Tribbles-1 regulates hepatic lipogenesis through posttranscriptional regulation of C/EBPα

Robert C. Bauer,1 Makoto Sasaki,1 Daniel M. Cohen,2,3 Jian Cui,1 Mikhaila A. Smith,1 Batuhan O. Yenilmez,1 David J. Steger,2,3 and Daniel J. Rader1,3,4

1Division of Translational Medicine and Human Genetics and 2Endocrinology, Diabetes, and Metabolism of the Department of Medicine, 3The Institute for Diabetes, Obesity, and Metabolism, and 4Department of Genetics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, USA.

Introduction

Genome-wide association studies have identified over 150 genomic loci that are associated with plasma lipid traits in humans, and more than half of these loci have no previously appreciated role in lipid metabolism (1–3). One of the most interesting of these lipid loci is the genomic region 8q24, at which a cluster of noncoding variants are significantly associated with all major plasma lipid traits, including triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density cholesterol (HDL-C) (2, 3). The same variants are also significantly associated with plasma concentrations of alanine transaminase (ALT) (4) and with coronary artery disease (5). However, the molecular mechanisms underpinning these genetic associations are poorly understood.

The 8q24 genomic locus harbors the gene TRIB1, which encodes the protein tribbles-1 (TRIB1). We previously reported that hepatic overexpression of C/EBPα in mice reduced lipogenesis, VLDL secretion, and plasma lipid levels (6). However, the mechanism through which gain-of-function of hepatic TRIB1 regulated lipid metabolism was not determined. Here, we reported the investigation of mice with a liver-specific deletion of TRIB1 (Trib1_LSKO) to elucidate the role of tribbles-1 in mammalian hepatic lipid metabolism. These mice exhibited increased hepatic triglyceride (TG) content, lipogenic gene transcription, and de novo lipogenesis. Microarray analysis revealed altered transcription of genes that are downstream of the transcription factor C/EBPα, and Trib1_LSKO mice had increased hepatic C/EBPα protein. Hepatic overexpression of C/EBPα in WT mice phenocopied Trib1_LSKO livers, and hepatic knockout of Cebpa in Trib1_LSKO mice revealed that C/EBPα is required for the increased lipogenesis. Using ChIP-Seq, we found that Trib1_LSKO mice had increased DNA-bound C/EBPα near lipogenic genes and the Trib1 gene, which itself was transcriptionally upregulated by C/EBPα overexpression. Moreover, we have shown that Trib1 regulates hepatic lipogenesis through a novel posttranscriptional regulation of C/EBPα, which in turn transcriptionally upregulates Trib1. These data suggest an important role for C/EBPα in mediating the lipogenic effects of hepatic Trib1 deletion and provide insight into the association between TRIB1 and plasma lipids, and liver traits in humans.

Results

The liver-specific Trib1 KO mouse has elevated plasma ALT and lipids. We obtained mice on a C57BL/6 background with flanking loxP sites around the second exon of Trib1 (Supplemental Figure 1A; supplemental material available online with this article; doi:10.1172/JCI77095DS1). Since the liver is the main site of lipid and lipoprotein metabolism, we pursued tissue-specific deletion of hepatic Trib1. Trib1 was deleted in the liver of Trib1fl/fl mice using 2 complementary approaches. Trib1fl/fl mice were either injected with adeno-associated viral vector serotype 8 (AAV8) expressing Cre recombinase under the control of the liver-specific thyroxine-binding globulin (TBG) promoter (AAV-TBG-Cre), or they were crossed with transgenic mice expressing Cre under the control of the albumin promoter. Trib1fl/fl mice receiving AAV8-TBG-Cre (herein referred to as Trib1_LSKO) displayed >95% deletion of the allele as early as 1 week after injection, as compared with Trib1fl/fl mice treated with AAV containing empty vector (herein referred to as Trib1_fl/fl) (Figure 1A). Trib1fl/fl crossed with albumin-Cre mice (Trib1fl/fl Alb-Cre +) had similar levels of Trib1 deletion (Figure 1B). In the setting of Trib1 deletion, we observed no compensatory upregulation of Trib2 (not detectable in liver) or Trib3 (Figure 1A). Interestingly, hepatic deletion of Trib1 caused significantly increased Trib1 message in brown adipose tissue and trends toward increased expression in white adipose tissue and skeletal muscle of Trib1_LSKO mice (Supplemental Figure 1B). Trib1_LSKO mice also had significant increases in plasma ALT levels, and these were more notable in males than females. This observation was made in both AAV-Cre mice (Figure 1C) and Alb-Cre mice (Figure 1D), indicating that this finding was not due to the administration of the viral vector.
Trib1_LSKO animals had significantly increased plasma TC (23%), non-HDL cholesterol (37%), and TG (52%) 4 weeks after AAV-Cre injection as compared with control Trib1fl/fl animals treated with AAV-Null (Figure 2, A and B). The cholesterol and non-HDL cholesterol levels in Trib1_LSKO mice increased over time to at least 20 weeks after injection (Figure 2, C and D). Similar plasma lipid results were also observed in Trib1fl/fl Alb-Cre + mice (Supplemental Figure 2).

