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Complement Factor B Is a Determinant of Both Metabolic and Cardiovascular Features of Metabolic Syndrome

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Abstract—CFB (complement factor B) is elevated in adipose tissue and serum from patients with type 2 diabetes mellitus and cardiovascular disease, but the causal relationship to disease pathogenesis is unclear. Cfb is also elevated in adipose tissue and serum of the spontaneously hypertensive rat, a well-characterized model of metabolic syndrome. To establish the role of CFB in metabolic syndrome, we knocked out the Cfb gene in the spontaneously hypertensive rat. Cfb−/− rats showed improved glucose tolerance and insulin sensitivity, redistribution of visceral to subcutaneous fat, increased adipocyte mitochondrial respiration, and marked changes in gene expression. Cfb−/− rats also had lower blood pressure, increased ejection fraction and fractional shortening, and reduced left ventricular mass. These changes in metabolism and gene expression, in adipose tissue and left ventricle, suggest new adipose tissue-intrinsic and blood pressure-independent mechanisms for insulin resistance and cardiac hypertrophy in the spontaneously hypertensive rat. In silico analysis of the human CFB locus revealed 2 cis-regulated expression quantitative trait loci for CFB expression significantly associated with visceral fat, circulating triglycerides and hypertension in genome-wide association studies. Together, these data demonstrate a key role for CFB in the development of spontaneously hypertensive rat metabolic syndrome phenotypes and of related traits in humans and indicate the potential for CFB as a novel target for treatment of cardiometabolic disease. (Hypertension. 2017;70:00-00. DOI: 10.1161/HYPERTENSIONAHA.117.09242.) • Online Data Supplement

Key Words: adipose tissue ▪ blood pressure ▪ complement system proteins ▪ glucose ▪ hypertension

Metabolic syndrome (MetS) represents a complex clustering of cardiometabolic traits, including hypertension, insulin resistance, glucose intolerance, and dyslipidemia, all of which increase the risk of developing type 2 diabetes mellitus and cardiovascular disease. * Despite established environmental risk factors and genome-wide association study (GWAS) hits that link genetic variation to MetS constituents, the molecular and cellular events underlying its development remain incompletely understood.2,3 Chronic low-grade inflammation and innate immune system overactivation are now recognized causes of type 2 diabetes mellitus and MetS.4,5 In particular, the alternative pathway (AP) has received attention for its potential causal role in cardiometabolic disease.6 AP activation requires CFB (complement factor B) to bind C3 to form C3B, which opsonizes pathogens and contributes to the formation of the membrane attack complex.7 Thus, CFB is fundamental to pathogen clearance and host cell apoptosis. However, increased circulating CFB has been found in patients with type 2 diabetes mellitus,7 and expression of adipose tissue CFB correlates significantly with fasting glucose and circulating lipids.8 Elevated circulating CFB has also been found to increase the risk of endothelial dysfunction9 and coronary heart disease.10

Because of the complex genetic basis of human MetS, the spontaneously hypertensive rat (SHR), which exhibits hypertension, insulin resistance, and dyslipidemia, has been extensively studied as a MetS model.11-13 Multiple studies have identified SHR genes associated with features of MetS, many of which show conserved pathologies in humans.14-17

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The rat Cfb gene resides within the major histocompatibility region on chromosome 20p12. In SHR, this region has been demonstrated to be important in blood pressure regulation, serum cholesterol, adiposity, and glucose tolerance. In this study, we knocked out Cfb in SHR to test the hypothesis that Cfb is necessary for the full expression of cardiometabolic pathophysiological traits in this model of MetS.

Methods

Detailed methods are available in the online-only Data Supplement.

Rats

Cfb−/− rats were generated using SHR/NCrl rats (Charles River, Margate, United Kingdom), by microinjecting Zinc-finger nuclease (ZFN) mRNA (Sigma), targeted to exon 6 of Cfb (target sequence: CCGGCTCCATGaatatcTACATGGTGCTGGATG), into 1-cell stage SHR/NCrl embryos that were implanted into pseudopregnant rats. Heterozygous progeny, from a founder harboring a 19-base pair deletion in Cfb, were intercrossed to homozygosity. A search for off-target events, conducted by whole genome sequencing confirmed the 19-base pair deletion. Six additional putative mutations, analyzed by Sanger Sequencing, were determined to be false positives (Table S1). Rats were housed with free access to food and water. All procedures were performed in accordance with UK Home Office regulations.

Statistics

Unpaired t test or 2-way ANOVA (Minitab Express) were used to assess differences between genotype and treatment. All results are mean±SEM. *P<0.05 was considered significant.

