Published in final edited form as:

Diabetes Res Clin Pract. 2011 April ; 92(1): 82–91. doi:10.1016/j.diabres.2011.01.005.

Polymorphisms in Fatty Acid Binding Protein 5 Show Association with Type 2 Diabetes

Liming Bu, Lorena M. Salto, Kevin J De Leon, and Marino De Leon[§]

Center for Health Disparities and Molecular Medicine and Department of Basic Sciences, Loma Linda University, School of Medicine, Loma Linda, California 92350

Abstract

Genes for the fatty acid binding protein (FABP) family encode small 14–15 kDa cytosolic proteins and can be regulated during type 2 diabetes mellitus (T2DM) and obesity. This study compared association of single nucleotide polymorphisms (SNPs) in FABP1-5 with T2DM in different ethnic groups. Associations with T2DM of SNPs in these proteins were assessed in African American (AA), non-Hispanic White (NHW), and Hispanic American (HA) individuals. A total of 650 DNA samples were genotyped; control samples were obtained from Coriell's North American Human Variation Panel Repository (NAVP) of apparently healthy individuals and T2DM cases were taken from the American Diabetes Association GENNID Study. The rs454550 SNP of FABP5 showed a significant association with T2DM in NHW (OR: 9.03, 95% CI: 1.13–71.73, $p=0.014$). Our analysis also identified a new FABP5 SNP (nSNP) that showed a significant association with T2DM in NHW (OR: 0.44, 95% CI: 0.19–0.99, $p=0.045$) and AA (OR: 0.17, 95% CI: 0.03–0.80, $p=0.016$). The Ala54Thr FABP2 polymorphism was significantly associated with T2DM in HA individuals only (OR: 1.85, 95% CI: 1.05–3.27, $p=0.032$). All other FABP SNPs did not show association with T2DM. These findings suggest a potential distinct role of SNPs in FABP5, 2 genes in T2DM in different populations.

Keywords

Fatty Acid Binding Protein (FABP); Single Nucleotide Polymorphism (SNP) and Type 2 Diabetes (T2DM)

Introduction

Type 2 Diabetes Mellitus (T2DM) is a complex, multi-factorial metabolic disorder that exhibits a wide range of disparities among different ethnic groups (1–4). Genetic linkage analyses and T2DM association studies have shown a dynamic interplay between genetic and environmental components (5). In the United States alone, about 11% of the population 20 years of age and older has T2DM and 40% of these cases may be undiagnosed (1,4). Hispanic American (HA) and African American (AA) individuals exhibit twice the incidence of T2DM when compared to non-Hispanic whites (NHW). Due to the strong

[§]Corresponding author: LB: bliming@llu.edu, KDL: kdeleon@llu.edu, LS: lsalto@llu.edu, MDL: madeleon@llu.edu.

Conflict of Interest

The authors declare that they have no conflict of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

genetic component of T2DM, there is a need to identify promising target genes to develop adequate therapies and preventive educational interventions.

The fatty acid binding protein 5 (FABP5), also known as E-FABP, DA11, mal1 and PAFABP, belongs to the family of fatty acid-binding proteins (FABPs), a group of small intracellular 14–15 kDa cytoplasmic proteins that bind and transport long-chain free fatty acids. FABP5 is expressed in different tissues and organs including the skin, liver and the nervous system (6). The unique properties of FABPs as free fatty acids carriers in the cytoplasm have been the focus of intense studies because fatty acids can affect glucose metabolism by altering insulin receptor sensitivity (10). For instance, high levels of free fatty acids may result in insulin resistance and in glucose intake capacity reduction (7); members of the FABP family can be involved in regulating this process in cells. FABP5/E-FABP/mal1 may play a role in T2DM and obesity by protecting myocytes from the cellular toxicity of cholesterol, free fatty acids and lipid oxidants (8,9). Studies using the mal1/FABP5^{-/-} mice have shown that these animals exhibit an increased systemic insulin sensitivity (10). This and other findings reported independently by several groups, prompted studies to determine if single nucleotide polymorphisms (SNP) in members of FABP family of proteins are important regulators of diseases such as T2DM and obesity. Genetic association studies analyzing FABP1, FABP2, FABP3 and FABP4 genes have identified SNPs associated with an increased risk of developing T2DM and/or obesity in several populations (11–15). However, while FABP5 expression has been increasingly associated with T2DM and obesity, the literature does not report studies examining significant polymorphism associations.

The initial objective of this study was to identify and characterize SNPs in the FABP5 gene and to determine if significant associations with T2DM exist. To have a more comprehensive view of our findings, SNPs reported for other FABPs were also genotyped and used for comparison. The potential polymorphisms were characterized using established DNA collections from different ethnic groups and validated using more than one method of analysis. Our results support several key findings: (1) two SNPs in the FABP5 gene show significant associations with T2DM. The FABP5 rs454550 SNP association with T2DM was significant in NHW, but not in the HA and AA cohorts. A new SNP, nSNP, was identified in the regulatory region of the FABP5 gene. The results show an association between the minor allele of the nSNP and absence of T2DM in AA and NHW but not in HA. (2) The Ala54Thr SNP in the FABP2 gene is associated with T2DM in HA but not in AA or NHW.

Materials and methods

The study analyzed a total of 650 DNA samples obtained from the Coriell Institute for Medical Research Repository, Camden NJ, USA. Three hundred thirty (330) DNA samples, 110 for each ethnic group respectively, were obtained from the North American Human Variation Panel (NAHVP) collection of apparently healthy individuals as the control group. DNA samples from participants of the Genetics of Non-Insulin Dependent Diabetes Mellitus (GENNID) Study repository (16) were selected at random from non-related individuals of AA (n=100), NHW (n=100) and HA (n=120) ethnicities for the type 2 diabetes mellitus cases. The Coriell Institute for Medical Research Repository obtains DNA samples from clinicians and researchers who use a standardized National Institute of General Medical Sciences informed consent for all donors/subjects. The DNA samples are anonymized and all donors have provided informed consent for the use of their DNA in research. The GENNID study collected informed consents for all sib-pairs (index cases) and family members enrolled in the study once the complete family pedigree was admitted into the study (17). According to the GENNID study, the criteria used to define type 2 diabetes mellitus cases is based on the criteria set forth by the National Diabetes Data Group at the