Trib1_LSKO mice have increased liver TG and increased de novo lipogenesis. Trib1_LSKO animals exhibited a 25% increase in liver weight (P < 0.001) relative to body weight (Figure 3A). Examination of hepatic lipids revealed a 78% increase in hepatic TG content (P < 0.001) of Trib1_LSKO mouse livers compared with controls 4 weeks after AAV injection. A total of 2,095 genes (1,478 upregulated, 617 downregulated) were significantly altered between the 2 groups (absolute fold change>1.5, FDR<10%). Gene set enrichment analysis of this array data suggested that the altered gene set was enriched for genes downstream of multiple transcriptional effectors, including the transcription factor C/EBPα (P = 6.22 × 10−12) (Table 1). Western blot analysis of liver extracts showed a dramatic increase in C/EBPα protein in Trib1_LSKO mice compared with controls, while mRNA abundance of Cebpa transcript was in fact decreased –37%, P = 0.01), suggesting a posttranscriptional effect of Trib1 deletion on turnover of C/EBPα (Figure 4). We observed similar patterns with C/EBPβ protein and message as well, albeit to a lesser extent (Figure 4). These observations suggest that the increase in C/EBPα protein is, at least in part, contributing to the phenotypes observed in the Trib1_LSKO mice.
Overexpression of mCebpa is sufficient to phenocopy the lipogenesis phenotype of the Trib1_LSKO mice but not the plasma lipid findings. To determine if increased C/EBP$\alpha$ protein was sufficient to drive increased de novo lipogenesis, we overexpressed murine C/EBP$\alpha$ (mCebpa) via AAV8 in the livers of C57BL/6 mice (Supplemental Figure 5A). AAV_mCebpa mice had increased hepatic TG content as compared with control mice receiving AAV_Null 2 weeks after injection (Figure 5A). Mice overexpressing mCebpa also had a similar gene expression profile to Trib1_LSKO mice with respect to lipogenic genes; Fasn, Scd1, and Acaca were all significantly increased, while Chrebp and Srebf1 were not (Figure 5B). De novo lipogenesis in mCebpa-overexpressing mice was significantly increased in production of fatty acids (1.7-fold, $P < 0.001$), diacylglycerol (1.5-fold, $P < 0.01$), and TG (3.5-fold, $P < 0.001$) as compared with control mice receiving AAV_Null (Figure 5C). This closely mimics the phenotype seen in the Trib1_LSKO mice and strongly suggests that increased levels of C/EBP$\alpha$ are responsible for the changes in lipogenic gene expression, hepatic TG content, and de novo lipogenesis observed in the setting of hepatic loss of Trib1. AAV_mCebpa mice displayed increased plasma ALT levels, as well (Supplemental Figure 5B); however, in contrast to the Trib1_LSKO mice, they surprisingly did not exhibit increased plasma lipids, even 10 weeks after injection (Figure 5D). We observed no significant differences in plasma TC or TG between the groups, along with a small but significant decrease in plasma non-HDL levels in the AAV_mCebpa animals (Figure 5D). The lack of increased plasma lipids in the AAV_mCebpa mice suggests that the plasma lipid phenotype in Trib1_LSKO mice is due to an alternative mechanism independent of C/EBP$\alpha$.