Results

Generation of a Cfb Knockout Rat

Using data from a quantitative trait transcript analysis of recombinant inbred strains derived from a SHR×Brown Norway (BN-Lv/Cub) cross, we identified Cfb transcript levels as uniquely and strongly correlated significantly across the recombinant inbred strains for metabolically relevant traits (glucose uptake in isolated adipocytes, r2=−0.65, P<0.0003; basal lipogenesis in epididymal fat, r2=−0.64, P<0.0002; serum high-density lipoprotein cholesterol, r2=−0.64, P<0.0005) and significantly differentially expressed in adipose tissue between parental strains (SHR versus Brown Norway, 1.47-fold P<0.05). Overexpression in SHR adipose tissue was confirmed by quantitative polymerase chain reaction by comparing a further insulin sensitive/normotensive Wistar Kyoto strain (WKY/NCrl; Figure S1A). Cfb was also overexpressed in SHR left ventricle (LV), but not liver, compared with WKY (Figure S1A). Cfb overexpression in SHR was associated with increased AP activity compared with WKY (Figure S1B). Analysis of the Cfb gene and its adjacent region revealed 14 variants unique to SHR, not present in Brown Norway or WKY; 2 variants reside upstream of the transcription start site (Figure S1C). To investigate the potential causative role of Cfb in the cardiometabolic traits of SHR, a 19-base pair deletion in exon 6 of the Cfb gene in the SHR germline was made using ZFNs (Figure S1D). Abolition of Cfb expression was confirmed by quantitative polymerase chain reaction and immunoblot (Figure S1E), and loss of Cfb function was confirmed by ablation of serum AP activity (Figure S1F).

Glucose Homeostasis

To test whether Cfb ablation affected glucose homeostasis in SHR, oral glucose tolerance and insulin sensitivity (IVITT

[intravenous insulin tolerance test]) were assessed. Fasting plasma glucose concentration in Cfb−/− was significantly lower than SHR (Figure 1A; SHR, 4.62±0.10 versus Cfb−/−, 4.25±0.09; P=0.013). Throughout the oral glucose tolerance, blood glucose remained lower, and area under the glucose curve was significantly reduced in Cfb−/− compared with SHR; insulin concentrations were similar in both groups (Figure 1A and 1B). Together with the G:I ratio (ratio of area under the curve of plasma glucose concentration to area under the curve of plasma insulin concentration; Figure 1C), this indicated an improvement in insulin sensitivity, further demonstrated in IVITTs by a significant 48% increase in insulin-stimulated glucose disposal (Km in Cfb−/− compared with SHR (Figure 1D).

Adipose Tissue Function

To determine whether Cfb affects adipose function, as suggested by our previous quantitative trait transcript analysis and metabolic phenotyping, we measured adipose tissue depot masses. Relative wet masses of visceral (epididymal adipose tissue [EAT]; mesenteric adipose tissue [MAT]; and retroperitoneal adipose tissue) and brown fat (brown adipose tissue [BAT]) were significantly reduced in Cfb−/− rats compared with SHR, despite similar total body mass (269±20 versus 265±11 g; P>0.05; Figure 2A); however, Cfb−/− had significantly more relative subcutaneous fat (SAT; Figure 2A). Overall, total fat mass was similar (Figure 2B). Stereological analysis of EAT showed that Cfb−/− had significantly fewer, similar-sized adipocytes than SHR (SHR, 4.06±0.21 versus Cfb−/−, 4.13±0.14 g/kg; P<0.05). Further, serum analysis of circulating lipids and adipokines demonstrated significant decreases in levels of cholesterol, triglycerides, and high molecular-weight adiponectin (−Δ48%), in Cfb−/− compared with SHR; however, circulating total adiponectin and leptin were similar (Table S4).

Given the varied metabolic contributions of different fat depots found in the Cfb−/− rat, we analyzed transcript abundance for markers of oxidation (Cpt1 and Aco1), bieging (Ucp1 and Pgc1a), insulin sensitivity (Slc2a4), lipid metabolism (fatty acid synthase [Fasn]), and adipokines (Adipoq and Lep). In EAT, Pgc1a, Cpt1, Aco1, and Slc2a4 were significantly increased in Cfb−/− compared with SHR (Figure 2C). In SAT, Aco1, Ucp1, Fasn, and Adipoq were significantly elevated, whereas Pgc1a was reduced, in Cfb−/− compared with SHR (Figure 2D). In BAT, Pgc1a and Slc2a4 were significantly increased in Cfb−/− compared with SHR, whereas Ucp1 and Fasn were significantly decreased (Figure 2E). Lep was significantly reduced in all Cfb−/− depots compared with SHR (Figure 2F through 2E).

