin

resistance, beta cell function and leptin, after which the data showed less skew and kurtosis and no considerable deviation from a normal distribution in most cases. The transformed data were used for parametric statistical analyses and the original data were used for non-parametric statistical analyses and presentation purposes.

Analysis of variance (ANOVA) was performed using the General Linear Models procedure, with and without covariance models for each of age, gender, BMI and *APOE* genotype. Additional covariates were tested if they demonstrated a correlation with the dependent variable; for example, insulin and insulin resistance were modelled as covariates for TGs. The type III sums of squares, computed from F tests, was used, as it is applicable to unbalanced study designs. Analysis of covariance (ANCOVA) revealed significant interactions for some variables. If the significant interaction occurred with a categorical variable, ANOVA was conducted within each category. However, when the interaction occurred with a continuous variable, it indicated that the slopes of the lines relating the dependent and independent variables were significantly different for the genotype groups. To further assess these interactions, specific Pearson product-moment correlations were performed within each *FABP2* genotype group. Spearman's rank correlations were also performed to corroborate the results of the parametric correlations and substantiate that the results were not driven by outlying data points.

## RESULTS

### Subject phenotypes

The mean ( $\pm$ standard deviation [SD]) age of subjects was  $31.7 \pm 9.6$  years with the age range spanning 19 to 51 years for women and 20 to 58 years for men. The mean BMI was  $23.8 \pm 3.0$  kg/m<sup>2</sup>, representing a mostly lean sample (i.e. 37 subjects with BMI < 25, 13 subjects with BMI = 25 to < 30, and only 2 subjects with BMI = 30 to 31). The frequency of the *FABP2* T54 allele in this sample population was 0.22 (98% confidence interval [CI], 0.14 to 0.30), with no deviation from the Hardy-Weinberg law. T54 homozygotes are uncommon in the general population. There were only 2 T54 homozygotes in this sample so they were grouped with the heterozygotes for purposes of statistical analysis; this group is represented as T54. Alanine homozygotes are represented as A54.

### Genotype-phenotype associations

The phenotypes of subjects according to *FABP2* genotype are presented in Table 1. There were no significant differences between *FABP2* genotype groups in *APOE* genotype, age, gender or BMI; however, between group differences in gender and BMI approached significance with p values of 0.063 and 0.089, respectively. Similarly, TC, TGs, LDL-C, HDL-C, FFAs, FPG, HbA<sub>1c</sub>, insulin, insulin resistance, beta cell function and leptin did not differ between groups. However, when ANCOVAs were performed with age, gender, BMI and *APOE* as covariates, there were significant interaction terms

for some variables. That is, for LDL-C, there was a significant interaction with *FABP2* genotype and gender ( $p=0.015$ ). ANOVA within each gender revealed that T54 females had significantly higher TC and LDL-C than A54 females ( $4.91 \pm 1.08$  vs.  $4.13 \pm 0.59$  mmol/L,  $p=0.021$ , and  $2.93 \pm 0.93$  vs.  $2.11 \pm 0.53$  mmol/L,  $p=0.011$ , respectively) (Table 2); however, there were no significant differences between T54 and A54 males. Further analyses showed that A54 males had significantly higher LDL-C and significantly lower HDL-C than A54 females (Table 2), but there were no significant differences in LDL-C and HDL-C between T54 males and T54 females.

A significant interaction was also observed between *FABP2* and *APOE* genotypes with respect to FFAs. Analyses within each *APOE* genotype demonstrated that FFAs were significantly higher in the T54 group than in the A54 group only in those subjects with the E2/3 genotype ( $0.461 \pm 0.029$  vs.  $0.180 \pm 0.111$  mmol/L,  $p=0.040$ , respectively) (Table 3).

**Table 1. Metabolic phenotypes of subjects**

	<b>FABP2 codon 54 genotype</b>	
	<b>T54</b>	<b>A54</b>
n	21	31
Gender (female/male)	8/13	20/11
Age (years)	$32.7 \pm 8.8$	$31.0 \pm 10.2$
BMI (kg/m <sup>2</sup> )	$24.6 \pm 3.4$	$23.2 \pm 2.6$
<i>APOE</i> genotype* (% E3/3)	52	61
TC (mmol/L)	$4.63 \pm 0.88$	$4.28 \pm 0.78$
TGs (mmol/L)	$1.36 \pm 0.87$	$1.22 \pm 0.51$
LDL-C (mmol/L)	$2.69 \pm 0.81$	$2.39 \pm 0.84$
HDL-C (mmol/L)	$1.32 \pm 0.33$	$1.33 \pm 0.30$
FFAs (mmol/L)	$0.349 \pm 0.15$	$0.381 \pm 0.21$
FPG (mmol/L)	$4.39 \pm 0.50$	$4.42 \pm 0.52$
HbA <sub>1c</sub> (%)	$5.2 \pm 0.4$	$5.2 \pm 0.3$
Insulin (pmol/L)	$47.3 \pm 26.8$	$53.1 \pm 30.1$
Relative insulin resistance (HOMA)	$1.3 \pm 0.8$	$1.4 \pm 0.7$
Beta cell function (HOMA) (%)	$155 \pm 89$	$192 \pm 230$
Leptin (ng/mL)	$8.85 \pm 11.43$	$8.61 \pm 6.40$

Values are mean  $\pm$  SD. All values are fasted ( $\geq 12$  hours).