Hepatic deletion of C/EBP$\alpha$ in Trib1_LSKO mice completely attenuates the lipogenic phenotype. While increased C/EBP$\alpha$ protein levels are sufficient to drive increased lipogenesis in Trib1_LSKO mice, we next sought to test if C/EBP$\alpha$ was required for this phenotype. We obtained a previously reported Cebpa$^{fl/fl}$ conditional KO mouse (7) and crossed this with our Trib1$^{fl/fl}$ mouse to generate 3 experimental groups: WT, Trib1$^{fl/fl}$, and Trib1$^{fl/fl}$Cebpa$^{fl/fl}$ mice. We administered AAV-TBG-Cre to all 3 groups to generate WT, Trib1_LSKO, and Trib1/Cebpa double-LSKO (herein referred to as Trib1/Cebpa_dLSKO) mice, the last of which had >95% deletion of Cebpa (Figure 6A). Trib1_LSKO mice exhibited the expected increases in lipogenic gene transcription, while Trib1/Cebpa_dLSKO exhibited transcription at WT levels or lower (Figure 6B). Production of fatty acids, diacylglycerol, and TG in Trib1/Cebpa_dLSKO mice were returned to WT levels as compared with Trib1_LSKO mice (Figure 6C). These data confirm that C/EBP$\alpha$ is required for the increased lipogenesis phenotype observed in Trib1_LSKO mice.

Deletion of hepatic Trib1 leads to increased C/EBP$\alpha$ binding near upregulated lipogenic genes, as well as Trib1 itself. While the above data reveal a causal role for C/EBP$\alpha$ in the lipogenic phenotype observed in the Trib1_LSKO mice, they do not address whether it functions directly or indirectly through the regulation of another transcription factor to induce lipogenic gene

Figure 2. Trib1_LSKO mice have increased plasma TC, HDL and non-HDL cholesterol, and plasma TGs. (A) Plasma TC, non-HDL cholesterol, and TG in male Trib1$^{fl/fl}$ or Trib1_LSKO mice (n = 10) 4 weeks after injection. Plasma samples were collected after mice were fasted for 4 hours. (B) Plasma TC, non-HDL cholesterol, and TG after 4-hour fast in female Trib1$^{fl/fl}$ and Trib1_LSKO mice (n = 10) 4 weeks after injection. (C) Plasma TC in male Trib1$^{fl/fl}$ and Trib1_LSKO mice (n = 5) out to 20 weeks after injection. (D) Change from baseline for non-HDL cholesterol in the same male Trib1$^{fl/fl}$ and Trib1_LSKO mice from C. All plasma levels were measured by Cobas-Mira autoanalyzer with commercially available reagents. Significance was determined in all panels by Student’s t test (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$).
entially expressed genes in the Trib1_LSKO mice were highly correlated with the direction of altered C/EBPα occupancy (Figure 7C), and differentially expressed genes had 1.8-fold higher frequency of association with at least one differential C/EBPα peak than nondifferential genes (Supplemental Figure 6B). Multiple genes involved in fatty acid and triglyceride synthesis showed both upregulated expression in the livers of Trib1_LSKO mice and the presence of at least one substantially enriched C/EBPα-binding site within 100 kb of their transcription start sites (TSSs) (Figure 7D). As a whole, these data demonstrate a strong association between the lipogenic transcriptome and C/EBPα cistrome in liver, and suggest a direct role for C/EBPα in inducing lipogenic genes in the absence of Trib1.