collection of the samples. At this time, type 2 diabetes was defined as having a fasting plasma glucose concentration of 140 mg/dl or greater on more than one occasion. Index cases could also be included in the study if they had a plasma glucose concentration of 200 mg/dl or greater, in the 2-hour sample and in at least one other sample during an oral glucose tolerance test. The criterion for defining type 2 diabetes mellitus using fasting plasma glucose concentration has changed. The National Diabetes Data Group now defines type 2 diabetes as having a fasting plasma glucose concentration of 126 mg/dl or greater on more than one occasion. The cut point for the oral glucose tolerance test concentration has remained the same. All of the cases defined as having type 2 diabetes mellitus in the original study would currently qualify as type 2 diabetes mellitus cases. According to the Coriell Institute, the DNA control samples used for the study are classified as “apparently healthy” by the investigators who submit the samples to the Repository. Along with each sample collected, a submission form is used to request available clinical, molecular, cytogenetic and biochemical data. (<http://ccr.coriell.org/Sections/Support/NIGMS>). These samples were first used to characterize seven putative SNPs in the FABP5 gene reported in the NCBI and SNPper (<http://www.ncbi.nlm.nih.gov/SNP> and <http://snpper.chip.org>) databases: rs454550(C/G), rs391842 (A/G), rs162115(C/T), rs408070 (A/G), rs11542504(C/T), rs3180883(C/T), and rs10098288 (G/T). Furthermore, genotyping and sequencing resulted in the identification of a new SNP in FABP5 (nSNP) that was also used to perform T2DM association studies. SNPs in FABP1, FABP2, FABP3 and FABP4 genes were selected from four published papers: rs52241883 in FABP1 (18); A154Thr in FABP2 (19); rs2271072 and rs2279885 in FABP3 (20); and SNP -87T > C in FABP4 (21). Procedures used included PCR combined with confronting two-pair primers (PCR-CTPP) (22) and TaqMan Allelic Discrimination Assay (Applied Biosystems Inc., Foster City, CA, USA). The SDS 2.3 Allelic Discrimination software was employed for genotyping characterization. We did a final confirmation using direct DNA sequencing of the SNP region of interest in FABP5. Each SNP was confirmed with at least two of these methods getting the same results. For the PCR-CTPP approach, two overlapping PCR products were amplified covering the selected SNP region. The primers used for the rs454550 SNP were: 5'-GGCAGGGACTTGAAATGAAA-3' and 5'-GGAAGTGATTGATCGGCTGT-3'; 5'-GAAACCACACTTCCCGAGAC-3' and 5'-GGGACCTAGGGACAGACGAC-3'. The primers used for the nSNP were: 5'-TGG GAG ATA GCA AAC CAA CC -3' and 5'-TGC CTG TTG GAA GTC AGA TG -3'; 5'-CTA TAA AGT GCC CAG GAA GCA CA -3' and 5'-TGA GTA GCT ACT AAG TGC TAG AC -3'. Power analysis for the study was calculated using the CaTS power calculator (23). The Linkage Disequilibrium (LD) value (D') was calculated using Haploview (24,25). Statistical analyses were performed using PASW Statistics v 18.0 (IBM: SPSS inc.). Distributions of genotypes, allele frequencies and their association with T2DM were evaluated by Pearson's chi-square and by Fisher's exact test. The ethnic groups were separated for the analysis. The minor allele genotypes were collapsed to compute odds ratios (OR) and 95% confidence intervals (95% CI). Due to poor age-matching between the cases and the controls, an odds ratio was computed, age-crude, for the full sample and for an ethnic-specific age-range subgroup, where the range in ages for the cases and controls overlapped. Logistic regression analysis was used to estimate an age-adjusted OR, where age was entered into the model as a categorical variable in the age range subgroup. The Hardy-Weinberg Equilibrium (HWE) was assessed for all genotypes in each ethnic group, and all except the FABP4 SNP 87T>C obeyed HWE. Statistical significance was accepted at $P < 0.05$.

Results

Table 1 shows the age and gender distribution of the cases and controls. For all 3 ethnic groups, the controls were significantly younger than the cases. Only the males in the African American comparison group were underrepresented. Table 2 shows the age, anthropometric

and blood chemistry characteristics for the T2DM cases by ethnic group; age, weight, fasting blood glucose (mg/dl), insulin (uU/ml), HDL cholesterol (mg/dl), and triglyceride (mg/dl) values were significantly different between the ethnic groups. Our analysis of the putative reported SNPs in FABP5 (see Methods) showed that only rs454550 exhibited polymorphism in all the DNA samples analyzed. Furthermore, analysis of the DNA sequencing of the region revealed the presence of an unreported, new SNP (nSNP) at genomic position 82354416, position -1031 upstream to the first ATG in exon 1 of FABP5. Figure 1A shows representative alignments of the DNA sequence in the region containing the nSNP, located between rs454550 and rs391842. The letter “N” in samples NA17033 and NA17040 indicated that the genotypes at this position were heterozygous but not homozygous (A/A) as observed in other samples. DNA sequence chromatograms from this genomic region (Figure 1B) demonstrate that overlapping peaks in this position were heterozygous A/G, while a single peak in this position representing homozygous A/A (Wild Type) or G/G. Figure 2A and 2B show examples of agarose gels confirming rs454550 and the nSNP using the PCR-CTPP approach by which two allele-specific bands and one consensus band were identified.