\*distribution of *APOE* is shown in Table 3

A54 = homozygous for alanine at *FABP2* codon 54

*APOE* = apolipoprotein E gene

BMI = body mass index

*FABP2* = intestinal fatty acid-binding protein 2 gene

FFA = free fatty acid

FPG = fasting plasma glucose

HDL-C = high-density lipoprotein cholesterol

HOMA = homeostasis model assessment

LDL-C = low-density lipoprotein cholesterol (calculated)

T54 = heterozygous or homozygous for threonine at *FABP2* codon 54

TC = total cholesterol

TG = triglyceride

Interactions between *FABP2* genotypes and continuous variables suggested that regression slopes between the dependent variable and the covariate were significantly different for T54 vs. A54 genotypes, i.e. TGs vs. BMI ( $p=0.006$ ; Figure 1A), TGs vs. insulin ( $p=0.014$ ; Figure 1B), TGs vs. insulin resistance ( $p=0.010$ ; Table 4), HDL-C vs. insulin ( $p=0.018$ ; Figure 1C), and HDL-C vs. insulin resistance ( $p=0.027$ ; Table 4).

### Within-genotype phenotypic associations

As a follow-up to the above results, specific correlations were performed within each genotype group (T54 and A54). There were significant phenotypic associations within the T54 group that were absent in the A54 group (Table 4). Specifically, there were strong positive associations between TGs and BMI (Pearson's  $r=0.72$ ,  $p<0.001$ ; Spearman's  $r=0.67$ ,  $p<0.001$ ), TGs and insulin (Pearson's  $r=0.67$ ,

$p<0.001$ ; Spearman's  $r=0.54$ ,  $p=0.01$ ), and TGs and insulin resistance (Pearson's  $r=0.69$ ,  $p<0.001$ ; Spearman's  $r=0.53$ ,  $p=0.01$ ), and strong negative associations between HDL-C and insulin (Pearson's  $r=-0.63$ ,  $p=0.002$ ; Spearman's  $r=-0.58$ ,  $p=0.006$ ) and HDL-C and insulin resistance (Pearson's  $r=-0.67$ ,  $p<0.001$ ; Spearman's  $r=-0.61$ ,  $p=0.003$ ) in the T54 group and no similar associations in the A54 group. A significant interaction between *FABP2* genotype and age for leptin was also observed; however, both parametric and non-parametric correlations indicated that the regression lines were not significantly different from 0 and the interaction was driven by 2 outlying data points (data not shown).

### DISCUSSION

The literature has suggested that individuals with a codon 54 polymorphism of the *FABP2* gene have abnormalities in carbohydrate and lipid metabolism (14); however, there have

**Table 2. Lipid parameters by gender and *FABP2* genotype**

	Females		Males	
	T54 n=8	A54 n=20	T54 n=13	A54 n=11
TC (mmol/L)	4.91±1.08*	4.13±0.59	4.46±0.73	4.56±1.01
TGs (mmol/L)	1.13±0.53	1.21±0.53	1.51±1.02	1.24±0.50
LDL-C (mmol/L)	2.93±0.93†	2.11±0.53	2.54±0.72	2.91±1.07‡
HDL-C (mmol/L)	1.47±0.22	1.46±0.25	1.24±0.37	1.09±0.24§

Values are mean±SD. All values are fasted ( $\geq 12$  hours).

\* $p=0.021$  vs. A54 females

† $p=0.011$  vs. A54 females

‡ $p=0.009$  vs. A54 females

§ $p<0.001$  vs. A54 females

A54 = homozygous for alanine at *FABP2* codon 54

*FABP2* = intestinal fatty acid-binding protein 2 gene

HDL-C = high-density lipoprotein cholesterol

LDL-C = low-density lipoprotein cholesterol (calculated)

T54 = heterozygous or homozygous for threonine at *FABP2* codon 54

TC = total cholesterol

TG = triglyceride

**Table 3. FFAs by APOE and *FABP2* genotypes**

APOE genotype	FFAs (mmol/L)		n (T54,A54)	p value
	T54	A54		
E2/4	0.190	0.142	2 (1,1)	-
E2/3	0.461±0.029	0.180±0.111	8 (3,5)	0.040
E3/3	0.320±0.127	0.425±0.184	30 (11,19)	0.075
E3/4 or E4/4	0.373±0.207	0.451±0.254	12 (6,6)	0.503

Values are mean±SD. All values are fasted ( $\geq 12$  hours).