To identify genes strongly associated with differential C/EBPα occupancy, we performed ChIP-Seq for C/EBPα in biological replicates of Trib1_LSKO mice, as well as for the AAV_mCebpa-treated mice, and compared the results to the respective controls from each experiment. We identified approximately 48,000 C/EBPα-binding sites in liver tissue from the Trib1_fl/fl and Trib1_LSKO mice. Of these, 4,480 and 1,361 sites had increased and decreased C/EBPα occupancy, respectively, in the Trib1_LSKO versus Trib1_fl/fl mice; C/EBPα occupancy was affected similarly at these sites by forced expression of C/EBPα (Figure 7A), suggesting that differential occupancy is a direct consequence of increased levels of C/EBPα. Motif analysis at sites with stronger C/EBPα binding in the Trib1_LSKO mice revealed robust enrichment for the canonical C/EBP motif (Figure 7B), and gene-set enrichment analysis of these peaks showed major enrichment of genes involved in lipid metabolism as one of the top findings (Supplemental Figure 6A). Moreover, differentially expressed genes in the Trib1_LSKO mice were highly correlated with the direction of altered C/EBPα occupancy (Figure 7C), and differentially expressed genes had 1.8-fold higher frequency of association with at least one differential C/EBPα peak than nondifferential genes (Supplemental Figure 6B). Multiple genes involved in fatty acid and triglyceride synthesis showed both upregulated expression in the livers of Trib1_LSKO mice and the presence of at least one substantially enriched C/EBPα-binding site within 100 kb of their transcription start sites (TSSs) (Figure 7D). As a whole, these data demonstrate a strong association between the lipogenic transcriptome and C/EBPα cistrome in liver, and suggest a direct role for C/EBPα in inducing lipogenic genes in the absence of Trib1.

To identify genes strongly associated with differential C/EBPα peaks, we mapped clusters of Trib1_LSKO-induced C/EBPα peaks that were within 50 kb of each other (Supplemen-
tal Table 1). Interestingly, the second-ranked cluster was approximately 40 kb downstream of the Trib1 locus, where 10 peaks in an approximately 20-kb region had substantially more C/EBPα binding in both the Trib1_LSKO and AAV mCebpa mice as compared with controls (Figure 8A). Indeed, Trib1 gene expression was significantly increased in livers of AAV_mCebpa mice (Figure 8B). These findings suggest that C/EBPα transcriptionally upregulates TRIB1, while the TRIB1 protein posttranscriptionally downregulates C/EBPα protein, and that these 2 proteins participate in a strong negative feedback loop (Figure 8C).

Discussion
Recent genome-wide association studies performed in extremely large patient cohorts have allowed for the identification of over 150 genomic loci as being implicated in human plasma lipid metabolism (3). One of the most strongly associated of these loci is nearest to the gene TRIB1, and this locus was subsequently associated with liver transaminases (4), as well as with coronary artery disease (5). Here, we used liver-specific deletion of Trib1 in mice to gain greater insight into the mechanisms by which TRIB1 influences hepatic lipid metabolism.

We previously reported that AAV8-mediated hepatic overexpression of Trib1 decreased hepatic lipogenesis and plasma lipids in mice (6). In this study, we report that hepatic-specific deletion of Trib1 resulted in upregulation of the transcription of genes involved in fatty acid synthesis, de novo lipogenesis, increased hepatic triglyceride and plasma transaminases, and elevated plasma lipids. We found a dramatic increase in the hepatic protein levels of C/EBPα, despite no increase in Cebpa mRNA abundance. Overexpression of C/EBPα in mice phenocopied the hepatic lipogenesis, steatosis, and increased transaminase phenotypes observed in the Trib1_LSKO mice. Deletion of Cebpa in the livers of Trib1_LSKO mice returned the levels of transcription of lipogenic genes and de novo lipogenesis back to WT levels or lower. ChIP-Seq verified that increased occupancy of C/EBPα occurred near lipogenic genes. These data strongly support a role for C/EBPα in de novo lipogenesis via upregulation of fatty acid–synthesis genes, and this regulation appears to be independent of canonical regulators of fatty acid synthesis, such as ChREBP, LXR, and SREBP1, the latter of which C/EBPα is reported to regulate the transcription of in adipose (8). C/EBPα protein abundance in liver is regulated by TRIB1, and thus the TRIB1-C/EBPα axis may be an underappreciated modulator of human hepatic lipogenesis.