The genotype and allele frequency distributions for FABP2 and FABP5 are shown in Table 3. Although the genotype distributions, across the three genotype categories, are not significantly different between ethnic-specific T2DM cases and controls, the allele frequencies mirror the same significant associations for FABP2 and FABP5 when the genotypes are collapsed (See Table 3 and Table 5). When the minor genotypes are collapsed, our results show (Table 5) that SNP rs454550 (G/C) and the novel nSNP (A/G) in the FABP5 gene show association with T2DM in AA and NHW cohorts, but not in the HA cohort. In the NHW cohort, the minor ‘C’ allele carriers of rs454550 polymorphism exhibited a 9.03 times higher risk of having T2DM compared to the “G” allele carriers (OR=9.03, 95% CI: 1.13–71.73, $p=0.014$). This association was not significant in a smaller, age-adjusted subgroup (OR: 4.94, 95% CI: 0.55–43.73, $p=0.151$) (See Table 6). In FABP5, the minor “G” allele of the nSNP shows a protective association against T2DM compared to the “A” allele in the AA (OR=0.17, 95%CI: 0.03–0.80, $p=0.016$) and NHW cohorts (OR=0.44, 95%CI: 0.19–0.99, $p=0.045$); or, presence of the minor allele is associated with an absence of T2DM (Table 5). This association remained significant when adjusted for age in the NHW group only (OR=0.30, 95% CI: 0.09–0.96, $p=0.044$) (Table 6). The Ala54Thr SNP in FABP2 showed a significant association with T2DM in the HA cohort. Hispanic American threonine carriers (allele A) were more likely to have T2DM (OR=1.85, 95% CI: 1.05–3.27, $p=0.032$) (Table 5). When adjusted for age (Table 6) in a smaller subgroup, the Ala54Thr association with T2DM remained significant for the HA group (OR=3.04, 95% CI: 1.33, 6.95, $p=0.008$). The rs52241883 (FABP1), rs2271072 and rs2279885 (FABP3) SNPs did not show association with T2DM in any of the groups studied and FABP4 (-87T>C) did not show polymorphism (Table 4).

The power of the present study varied according to the allelic frequencies of the rs454550 and the nSNP polymorphisms in FABP5. Under the dominant model, when the significance level and relative risk (RR) were set at 0.05 and 2.5 respectively, the power to determine an association between the nSNP and T2DM was highest in the NHW group (81%), followed by the HA group (73%) and the AA group (67%). In contrast, the power to determine an association between rs454550 and T2DM was 14% for AA, 58% for NHW and 57% for HA. The low power of rs454550 to predict an association in the AA group can be attributed to the minor allele frequency of rs454550 at 0.5% even though the prevalence of T2DM is 12.8% in this population group. Due to the low power of this study for the rs454550 AA analysis, future studies using larger DNA collections should confirm whether an association exists between rs454550 and T2DM in AA. In the NHW group, the wide confidence intervals for the age-crude odds ratio suggest that a larger sample size might be needed to

obtain a more precise point estimate. The power to explore the association of the Ala54Thr polymorphism with T2DM was greater than 85% among the AA, NHW and HA groups; suggesting that our sample size was adequate for determining this association. In the human genome, LD happens in large blocks of the genome with very low recombination, and the LD blocks can cover 5–20 kb. However, in some circumstances these blocks can expand from 3 to 92 kb. The rs454550 and nSNP polymorphisms do not show strong LD but rather an incomplete LD ($D' = 0.483$); suggesting that these alleles may have independent associations with T2DM.

Discussion

Our results suggest that DNA polymorphisms in the FABP5 gene can play distinct roles in the incidence and/or development of T2DM. This study investigated potential associations of SNPs in FABP5 with T2DM and reports for the first time the identification of a novel SNP (nSNP) in the regulatory region of FABP5. Interestingly, the characterization of putative SNPs in the FABP5 gene showed that only rs454550 and the nSNP exhibited polymorphisms and associations with T2DM in NHW and AA groups. The association of the FABP2 Ala54Thr SNP with T2DM in HA is consistent with previous studies reporting significant associations of this SNP with obesity and diabetes (26,27). The differences observed in the associations of these SNPs in different ethnic groups highlight the need to consider ethnicity when conducting this type of analysis. The differences in SNP frequencies among different ethnic groups may be an important factor in studies evaluating the genetic and environmental interaction and etiology of T2DM.

FABP5 nSNP and rs454550 localization in the regulatory region of the gene presents a new target to assess whether these allele changes are important in the transcriptional regulation of the gene in T2DM. Previous studies have shown that FABP5 exhibits a robust up regulation in response to different stressors' stimuli (28,29) and lipid metabolism (30). Polymorphisms in the promoter region of FABP5 may influence binding affinities for transcription factors such as PPARs, RXR α and Oct-1 as shown for FABP2 (31). Polymorphisms in the FABP2 promoter result in two promoters haplotypes (A and B), which exhibit different transcriptional properties. Activation of haplotypes by rosiglitazone-stimulated PPAR/RXR α reveals two-fold higher activity of haplotype B in comparison to haplotype A. This is a critical finding considering that the FABP2 promoter haplotype B can significantly decrease the risk of T2DM in comparison to haplotype A (32).

EFABP/DA11/FABP5 was originally identified in the skin (33), and independently after screening axotomized rat dorsal root ganglia (28,34–36). EFABP/FABP5/DA11 can protect cells against the cellular toxicity of cholesterol, free fatty acid and lipid oxidants (37). FABP5 gene expression shows a robust up regulation during free fatty acid absorption and metabolism, suggesting its involvement in lipid signaling cascades (38). FABP5 has also been associated with insulin resistance, cardiovascular disease, and hypertension (32,39–41). In nonalcoholic fatty liver patients, the expression level of FABP5 is increased more than two fold compared to normal tissue (42). In the liver of ob/ob mice, FABP5 is induced at least two-fold compared to lean mice. Moreover, mal11 (FABP5) deficient mice are protected from diet-induced obesity and accumulation of fat in the liver (43) and FABP5-deficient mice show improvement in insulin sensitivity associated with obesity. Interestingly, over expression of FABP5 in mice leads to a reduction in insulin sensitivity; adipocytes show an enhanced insulin-stimulated glucose transporting capacity (10). Thus, FABP5/mal1 can regulate not only fat metabolism, but it can also affect insulin resistance for glucose transport.

Numerous genetic studies have shown significant associations with polymorphism in the FABP2 gene and T2DM as well as other disorders. The common missense variation Ala54Thr in FABP2 has been previously associated with insulin resistance, T2DM and postprandial lipidemia (44,45). Genetic expression levels of FABP2 vary according to different alleles of the gene and also vary according to different levels of exercise, diet and other lifestyle factors. The FABP2 Ala54Thr SNP association with insulin resistance, obesity and T2DM has previously studied in the context of ethnic background. Similar to our findings, Lei et al (46) did not find an association between the Ala54Thr SNP and T2DM in AA. Furthermore, the Ala54Thr SNP was associated with obesity but not T2DM in a study of Greek volunteers (47). However, in a German cohort, the Thr54 carriers were associated with a lower risk of T2DM and obesity in women (48). Our findings showing that the FABP2 Ala54Thr SNP exhibits a significant association with T2DM in HA agrees with a previous study performed in Chilean older adults (49). This four-year longitudinal study conducted among the Aymara and Mapuche indigenous populations showed a strong association between the Ala54Thr polymorphism in FABP2 and diabetes (49). Of particular significance is that this FABP2 SNP seemed to play a more prominent role in the Aymara than in the Mapuche population (50) and suggest that the effect of this polymorphism on insulin resistance may be modulated by genetic and environmental factors.