To determine whether transcript changes were associated with altered adipose tissue respiration, we analyzed epididymal adipocyte metabolic rate. Maximal and basal respiratory rates were significantly greater in Cfb−/− than in SHR, +Δ1.64, and +Δ1.96-fold, respectively (Figure 2; Figure S2A). Further, reserve capacity and leak respiration were both significantly increased (Figure S2B and S2C). However, ATP-linked respiration and ATP-generation efficiency were similar (Figure S2D through S2E). CoxIV protein abundance—a mitochondrial marker—was similar in both Cfb−/− and SHR (Figure S2F).

There were no differences in body temperature or activity associated with Cfb deletion (Figure S3A and S3B).
Cardiovascular Analyses

Cfb deletion reduced relative LV mass and cardiomyocyte diameter by 10% compared with SHR; however, relative heart weight was similar between genotypes (Figure 3A and 3B; Figure S4A and S4B). Telemetrically measured systolic and diastolic blood pressures were significantly lower (−Δ7 mm Hg) in Cfb−/− than in SHR, and although heart rate was similar, rate pressure product was significantly reduced (Figure 3C and 3D; Figure S4C through S4F). Serum aldosterone and transcripts for renal renin and hepatic angiotensinogen were all significantly reduced in Cfb−/− rats (Table S4, Figure S5A and S5B).

Early structural and functional changes in the heart were investigated using echocardiography. We confirmed that relative LV mass was significantly reduced in Cfb−/− compared with SHR; however, at this stage, LV wall thickness was not significantly different (Table S5). Functionally fractional shortening and ejection fraction were significantly increased in Cfb−/− LV compared with SHR (Table S5). Given the similar heart rate and stroke volume, cardiac output was not significantly different (Table S5).

An acute hypertrophic challenge designed to investigate whether Cfb deletion conferred protection from cardiac stress, independent of blood pressure, showed that the rate pressure product was significantly reduced in Cfb−/− hearts in the 24 hours after isoproterenol treatment (Figure 3E and 3F; Figure S6A). Isoproterenol increased relative heart and LV mass similarly (Figure S6B and S6C). Transcripts related to cardiac hypertrophy were investigated in LV from isoproterenol and saline-treated rats. In saline-treated Cfb−/− rats, Nppa, Actc1, and Camk2d were significantly increased compared with SHR (Figure 4A, 4C, and 4E); whereas Nppb was significantly decreased (Figure 4B). In isoproterenol-treated rats, Nppb increased marginally in Cfb−/− rats compared with SHR (Figure 4B). Acta1 in isoproterenol-treated Cfb−/− rats was similar to both saline-treatment groups (Figure 4F). The ratio of Acta1:Acta1 was significantly greater in Cfb−/− compared with SHR, in saline-treated (317±43 versus 138±18; \(P<0.05\)) and isoproterenol-treated rats (256±37 versus 53±9; \(P<0.005\)). Myh6 and Myh7 expression was similar between genotypes (Figure 4D; Figure S7).

Serum Markers of Inflammation

Given the function of Cfb in inflammatory responses, we determined the effect of Cfb−/− on Th-1 mediated inflammation by quantifying serum concentrations of cytokines (II-2,
II-6, II-10, granulocyte macrophage colony stimulating factor, Ifn-γ, and Tnfα). We found significant decreases in serum concentrations of II-10 and Ifn-γ in Cfb−/− rats compared with SHR. In addition, whereas II-6 and Tnfα were detected in SHR, the cytokines were undetectable in sera from Cfb−/− rats. Granulocyte macrophage colony stimulating factor was similar in both groups, and in neither group was II-2 detected (Table S4).

Analysis of GWAS Hits and cis-Expression QTLs at the Human CFB Locus
To determine whether genetic variants near CFB are associated with metabolic and cardiovascular disorders relevant to MetS (Table S3), we mined the NHGRI GWAS catalog (National Human Genome Research Institute) and located 18 single-nucleotide polymorphisms (SNPs) associated with cardiometabolic traits ≤1 Mb from CFB (Figure 5; Table S6). Six SNPs were found to be associated with type 2 diabetes mellitus, MetS, or visceral fat. Six further SNPs were related to circulating lipids. The remaining SNPs were associated with coronary heart disease and hypertension (Table S6).