The tribbles protein was originally identified in Drosophila as a regulator of cell migration and mitosis during oogenesis (9–11), with its role in oogenesis determined to be regulating the proteasomal degradation of Slbo, the Drosophila homolog of the human gene C/EBPα (12). There are 3 mammalian tribbles genes (tribbles1-3), all of which are known as pseudokinases; they have a protein domain homologous to a kinase domain yet are lack-

Table 1. Trib1_LSKO mice have increased transcription of C/EBP target genes as identified by pathway analysis

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<th>Transcription factor</th>
<th>P value</th>
<th>Number of target genes</th>
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<tr>
<td>Ppara</td>
<td>5.60 × 10⁻²²</td>
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<tr>
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<td>Med13</td>
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Ingenuity analysis of the set of differentially regulated genes (±1.5 absolute fold change, FDR<0.1%) in Trib1_LSKO mice compared with Trib1_fl/fl mice (n = 4/group) as obtained from Agilent microarray analysis of liver total RNA 4 weeks after injection. List shown is upstream transcriptional regulators whose target genes are enriched in our differential gene set.
ing critical amino acids in the catalytic region that would allow for phosphorylation of proteins (13). Work in the myeloblast 32D cell line has shown that overexpression of TRIB1 or TRIB2 induces the proteasomal degradation of C/EBPα and C/EBPβ by promoting their ubiquitination by the E3 ligase COP1 through direct binding to both targets and the ligase (14, 15). This function is critical for tribbles-mediated leukemogenesis (16, 17) and also coordinates TRIB1 regulation of macrophage polarization and differentiation (18). Earlier work has identified partially defined roles for other tribbles proteins in human metabolism, particularly TRIB3, which is involved in regulation of insulin signaling with GAPDH housekeeping gene. (19, 20) Adipose TRIB3 can also facilitate the turnover of the protein acetyl–coenzyme A carboxylase (ACACA), the rate-limiting enzyme in fatty acid synthesis (22).

C/EBPα belongs to the C/EBP family of transcription factors known to play wide-ranging roles in transcriptional regulation in adipose and the liver (23). C/EBPα has been long known to regulate lipogenesis in adipocytes, and multiple studies have implicated C/EBPα in regulation of lipogenic genes in the setting of mice deficient for leptin signalling (24, 25). An age-associated isoform of C/EBPα has been shown to promote steatosis in older mice, as well through regulation of TG-synthetic enzymes (26). However, a role for C/EBPα in hepatic lipogenesis in WT mice, especially in the context of TRIB1-mediated regulation, has not been investigated. We showed that Trib1_LSKO mice exhibiting increased lipogenesis also have increased hepatic C/EBPα and C/EBPβ protein levels. C/EBPα and β have been shown experimentally to have nearly completely overlapping ChIP-Seq binding profiles in both mouse liver (27) and adipose (28), and thus we suspect any role for C/EBPβ in the Trib1_LSKO mice is redundant to that of C/EBPα. Through AAV-mediated overexpression, we showed that increased C/EBPα levels are sufficient to cause increased lipogenic gene expression and fatty acid synthesis. We also deleted Cebpa in the livers of Trib1_LSKO mice, which completely ablated the lipogenic effects of hepatic deficiency of Trib1, showing that C/EBPα is also necessary for the lipogenic effects of hepatic Trib1 deletion. It remains possible that TRIB1 affects the function of other drivers of lipogenic gene expression (i.e., SREBP, ChREBP) via increased C/EBPα DNA binding, which may in turn facilitate increased binding of other transcription factors. Interestingly, our AAV_mCebpa mice did not display increased plasma lipids, suggesting that the mechanism governing the increased plasma lipids in the Trib1_LSKO mice is independent of the increased C/EBPα protein levels, and the elucidation of this mechanism remains a focus of our ongoing studies. The AAV_mCebpa mice did, however, exhibit increased plasma ALT levels, in keeping with the steatotic phenotype of these mice.