Fisher et al (18) reported that the FABP1 T94A polymorphism is associated with fasting triglycerides and LDL-cholesterol in women, but did not find association with CVD or T2DM. Further, FABP1 T94A was associated with atherothrombotic cerebral infarction among Japanese individuals with metabolic syndrome (51) in contrast to our findings that did not show any association between FABP1 T94A and T2DM in AA, NHW and HA groups. In a different study, a decrease in H-FABP (FABP3) protein seems to normalize fasting glucose levels and improves insulin sensitivity in high fat diet-fed mice despite obesity (52). To further investigate FABP3 effect Shin et al.(20) reported that one insertion/deletion polymorphism in FABP3 had a weak association with an increased risk of T2DM, but other SNP's tested in his study did not show significant associations with T2DM. In our study, we tested two FABP3 SNPs, rs2271072 and rs2279885, but we did not find any association between those SNP's and T2DM in AA, NHW and HA cohorts. Our results do not support an association between SNP -87T>C in FABP4 with T2DM, although previous studies have reported an FABP4 (aP2) -87T>C polymorphism association with lower serum triglyceride levels and a reduced risk for coronary heart disease and type 2 diabetes (21). Our findings on the FABP4 -87T>C polymorphism should be interpreted with caution because the allele frequencies of SNP -87>C in our sample are low.

To our knowledge, the associations between polymorphisms for the FABP5 genes and T2DM have not been previously reported. However, some of the findings in our study should be interpreted with caution and may need to be confirmed using a larger collection. Primarily, in general the ages of the controls poorly match the ages of the T2DM cases. We addressed this limitation by analyzing a subgroup in each ethnic group where the ages for the cases and controls overlapped (Age Range Subgroup, see Table 6). For AA, the age range was 27–58 years, for NHW, 30–79 years, and for HA, 36–62 years of age. In these respective subgroups, logistic regression analysis, adjusted for age, verified the results of the full sample for the HA FABP2 Thr carrier association with T2DM and the FABP5 nSNP minor allele association with absence of T2DM in NHW. Logistic regression analysis also yielded significant associations for the Ala54Thr polymorphism and T2DM in AA and NHW age range subgroups. Since these effects in AA and NHW are not seen in the larger sample, the significant associations in the smaller, age range subgroup might be due to selection bias, rather than to a true effect. Future studies should verify these results in all three ethnic groups. Also, our control samples did not have blood chemistry, weight, BMI or

other anthropometric data; this limited the analysis we could perform, since the cases and controls only share age, ethnicity and gender data.

In conclusion, our results show significant associations between polymorphisms in the FABP5 gene and T2DM. The rs454550 and nSNP (new SNP) p show a significant association with T2DM in DNA samples from NHW and AA but not from HA collections. The results also show a significant association between the Ala54Thr FABP2 SNP with T2DM in a HA but not in DNA samples from the AA or NHW collection. These significant associations with T2DM suggest that DNA polymorphisms in FABP5 and FABP2 genes may be important in understanding the incidence and/or development of T2DM. These results merit investigation of these associations in future studies using larger samples collections of diabetic and control populations. The knowledge gained may serve to enhance the prevention and treatment of this serious condition. Taken together these results highlight the need to further study the importance of significant associations between type 2 diabetes mellitus and single nucleotide polymorphisms in members of the FABP gene family.

Acknowledgments

We acknowledge the Coriell Institute for Medical Research for providing DNA samples (NAHVP) and the T2DM participants and the GENNID study (American Diabetes Association) and the GENNID study investigators. Lastly, we would like to thank Dr. Jo-Wen Liu for important discussions, Dr. Johnny D. Figueroa, Amelia Padilla and Dr. Anamika Basu, for their valuable scientific criticism in the course of the study.

This work was supported by NIH grants [5P20MD001632-05](#) and [2R25GM060507-09](#)

References

1. Mitchell P, Gopinath B, McMahon CM, Rochtchina E, Wang JJ, Boyages SC, Leeder SR. Relationship of Type 2 diabetes to the prevalence, incidence and progression of age-related hearing loss. *Diabet Med*. 2009; 26:483–488. [PubMed: 19646187]
2. Soria ML, Sy RG, Vega BS, Ty-Willing T, Abenir-Gallardo A, Velandria F, Punzalan FE. The incidence of type 2 diabetes mellitus in the Philippines: A 9-year cohort study. *Diabetes Res Clin Pract*. 2009; 86:130–133. [PubMed: 19766344]
3. Seifi S, Rahbar M, Lessan-Pezeshki M, Khatami MR, Abbasi MR, Mahdavi-Mazdeh M, Ahmadi F, Maziar S. Posttransplant diabetes mellitus: incidence and risk factors. *Transplant Proc*. 2009; 41:2811–2813. [PubMed: 19765442]
4. Evertsen J, Alemzadeh R, Wang X. Increasing incidence of pediatric type 1 diabetes mellitus in Southeastern Wisconsin: relationship with body weight at diagnosis. *PLoS One*. 2009; 4:e6873. [PubMed: 19727402]
5. Clement K, Dina C, Basdevant A, Chastang N, Pelloux V, Lahlou N, Berlan M, Langin D, Guy-Grand B, Froguel P. A sib-pair analysis study of 15 candidate genes in French families with morbid obesity: indication for linkage with islet 1 locus on chromosome 5q. *Diabetes*. 1999; 48:398–402. [PubMed: 10334320]
6. Storch J, McDermott L. Structural and functional analysis of fatty acid-binding proteins. *J Lipid Res*. 2009; 50(Suppl):S126–131. [PubMed: 19017610]
7. Ebbesson SO, Tejero ME, Lopez-Alvarenga JC, Harris WS, Ebbesson LO, Devereux RB, Maccluer JW, Wenger C, Laston S, Fabsitz RR, Howard BV, Comuzzie AG. Individual saturated fatty acids are associated with different components of insulin resistance and glucose metabolism: the GOCADAN study. *Int J Circumpolar Health*. 2010
8. Bennaars-Eiden A, Higgins L, Hertzel AV, Kapphahn RJ, Ferrington DA, Bernlohr DA. Covalent modification of epithelial fatty acid-binding protein by 4-hydroxynonenal in vitro and in vivo. Evidence for a role in antioxidant biology. *J Biol Chem*. 2002; 277:50693–50702. [PubMed: 12386159]