We also investigated whether variants at the CFB locus are associated with CFB expression by mining GTEx datasets (the Genotype-Tissue Expression project) for CFB cis-expression quantitative trait loci (QTLs). Fifty-three SNPs were associated with CFB expression in 4 tissues (Figure 5; Table S7). One SNP, rs76846904, close to the HLA-DRB5 gene, is highly correlated with CFB gene expression in subcutaneous...
adipose tissue (effect size, 0.78; \( P = 0.000015 \)) and within 100 kb of GWAS hits for visceral adiposity, serum cholesterol, and coronary heart disease.

The influence of the 18 GWAS SNPs, or any of their proxies (a total of 280 SNPs), on gene expression across 9 tissues was investigated using the GTEx Portal. Four SNPs were significantly associated (false discovery rate<0.05) with \( C_{FB} \) expression in tissues of interest (Figure 5; Tables S6 and S7). Two SNPs, correlating with \( C_{FB} \) expression in “adipose subcutaneous” and “artery aorta”, respectively, are proxies for rs13196329 and rs2247056, which are associated with visceral fat and triglycerides in the GWAS catalog (Table, Figure 5). Two further SNPs were significantly associated with increased \( C_{FB} \) expression in “heart LV” and correspond to the same SNP (rs805303) that is associated with increased systolic and diastolic blood pressure and hypertension in the GWAS catalog (Table; Figure 5).

**Discussion**

We tested the hypothesis that \( C_{fb} \) is necessary for the full expression of cardiometabolic pathophysiological traits in the SHR model of MetS. Through ZFN-mediated gene knockout, we showed that the \( C_{fb}^{-/-} \) SHR has improved glucose tolerance and insulin sensitivity, along with favorable adipose tissue distribution, adipose oxidative capacity, and reduced circulating lipids and proinflammatory cytokines compared with parental SHR. Further, \( C_{fb}^{-/-} \) rats had reduced blood pressure that was associated with increased ejection fraction and fractional shortening and reduced LV mass. The human \( C_{FB} \) locus—a gene-rich region within the major histocompatibility complex—contains several GWAS hits for cardiometabolic traits, including coronary heart disease, blood pressure, MetS, type 2 diabetes mellitus, serum lipids, and visceral fat. These colocalize with cis-expression QTLs associated with expression of \( C_{FB} \) in subcutaneous adipose tissue and other tissues, indicating that variation in \( C_{FB} \) expression may underlie, in part, the GWAS hits at this locus.

Glucose intolerance, insulin resistance, visceral adiposity, and dyslipidemia are the key metabolic features of MetS that increase the risk of type 2 diabetes mellitus.\(^{23} \) In our study, \( C_{fb}^{-/-} \) rats had reduced visceral but increased subcutaneous fat. To investigate potential molecular changes associated with favorably altered fat distribution and ameliorated glucose homeostasis in \( C_{fb}^{-/-} \) rats, we investigated transcripts central to adipose tissue metabolism. Reduced EAT mass in \( C_{fb}^{-/-} \) rats was because of reduced adipocyte number rather than altered adipocyte volume. \( Pgc1a, Cpt1, \) and \( Aco1 \) were upregulated in \( C_{fb}^{-/-} \) rats, suggestive of increased adipocyte oxidative phosphorylation, which we confirmed by Seahorse
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analysis. Cfb−/− rats exhibited a marked increase in basal and maximal respiration and had a 2-fold increased reserve respiratory capacity. Taken together with the reduction in adipocyte number, the data suggest that the elevation of mitochondrial respiratory capacity may provide an adipose tissue-intrinsic mechanism for reduced fat accumulation in Cfb−/− EAT.

In SAT, increased mass in Cfb−/− rats was associated with increased Fasn and reduced Pgc1a expression, consistent with the function of Fasn as an insulin-sensitive fatty acid synthase, the role of Pgc1a in stimulating fatty acid oxidation, and the known upregulation of FASN in human obesity and type 2 diabetes mellitus.24 These changes seemed to override the increases in Aco1 and Ucp1 expression observed in Cfb−/− rats, which would be expected to reduce adipocyte mass through increased trichloroacetic acid cycle activity and thermogenesis. The redistribution of visceral to subcutaneous