A54 = homozygous for alanine at *FABP2* codon 54

APOE = apolipoprotein E gene

*FABP2* = intestinal fatty acid-binding protein 2 gene

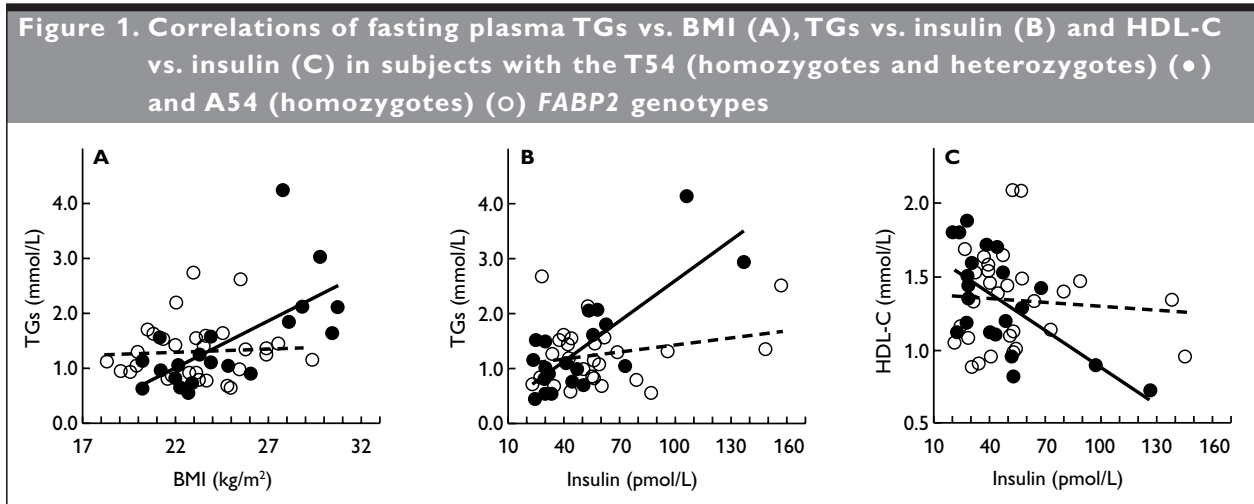
FFA = free fatty acid

T54 = heterozygous or homozygous for threonine at *FABP2* codon 54

been some inconsistencies in the reported associations. For example, some studies have shown higher BMI (2) or abdominal obesity (8) in subjects with the T54 allele, while other studies have not been able to support these findings (1,4). Similarly, 1 Japanese study demonstrated that the group with the T54 allele had greater insulin resistance (8), while another reported no significant difference in insulin sensitivity between *FABP2* genotypes (3). Some of these studies were conducted in populations that may be susceptible to metabolic

abnormalities due to enhanced ethnic risk or increased BMI (1,2). In a healthy population, we were not able to demonstrate a mean difference between *FABP2* genotypes, suggesting that this polymorphism is not a primary defect but is instead a susceptibility gene.

We were able to show that correlations between TGs and BMI were present in subjects with the T54 allele and absent in homozygotes for the A54 allele, suggesting that an elevated TG level may only be apparent in T54 individuals



The slopes of the regression lines (T54 — and A54 - -) were significantly different at  $p=0.006$  (A),  $p=0.014$  (B) and  $p=0.018$  (C).

A54 = homozygous for alanine at *FABP2* codon 54

BMI = body mass index

*FABP2* = intestinal fatty acid-binding protein 2 gene

HDL-C = high-density lipoprotein cholesterol

T54 = heterozygous or homozygous for threonine at *FABP2* codon 54

TG = triglyceride

**Table 4. Metabolic correlations within each *FABP2* genotype and the significant difference between their regression slopes**

	<b>T54 n=21</b>		<b>A54 n=31</b>		<b>Regression slopes*</b>
	<b>r</b>	<b>p value</b>	<b>r</b>	<b>p value</b>	
TGs and					
BMI	0.72	<0.001	0.07	NS	0.006
Insulin	0.67	<0.001	0.19	NS	0.014
Insulin resistance	0.69	<0.001	0.12	NS	0.010
HDL-C and					
Insulin	-0.63	0.002	0.00	NS	0.018
Insulin resistance	-0.67	<0.001	-0.08	NS	0.027

\*p value of the interaction term in ANCOVA, General Linear Models procedure (SAS)

A54 = homozygous for alanine at *FABP2* codon 54

ANCOVA = analysis of covariance

BMI = body mass index

*FABP2* = intestinal fatty acid-binding protein 2 gene

HDL-C = high-density lipoprotein cholesterol

NS = not significant

T54 = heterozygous or homozygous for threonine at *FABP2* codon 54

TG = triglyceride

when BMI is elevated. Similar phenomena have been reported in individuals with different *APOE* genotypes (15,16). In fact, these authors have suggested that traditional statistical analyses that adjust for gender, age and BMI may be inappropriate because the associations between lipid levels and anthropometrics may vary with genotype.