9. Kucharekova M, Vissers WH, Schalkwijk JJ, van de Kerkhof PC, van der Valk PG. Lack of upregulation of epidermal fatty acid binding protein in dithranol induced irritation. *Eur J Dermatol.* 2003; 13:254–257. [PubMed: 12804984]
10. Maeda K, Uysal KT, Makowski L, Gorgun CZ, Atsumi G, Parker RA, Bruning J, Hertz AV, Bernlohr DA, Hotamisligil GS. Role of the fatty acid binding protein mal1 in obesity and insulin resistance. *Diabetes.* 2003; 52:300–307. [PubMed: 12540600]
11. Chan KH, Song Y, Hsu YH, You NC, LFT, Liu S. Common Genetic Variants in Fatty Acid-Binding Protein-4 (FABP4) and Clinical Diabetes Risk in the Women's Health Initiative Observational Study. *Obesity (Silver Spring).* 2010
12. Hoashi S, Hinenoya T, Tanaka A, Ohsaki H, Sasazaki S, Taniguchi M, Oyama K, Mukai F, Mannen H. Association between fatty acid compositions and genotypes of FABP4 and LXR-alpha in Japanese black cattle. *BMC Genet.* 2008; 9:84. [PubMed: 19077218]
13. Cho S, Park TS, Yoon DH, Cheong HS, Namgoong S, Park BL, Lee HW, Han CS, Kim EM, Cheong IC, Kim H, Shin HD. Identification of genetic polymorphisms in FABP3 and FABP4 and putative association with back fat thickness in Korean native cattle. *BMB Rep.* 2008; 41:29–34. [PubMed: 18304447]
14. Coll B, Cabre A, Alonso-Villaverde C, Lazaro I, Aragonés G, Parra S, Girona J, Masana L. The fatty acid binding protein-4 (FABP4) is a strong biomarker of metabolic syndrome and lipodystrophy in HIV-infected patients. *Atherosclerosis.* 2008; 199:147–153. [PubMed: 17983623]
15. Maclaren R, Cui W, Lu H, Simard S, Cianflone K. Association of adipocyte genes with ASP expression: a microarray analysis of subcutaneous and omental adipose tissue in morbidly obese subjects. *BMC Med Genomics.* 2010; 3:3. [PubMed: 20105310]
16. Malhotra A, Wolford JK. Analysis of quantitative lipid traits in the genetics of NIDDM (GENNID) study. *Diabetes.* 2005; 54:3007–3014. [PubMed: 16186406]
17. Raffel LJ, Robbins DC, Norris JM, Boerwinkle E, DeFronzo RA, Elbein SC, Fujimoto W, Hanis CL, Kahn SE, Permutt MA, Chiu KC, Cruz J, Ehrmann DA, Robertson RP, Rotter JJ, Buse J. The GENNID Study. A resource for mapping the genes that cause NIDDM. *Diabetes Care.* 1996; 19:864–872. [PubMed: 8842605]
18. Fisher E, Weikert C, Klapper M, Lindner I, Mohlig M, Spranger J, Boeing H, Schrezenmeier J, Doring F. L-FABP T94A is associated with fasting triglycerides and LDL-cholesterol in women. *Mol Genet Metab.* 2007; 91:278–284. [PubMed: 17485234]
19. Pratley RE, Baier L, Pan DA, Salbe AD, Storlien L, Ravussin E, Bogardus C. Effects of an Ala54Thr polymorphism in the intestinal fatty acid-binding protein on responses to dietary fat in humans. *J Lipid Res.* 2000; 41:2002–2008. [PubMed: 11108733]
20. Shin HD, Kim LH, Park BL, Jung HS, Cho YM, Moon MK, Lee HK, Park KS. Polymorphisms in fatty acid-binding protein-3 (FABP3) -putative association with type 2 diabetes mellitus. *Hum Mutat.* 2003; 22:180. [PubMed: 12872269]
21. Tuncman G, Erbay E, Hom X, De Vivo I, Campos H, Rimm EB, Hotamisligil GS. A genetic variant at the fatty acid-binding protein aP2 locus reduces the risk for hypertriglyceridemia, type 2 diabetes, and cardiovascular disease. *Proc Natl Acad Sci U S A.* 2006; 103:6970–6975. [PubMed: 16641093]
22. Hamajima N, Saito T, Matsuo K, Kozaki K, Takahashi T, Tajima K. Polymerase chain reaction with confronting two-pair primers for polymorphism genotyping. *Jpn J Cancer Res.* 2000; 91:865–868. [PubMed: 11011111]
23. Skol AD, Scott LJ, Abecasis GR, Boehnke M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet.* 2006; 38:209–213. [PubMed: 16415888]
24. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics.* 2005; 21:263–265. [PubMed: 15297300]
25. Ardlie KG, Kruglyak L, Seielstad M. Patterns of linkage disequilibrium in the human genome. *Nat Rev Genet.* 2002; 3:299–309. [PubMed: 11967554]
26. Formanack ML, Baier LJ. Variation in the FABP2 promoter affects gene expression: implications for prior association studies. *Diabetologia.* 2004; 47:349–351. [PubMed: 14666368]