Figure 5. Overexpression of mCebpa in livers of mice phenocopies the increased hepatic TG synthesis observed in Trib1_LSKO mice. (A) Liver TG content in mice receiving 1 × 10^12 AAV_Null or AAV_mCebpa 2 weeks after injection after overnight fast (n = 5). (B) Hepatic lipogenic gene expression in AAV_Null and AAV_mCebpa mice 2 weeks after injection, as measured by TaqMan real-time RT-PCR from 1 μg total RNA. Values are relative expression compared with GAPDH housekeeping gene. (C) De novo lipogenesis in mice from A after overnight fast, refeeding, and fatty acid labeling with [3H]-Acetate. (D) Plasma TC, non-HDL cholesterol, and TG were measured after a 4-hour fast in mice (n = 6) 10 weeks after AAV_Null or AAV_mCebpa injection. Significance was determined in all panels by Student’s t test (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001).
We also observed that overexpression of C/EBPα induces the transcription of Trib1 in WT mice. A previous report investigating C/EBPα genomic binding sites that are highly conserved between 5 different species identified 4 sites near TRIB1 that ranked in the top 25 most evolutionarily conserved (29). This data is bolstered by our own ChIP-Seq findings that, in Trib1_LSKO mice and AAV_mCebpa mice, some of the most highly enriched binding peaks for C/EBPα are in fact downstream of the Trib1 gene. Given these data, it appears that TRIB1 and C/EBPα participate in a tight feedback loop, wherein more C/EBPα protein leads to increased Trib1 transcription, whereupon TRIB1 targets C/EBPα for degradation (Figure 8C). This feedback loop is completely posttranscriptional, with respect to C/EBPα, and would not be identified by looking solely at transcript levels. Interestingly, Trib1 has a circadian pattern of expression, with message levels peaking at the start of dark hours (30). It follows then that C/EBPα protein levels, and therefore C/EBPα-mediated lipogenesis, are likely circadian, as well, and hepatic lipogenesis is in fact known to be circadian (31). The highly induced C/EBPα binding peaks near the Trib1 gene in our Trib1_LSKO mice are in a region with high synteny to the downstream region of TRIB1 in humans, where the plasma lipid GWAS signal is located (3). It is an attractive hypothesis that common variation in this region is causing defects in this circadian rhythm, and our group hopes to elucidate the functionally relevant SNPs in this region with further studies that are already underway.

The same variants at the TRIB1 locus associated with plasma lipid traits are also associated with circulating liver transaminases (4). Elevated ALT and AST levels are some of the strongest indicators of hepatic steatosis and nonalcoholic fatty liver disease (NAFLD) in humans (32), as the increased lipid deposition causes hepatocellular damage, which in turn increased serum transaminase levels (33). Interestingly, our Trib1_LSKO mice have increased ALTs, a finding replicated in the Trib1fl/fl Alb-Cre mice, suggesting that increased ALTs are a consequence of decreased Trib1 levels and not an artifact of the virus administration. The observation that Trib1_LSKO mice have increased histopathological findings consistent with steatohepatitis as well as elevated plasma ALT and AST levels, combined with the genetic association between TRIB1 and ALTs in humans, suggests that there is a role for altered TRIB1 levels in NAFLD etiology. It has been previously shown that increased hepatic de novo lipogenesis can contribute to NAFLD (34), and thus TRIB1 regulation of hepatic TG synthesis could be a part of disease pathology. Genome-wide scans for genes associated with NAFLD in humans have not identified TRIB1 as a disease locus. Some of these studies, however, have relied on coding variation that would not capture the noncoding SNPs downstream of TRIB1 (35, 36), and all of these studies were performed in patient cohorts significantly smaller than those used to identify the association of TRIB1 with transaminases (37). More recently, a targeted study investigating the association of 3 SNPs in the region downstream of TRIB1
Methods