Studies conducted in populations at increased risk for type 2 diabetes mellitus may demonstrate greater metabolic differences between the *FABP2* genotypes than would otherwise be observed in a nonrisk population. One of the first studies to show that the T54 allele of *FABP2* was associated with hyperinsulinemia, insulin resistance and increased fat oxidation was conducted in the Pima Indians (1), a population with a high prevalence of diabetes and abnormalities in carbohydrate and lipid metabolism. Similarly, a study in aboriginal Canadians demonstrated higher BMI and TGs in the group with the T54 allele (2); this is also a high-risk population with respect to diabetes. These subjects had a mean BMI of  $28.1 \pm 5.26$  kg/m<sup>2</sup>, which is consistent with our hypothesis that the difference between TG levels is only present when BMI is elevated.

Previously published cross sectional data from the Canadian Heart Health Survey demonstrated a moderate association between TGs and BMI ( $r=0.39$  for men and  $r=0.37$  for women) (17). We speculate that moderate correlations such as these may have been driven by potentially strong correlations present in an estimated 40% of the population with the T54 allele. A much larger cross sectional study would be necessary to confirm this speculation.

Conversely, Ågren and colleagues (9) reported a strong positive correlation between BMI and TGs in obese A54 homozygotes (BMI:  $29.7 \pm 6.0$  kg/m<sup>2</sup>;  $r=0.93$ ,  $p=0.01$ ) and no significant association in T54 homozygotes (BMI:  $32.8 \pm 4.2$  kg/m<sup>2</sup>;  $r=0.07$ ). The correlations we observed between TGs and BMI in the T54 group occurred at a lower BMI ( $24.6 \pm 3.4$  kg/m<sup>2</sup>), which may suggest that individuals with the T54 genotype are susceptible to the metabolic defects of obesity at a lower BMI than those with the A54 genotype.

TC and LDL-C were significantly higher in the T54 females compared to the A54 females, as previously reported (18). It may be possible that the premenopausal benefits that women normally experience with respect to lower levels of TC and LDL-C are abolished with this polymorphism; however, more evidence is required to substantiate this hypothesis.

The finding of higher FFAs in the T54 *APOE* 2/3 subjects than in the A54 *APOE* 2/3 subjects poses an interesting, but difficult to explain, result. While *APOE* 2 has been associated with defective removal of chylomicron remnants in the postprandial state and could potentially lead to increased FFAs, this has been limited to homozygotes (19) and our data are in the fasting state. A greater number of subjects would be required to corroborate this result.

Possible mechanisms for the T54 gene product, IFABP, relate to its increased binding affinity for dietary long-chain

fatty acids (1) and its potential ability to facilitate a greater flux of fatty acids across the intestinal mucosa (20). This has been proposed to cause increased absorption and/or modification of TG synthesis in the intestinal enterocyte, resulting in increased postprandial chylomicron TGs. Two studies have demonstrated increased postprandial chylomicron TGs in subjects homozygous for the T54 allele, 1 in obese subjects with diabetes (21) and another in obese subjects without diabetes (9).

Increased postprandial chylomicron TGs may result in increased availability of FFAs at the level of the adipocyte. This might normally be handled by physiologic adaptations; however, in the presence of obesity it is possible that there is a decreased adipocyte uptake of FFAs and increased shunting of FFAs to the liver. Elevated plasma FFAs and increased FFA uptake by the liver may lead to increased hepatic TG production, increased plasma TGs and increased HDL-TGs (22-24). This is supported by an increased HDL-TG level observed in hyperlipidemic patients with the T54 allele (25). Furthermore, HDL particles that take up more TGs will likely have an altered TG:cholesterol ratio and may result in increased clearance and decreased circulating HDL-C (26), i.e. potentially creating a negative correlation between TGs and HDL-C, as we have observed. While the results of the present study cannot definitively support the above hypotheses, they do raise questions that warrant further investigation with a larger sample size.

In conclusion, the aggregate of data is consistent with the hypothesis that the *FABP2* T54 allele is a susceptibility allele for abnormalities in carbohydrate and lipid metabolism. While the T54 allele may not be directly causative of abnormal phenotypes, it may increase the risk of developing a deleterious trait in the presence of other factors, such as obesity. Such a relationship may explain the disparate associations between *FABP2* genotypes and adverse phenotypes in various study samples.

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