27. de Luis DA, Aller R, Izaola O, Sagrado MG, Conde R. Influence of Ala54Thr polymorphism of fatty acid-binding protein 2 on weight loss and insulin levels secondary to two hypocaloric diets: a randomized clinical trial. *Diabetes Res Clin Pract.* 2008; 82:113–118. [PubMed: 18701184]
28. De Leon M, Welcher AA, Nahin RH, Liu Y, Ruda MA, Shooter EM, Molina CA. Fatty acid binding protein is induced in neurons of the dorsal root ganglia after peripheral nerve injury. *J Neurosci Res.* 1996; 44:283–292. [PubMed: 8723767]
29. Biron-Shental T, Schaiff WT, Ratajczak CK, Bildirici I, Nelson DM, Sadovsky Y. Hypoxia regulates the expression of fatty acid-binding proteins in primary term human trophoblasts. *Am J Obstet Gynecol.* 2007; 197:516e511–516. [PubMed: 17826730]
30. Storch J, Thumser AE. Tissue-specific functions in the FABP (fatty acid-binding protein) family. *J Biol Chem.* 2010
31. Bohme M, Nitz I, Doring F, Klapper M. Analysis of the transcriptional regulation of the FABP2 promoter haplotypes by PPARGgamma/RXRalpha and Oct-1. *Biochim Biophys Acta.* 2008; 1779:616–621. [PubMed: 18634911]
32. Li Y, Fisher E, Klapper M, Boeing H, Pfeiffer A, Hampe J, Schreiber S, Burwinkel B, Schrezenmeir J, Doring F. Association between functional FABP2 promoter haplotype and type 2 diabetes. *Horm Metab Res.* 2006; 38:300–307. [PubMed: 16718625]
33. Watanabe R, Fujii H, Odani S, Sakakibara J, Yamamoto A, Ito M, Ono T. Molecular cloning of a cDNA encoding a novel fatty acid-binding protein from rat skin. *Biochem Biophys Res Commun.* 1994; 200:253–259. [PubMed: 8166694]
34. Liu JW, Almaguel FG, Bu L, De Leon DD, De Leon M. Expression of E-FABP in PC12 cells increases neurite extension during differentiation: involvement of n-3 and n-6 fatty acids. *J Neurochem.* 2008; 106:2015–2029. [PubMed: 18513372]
35. Liu Y, Longo LD, De Leon M. In situ and immunocytochemical localization of E-FABP mRNA and protein during neuronal migration and differentiation in the rat brain. *Brain Res.* 2000; 852:16–27. [PubMed: 10661491]
36. Liu Y, Molina CA, Welcher AA, Longo LD, De Leon M. Expression of DA11, a neuronal-injury-induced fatty acid binding protein, coincides with axon growth and neuronal differentiation during central nervous system development. *J Neurosci Res.* 1997; 48:551–562. [PubMed: 9210525]
37. Hoekstra M, Stitzinger M, van Wanrooij EJ, Michon IN, Kruijt JK, Kamphorst J, Van Eck M, Vreugdenhil E, Van Berkel TJ, Kuiper J. Microarray analysis indicates an important role for FABP5 and putative novel FABPs on a Western-type diet. *J Lipid Res.* 2006; 47:2198–2207. [PubMed: 16885566]
38. van der Horst DJ, van Doorn JM, Passier PC, Vork MM, Glatz JF. Role of fatty acid-binding protein in lipid metabolism of insect flight muscle. *Mol Cell Biochem.* 1993; 123:145–152. [PubMed: 8232256]
39. Li Z, Huang C, Bai S, Pan X, Zhou R, Wei Y, Zhao X. Prognostic evaluation of epidermal fatty acid-binding protein and calcyphosine, two proteins implicated in endometrial cancer using a proteomic approach. *Int J Cancer.* 2008; 123:2377–2383. [PubMed: 18729184]
40. Yamamoto N, Kaneko I, Motohashi K, Sakagami H, Adachi Y, Tokuda N, Sawada T, Furukawa H, Ueyama Y, Fukunaga K, Ono M, Kondo H, Owada Y. Fatty acid-binding protein regulates LPS-induced TNF-alpha production in mast cells. *Prostaglandins Leukot Essent Fatty Acids.* 2008; 79:21–26. [PubMed: 18678477]
41. Yeung DC, Xu A, Tso AW, Chow WS, Wat NM, Fong CH, Tam S, Sham PC, Lam KS. Circulating levels of adipocyte and epidermal fatty acid-binding proteins in relation to nephropathy staging and macrovascular complications in type 2 diabetic patients. *Diabetes Care.* 2009; 32:132–134. [PubMed: 18931100]
42. Westerbacka J, Kolak M, Kiviluoto T, Arkkila P, Siren J, Hamsten A, Fisher RM, Yki-Jarvinen H. Genes involved in fatty acid partitioning and binding, lipolysis, monocyte/macrophage recruitment, and inflammation are overexpressed in the human fatty liver of insulin-resistant subjects. *Diabetes.* 2007; 56:2759–2765. [PubMed: 17704301]
43. Maeda K, Cao H, Kono K, Gorgun CZ, Furuhashi M, Uysal KT, Cao Q, Atsumi G, Malone H, Krishnan B, Minokoshi Y, Kahn BB, Parker RA, Hotamisligil GS. Adipocyte/macrophage fatty

- acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metab.* 2005; 1:107–119. [PubMed: 16054052]
44. Silver K. Identities and locations of 12 uranium mills in 1959 Atomic Energy Commission report. *Arch Environ Health.* 2000; 55:361–362. [PubMed: 11063412]
 45. Weiss EP, Brown MD, Shuldiner AR, Hagberg JM. Fatty acid binding protein-2 gene variants and insulin resistance: gene and gene-environment interaction effects. *Physiol Genomics.* 2002; 10:145–157. [PubMed: 12209017]
 46. Lei HH, Coresh J, Shuldiner AR, Boerwinkle E, Brancati FL. Variants of the insulin receptor substrate-1 and fatty acid binding protein 2 genes and the risk of type 2 diabetes, obesity, and hyperinsulinemia in African-Americans: the Atherosclerosis Risk in Communities Study. *Diabetes.* 1999; 48:1868–1872. [PubMed: 10480621]
 47. Tavidou A, Arvanitidis KI, Tiptiri-Kourpeti A, Petridis I, Ragia G, Kyroglou S, Christakidis D, Manolopoulos VG. Thr54 allele of fatty-acid binding protein 2 gene is associated with obesity but not type 2 diabetes mellitus in a Caucasian population. *Diabetes Res Clin Pract.* 2009; 84:132–137. [PubMed: 19324445]
 48. Fisher E, Li Y, Burwinkel B, Kuhr V, Hoffmann K, Mohlig M, Spranger J, Pfeiffer A, Boeing H, Schrezenmeir J, Doring F. Preliminary evidence of FABP2 A54T polymorphism associated with reduced risk of type 2 diabetes and obesity in women from a German cohort. *Horm Metab Res.* 2006; 38:341–345. [PubMed: 16718632]
 49. Albala C, Villarroel A, Santos JL, Angel B, Lera L, Liberman C, Sanchez H, Perez-Bravo F. FABP2 Ala54Thr polymorphism and diabetes in Chilean elders. *Diabetes Res Clin Pract.* 2007; 77:245–250. [PubMed: 17292994]
 50. Perez-Bravo F, Fuentes M, Angel B, Sanchez H, Carrasco E, Santos JL, Lera L, Albala C. Lack of association between the fatty acid binding protein 2 (FABP2) polymorphism with obesity and insulin resistance in two aboriginal populations from Chile. *Acta Diabetol.* 2006; 43:93–98. [PubMed: 17211557]
 51. Yamada Y, Kato K, Oguri M, Yoshida T, Yokoi K, Watanabe S, Metoki N, Yoshida H, Satoh K, Ichihara S, Aoyagi Y, Yasunaga A, Park H, Tanaka M, Nozawa Y. Association of genetic variants with atherothrombotic cerebral infarction in Japanese individuals with metabolic syndrome. *Int J Mol Med.* 2008; 21:801–808. [PubMed: 18506375]
 52. Shearer J, Fueger PT, Bracy DP, Wasserman DH, Rottman JN. Partial gene deletion of heart-type fatty acid-binding protein limits the severity of dietary-induced insulin resistance. *Diabetes.* 2005; 54:3133–3139. [PubMed: 16249436]