Animals. Trib1 genetically engineered mice C57BL/6-Trib1tm1.1mrl were provided by Merck and were produced for Merck by contract with Taconic. Details of the design of the Trib1 mice may be found at http://www.taconic.com/10265. Previously reported mice harboring a conditional allele of Cebpa (Cebpa tm1Dgt /J) were obtained from The Jackson Laboratory (stock number 006447) (7). Albumin-Cre mice were also obtained from The Jackson Laboratory. All mice were fed ad libitum with chow diet. All mice were fasted for 4 hours prior to collection of plasma for lipid measurements, unless stated otherwise. Blood was collected retroorbitally and centrifuged, and plasma was obtained. Fasting plasma lipids and ALTs were analyzed on a Roche Cobas Mira autoanalyzer using commercially available reagents (Sigma-Aldrich). Plasma ASTs (MAK055, Sigma-Aldrich) and GGTs with NAFLD in a Japanese cohort of patients (n = ~2,200) found significant associations between that region and hepatic TG content in those patients (38). Future genome-wide studies of increasing power performed on larger subject cohorts will likely identify TRIB1 as an NAFLD locus. Regardless of the exact role for TRIB1 in NAFLD patholgy, it is an attractive therapeutic target for the treatment of such disorders, as one might anticipate that, in the setting of fatty liver, increased levels of TRIB1 might mitigate the excess of hepatic lipids. Indeed, a study aimed at identifying compounds that upregulate transcript levels of TRIB1 in hepatocytes was recently published, suggesting that this may be a viable therapeutic mechanism (39).

In summary, our loss-of-function studies indicate that hepatic TRIB1 is a key posttranscriptional regulator of C/EBPα protein, which in turn is a major regulator of hepatic lipogenesis. In addition, C/EBPα strongly promotes the transcription of its regulator, TRIB1. TRIB1 likely plays many roles in human metabolic diseases, and further research in other TRIB1 mechanisms of action in the liver and in other tissues may present even more innovative avenues for intervention in cardiovascular disease and metabolic disorders.
The relative quantity of each mRNA was calculated using the delta CT method with Gapdh as the housekeeping gene. Total liver RNA was used for microarray analysis on the Agilent 4 × 44 Whole Mouse Genome Array. Microarray and data analysis were performed by the Penn Functional Genomics Core (Philadelphia, Pennsylvania, USA). Differential expression was determined using a False Discovery Rate of 10% and a minimum fold change of 1.5. All array data is publicly available on the NCBI Gene Expression Omnibus (GEO) database under accession number GSE70848.

ChIP-Seq analysis of C/EBPα binding in mouse liver. C/EBPα ChIP was performed with sonicated, nuclear extract prepared from formaldehyde-crosslinked liver and 14AA (sc-61, Santa Cruz Biotechnology Inc.) antibody. Each experimental condition was assayed in duplicate with separate mice. ChIP-Seq libraries were produced and sequenced according to Illumina protocols as previously described (41), with the addition that libraries were multiplexed for sequencing. Peak calling and de novo motif analyses were performed using HOMER (42). Peaks were required to meet a cutoff of 1 RPM (average tag density of the replicate conditions) in either the Trib1_fl/fl or Trib1_LSKO condition. Peak mapping to annotated genes and gene ontology analyses were conducted with CEAS (43) and GREAT (44). Data generated from these ChIP-Seq experiments are publicly available in the NCBI GEO online resource under accession number GSE70848.

Statistics. All data are reported as the mean ± SD except for gene expression results, which are mean ± SEM. Results were analyzed by 2-tailed Student’s t test, or ANOVA with Tukey’s post-hoc test when multiple comparisons were made. Statistical significance was defined as $P < 0.05$.

Study approval. Animal experiments were reviewed and approved by the Institutional Animal Care and Use Committees of the University of Pennsylvania, Philadelphia, Pennsylvania, USA.

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Address correspondence to: Daniel J. Rader, Perelman School of Medicine at the University of Pennsylvania, 3400 Civic Center Blvd, Building 421, 11th Floor, Room 125, Philadelphia, Pennsylvania 19104-5159, USA. Phone: 215.573.4176; E-mail: rader@mail.med.upenn.edu.