Figure 1. Example of DNA sequences showing polymorphism of nSNP in promoter region of FABP5 (EFABP). **A:** Sequences alignment of FABP5 (EFABP) for genotyping. The N in the box indicates a new variant that has been temporary named nSNP (A/G). **B:** Sequencing chromatography. Homozygous A/A and GG, heterozygous A/G.

Figure 2.

Characterization of two SNPs in the FABP5 gene using PCR-CTPP. Panels **A** and **B** show two typical agarose gels used to initially identify genotypes for SNPs rs454550 and nSNP. Panel **A** shows specific bands for allele C (221bp), allele G (167bp) and the consensus sequence (349 bp) of rs454550 SNP. Panel **B** shows bands for allele A (104 bp), allele G (292 bp) and the consensus sequence (395 bp) of nSNP. All genotypes in the study were further confirmed by DNA sequencing.

Table 1
Ethnic Specific Age and Gender Characteristics for Type 2 Diabetes Cases and Controls

	Age		Gender	
	Mean $\pm$ SD ^a	p-value ^b	Males (n)(%) ^a	Females (n)(%) ^a p-value ^c
African Americans				
CTL (n=105)	29.2 $\pm$ 9.4		16 (15.2%)	89 (84.8%)
T2DM (n=101)	54.8 $\pm$ 11.1	< 0.001**	34 (34.7%)	64 (65.3%)
Non-Hispanic Whites				
CTL (n=106)	37.1 $\pm$ 15.8		52 (49.1%)	54 (50.9%)
T2DM (n=100)	61.8 $\pm$ 10.9	< 0.001**	40 (46.5%)	46 (53.5%)
Hispanic Americans				
CTL (n=106)	30.5 $\pm$ 13.2		49 (46.2%)	57 (53.8%)
T2DM (n=119)	57.5 $\pm$ 10.8	< 0.001**	43 (36.1%)	76 (63.9%)

^a Mean, SD and percentages based on valid cases, excluding missing data

^b p-value based on the Mann Whitney U statistic

^c p-value based on the Chi-square test

* p-value < 0.01,

** p-value < 0.001

Table 2

Ethnic Specific Age, Anthropometric, and Blood Chemistry Characteristics^a for Type 2 Diabetes Cases

	AA (n= 101)	NHW (n= 100)	HA (n= 119)	p-value ^b
Age (yrs)	54.8 ± 11.1	61.8 ± 10.9	57.5 ± 10.8	< 0.001 **
Weight (kg)	92.5 ± 23.3	88.0 ± 16.5	78.3 ± 14.7	< 0.001 **
BMI (kg/m²)	33.0 ± 8.3	30.9 ± 5.3	30.5 ± 6.5	0.101
FB G (mg/dL)	174.1 ± 70.8	182.2 ± 54.4	204.5 ± 79.4	0.014 *
Insulin (uU/mL)	106.0 ± 96.4	114.9 ± 90.1	21.5 ± 17.2	< 0.001 **
Total Chol. (mg/dL)	196.7 ± 43.1	199.3 ± 45.8	199.9 ± 41.2	0.663
HDL Chol. (mg/dL)	45.2 ± 12.7	34.9 ± 11.2	37.7 ± 10.9	< 0.001 **
LDL Chol. (mg/dL)	127.8 ± 37.8	118.0 ± 37.3	124.6 ± 38.6	0.444
Triglycerides (mg/dL)	117.9 ± 87.7	216.4 ± 165.0	187.0 ± 113.4	< 0.001 **

AA: African Americans, NHW: Non-Hispanic Whites, HA: Hispanic Americans, BMI: Body Mass Index, FBG: Fasting Blood Glucose, Chol.: Cholesterol, HDL: High-density Lipoprotein, LDL: Low-density Lipoprotein

^aThe data are presented as Means ± Standard Deviation and based on corresponding sample sizes (n), excluding missing data

^bp-values based on the Kruskal-Wallis Statistic

* p² 0.05,

** p< 0.001

Table 3

Association of SNPs in FABP2 and FABP5 with T2DM in AA, NHW and HA Cohorts

		Genotypes			p	Allele Frequencies		OR	95%CI	p
FABP2(Ala54Thr)		G/G	G/A	A/A		G	A			
AA	CTL	61	33	6		155	45			0.636
	T2DM	56	34	7	0.878	146	48			
NHW	CTL	65	29	5		159	39			0.710
	T2DM	62	31	6	0.892	155	43			
HA	CTL	61	32	6		154	44			0.031
	T2DM	45	42	10	0.093	132	62	1.64	1.05–2.58	
FABP5(rs454550)		G/G	G/C	C/C		G	C			
AA	CTL	89	0	0		178	0			0.016
	T2DM	93	2	0	0.498	188	2			
NHW	CTL	69	1	0		139	1			0.036
	T2DM	84	11	0	0.014	179	11	4.24	1.32–13.58	
HA	CTL	80	4	1		164	6			0.130
	T2DM	112	7	0	1.000	231	7			
FABP5(rs454550)		A/A	A/G	G/G		A	G			
AA	CTL	80	9	1		169	11			0.009
	T2DM	93	2	0	0.016	188	2	0.22	0.07–0.67	
NHW	CTL	65	19	0		149	19			0.036
	T2DM	85	11	0	0.036	181	11	0.48	0.22–1.00	
HA	CTL	77	5	0		159	5			0.160
	T2DM	104	15	0	0.130	223	15			

Table 4

Association of SNPs in FABP1, FABP3 and FABP4 with T2DM in AA, NHW and HA Cohorts

	Genotypes			Allele Frequencies			p
	C/C	C/T	T/T	C	T		
FABP1(rs52241883)							
AA CTL	1	36	63	38	162		
T2DM	1	25	71	27	167	0.178	
NHW CTL	2	34	60	38	154		
T2DM	1	36	61	38	158	1.000	
HA CTL	9	39	52	57	143		
T2DM	8	46	66	62	178	0.590	
FABP3(rs2271072)	C/C	C/G	G/G	C	G		
AA CTL	32	45	22	109	89		
T2DM	30	39	24	99	87	0.759	
NHW CTL	26	52	20	104	92		
T2DM	25	55	18	105	91	1.000	
HA CTL	25	55	20	105	95		
T2DM	24	60	32	108	124	0.247	
FABP3(rs2279885)	C/C	C/G	G/G	C	G		
AA CTL	86	14	0	186	14		
T2DM	80	14	3	174	20	0.283	
NHW CTL	82	13	3	177	19		
T2DM	81	11	5	173	21	0.741	
HA CTL	82	17	1	181	19		
T2DM	102	17	0	221	17	0.388	
FABP4(-87T>C)	T/T	T/C	C/C	T	C		
AA CTL	97	0	0	194	0		
T2DM	98	0	0	196	0	1.000	
NHW CTL	96	0	0	182	0		
T2DM	99	0	0	198	0	1.000	
HA CTL	98	1	0	197	1		
T2DM	96	0	0	192	0	1.000	

Table 5

FABP2 & FABP5 Ethnic Specific, Age Crude Odds Ratios for Complete Sample (Collapsed Genotypes).

		Full Sample		
		Genotypes	Age Crude OR (95% CI)	p-value
FABP2 (Ala54Thr)	G/G	G/A + A/A		
AA	CTL	61	1.14 (0.64, 2.02)	0.641
	T2DM	56		
NHW	CTL	66	1.14 (0.63, 2.04)	0.657
	T2DM	62		
HA	CTL	61	1.85 (1.05, 3.27)	0.032*
	T2DM	45		
FABP5 (rs45450)	G/G	G/C + C/C		
AA	CTL	89	1.02 (0.99, 1.05)	0.498 [#]
	T2DM	93		
NHW	CTL	69	9.03 (1.13, 71.73)	0.014*[#]
	T2DM	84		
HA	CTL	80	1.00 (0.30, 3.26)	1.000
	T2DM	112		
FABP5 (nSNP)	A/A	A/G + G/G		
AA	CTL	80	0.17 (0.03, 0.80)	0.016*[#]
	T2DM	93		
NHW	CTL	65	0.44 (0.19, 0.99)	0.045*
	T2DM	85		
HA	CTL	77	2.22 (0.77, 6.37)	0.155 [#]
	T2DM	104		

AA: African Americans, NHW: Non-Hispanic Whites, HA: Hispanic Americans, OR: Odds Ratio

* p-value² 0.05[#] P-value is based approximately on Fisher's Exact Test, otherwise, p-value is based on Pearson's Chi-square

Table 6

FABP2 & FABP5 Ethnic Specific, Age Crude and Age Adjusted Odds Ratios for Age Compatible Subgroup (Collapsed Genotypes)

Genotypes		Age Range Subgroup ^d		Logistic Regression Model ^e		
		Age Crude OR (95% CI)	p-value	Age Adj. ^b OR (95% CI)	p-value	
FABP2 (Ala54Thr)						
AA	CTL	37	18			
	T2DM	35	25	1.46 (0.68, 3.14)	0.322	2.75 (1.26, 6.01) 0.011*
NHW	CTL	23	20		0.103	
	T2DM	56	26	0.53 (0.25, 1.14)		0.42 (0.18, 0.95) 0.039*
HA	CTL	19	13		0.105	
	T2DM	25	35	2.04 (0.85, 4.89)		3.04 (1.33, 6.95) 0.008*
FABP5 (rs45450)						
G/G		G/C+ C/C				
AA	CTL	46	0		1.000 [#]	Unstable OR ^c NA
	T2DM	55	1	1.01 (0.98, 1.05)		
NHW	CTL	41	1		0.059 [#]	
	T2DM	68	10	6.02 (0.74, 48.83)		4.94 (0.55, 43.73) 0.151
HA	CTL	27	1		1.000 [#]	
	T2DM	76	3	1.06 (0.10, 10.68)		0.95 (0.09, 9.87) 0.969
FABP5 (nSNP)						
A/A		A/G+ G/G				
AA	CTL	42	4		0.172 [#]	
	T2DM	55	1	0.19 (0.02, 1.77)		0.370 (0.03, 4.47) 0.434
NHW	CTL	43	9		0.178 [#]	
	T2DM	72	7	0.46 (0.16, 1.33)		0.30 (0.09, 0.96) 0.044*
HA	CTL	24	2		0.726 [#]	
	T2DM	69	10	1.73 (0.35, 8.50)		1.26 (0.24, 6.61) 0.781

AA: African Americans, NHW: Non-Hispanic Whites, HA: Hispanic Americans, OR: Odds Ratio

^aIn compatible age range subgroup, for AA: 27–58 years, NHW: 30–79 years, HA: 36–62 years^bAge adjusted model enters age as a categorical variable where: 20–29 yrs =1, 30–39 yrs=2, 40–49 yrs=3, 50–59 yrs=4, 60–69 yrs=5, 70–79 yrs=6 and the first category is the reference in each ethnic specific model^cUnstable Odds Ratio due to low event count, no p-value available

* p-value² 0.05

P-value is approximately based on Fisher's Exact Test, otherwise, p-value is based on Pearson's Chi-square