Figure 4. Gene expression levels in left ventricles after 72-h isoproterenol or saline treatment. A, Nppa, natriuretic peptide a, (B) Nppb, brain natriuretic peptide, (C) Camk2d, calcium/calmodulin dependent protein kinase II delta Myh6, (D) Myh7, myosin heavy polypeptide 7, (E) Actc1, α-cardiac actin, (F) Acta1, α-skeletal actin. Black-filled bars, SHR, saline-treated; stripe-filled bars, SHR, isoproterenol-treated; white-filled bars, Cfb−/−, saline-treated; hatch-filled bars, Cfb−/−, isoproterenol-treated. Differences in genotype *P<0.05, **P<0.005, ***P<0.0005 or treatment †P<0.05, ††P<0.005, †††P<0.0005.
fat marked changes in gene expression, and adipose respiratory capacity are likely to be the key to improvements in whole-body glucose homeostasis and metabolic function in Cfb−/− rats. Reduced BAT mass in Cfb−/− rats was associated with increased Pgc1a and Slc2a4 and decreased Ucp1 and FASN expression. This fat reduction may be consistent with increased Pgc1a driving lipolysis although inhibiting fatty acid synthesis; however, further experiments in Cfb−/− rats will be required to understand the BAT energy-substrate balance resulting from Cfb deficiency.

To further investigate altered adipose function in the Cfb−/− rat, we quantified Lep and Adipoq transcripts in EAT, SAT, and BAT. Although adipose Lep expression was reduced, circulating leptin was comparable in Cfb−/− and SHR. Although incompletely explained here, this could be accounted for by differences in post-translational processing and release, or peripheral metabolism, of leptin. Despite increased Adipoq expression in SAT alone, circulating high molecular-weight adiponectin was reduced in Cfb−/− rats. Conversely, high molecular-weight adiponectin in humans is lower in obese, insulin-resistant compared with lean, insulin-sensitive individuals.25 However, adiponectin deficiency in mice has been shown to have no effect on glucose homeostasis on a normal diet.26,27 Further, infusion of adiponectin in high-fat fed SHRs only marginally reduced insulin levels without affecting energy expenditure or hypertension.28 Taken together with the observed metabolic improvements, this suggests other mechanisms, besides adiponectin, drive insulin sensitization in the Cfb−/− rat.

We also tested the hypothesis that deletion of Cfb in SHR would affect the expression of SHR cardiovascular phenotypes. In this study, we showed that Cfb−/− rats had reduced systolic and diastolic blood pressure, reduced LV mass and cardiomyocyte diameter, and an abrogated isoproterenol-induced increase in rate pressure product. These alterations represent a marked amelioration in several of the key cardiovascular features of MetS manifested in SHR.

Figure 5. Cardiometabolic genome-wide association study (GWAS) hits and cis-eQTLs (quantitative trait loci) located in the human the complement factor B (CFB) locus. Eighteen relevant cardiometabolic single-nucleotide polymorphisms (SNPs) located <1 Mb from the boundaries of the human CFB gene (upper; red). Twenty-six SNPs were retrieved from the GTEx Portal that were found to be significantly associated with CFB expression (P<0.05), blue SNPs are associated with a significant negative effect, whereas red SNPs are associated with a significant positive effect. Four SNPs (with 1 overlapping) were determined to be correlated to both CFB expression, as well as being GWAS hits for relevant cardiometabolic traits (lower; red/blue). See Table S8 for a list of genes located in the CFB locus.
The reduction in blood pressure was associated with reductions in renin–angiotensin system components, suggesting that Cfb may have a direct effect, yet unexplained, on this system, mediating blood pressure and subsequently LV mass. Although Cfb deletion leads to lower blood pressure in SHR, our experiments do not distinguish whether Cfb is responsible for increasing above or maintaining basal blood pressure. Further detailed experiments are required to distinguish these 2 possible mechanisms.

To gain further insight into the molecular changes caused by Cfb deficiency in the heart, we investigated the effect of Cfb deletion on cardiomyogenic genes (ie, Nppa, Nppb, Myh6, Myh7, Acta1, and Camk2d), which are activated in response to stress. Our study showed that despite reduced LV mass, Camk2d expression was significantly increased in saline-treated Cfb−/−. CaMKII (calcium/calmodulin-dependent protein kinase type 2) is proposed to regulate inflammation (Cfb, Tnfa, and Il-6) and cardiomyogenesis in response to hypertension-related pressure overload, β-adrenergic agonists, or myocardial infarction-induced cell injury.45 Thus, Cfb may contribute to both cardiac inflammation and hypertrophy in response to stress, possibly through regulation of cardiomyogenic gene expression. For example, we showed complete or near complete abrogation in Cfb−/− of the metabolic and immune parameters that we measured in a mouse, although no cardiovascular measurements have been reported. Like the Cfb−/− rat, the Cfb−/− mouse lacks AP activity and has reduced Tnftc, II-6, and Ifn-γ.32,33 Although having some immune similarities to the Cfb−/− rat, Cfb−/− mice compared with WT mice are more glucose intolerant and have higher circulating triglycerides.34 The differences between these 2 models could be because of several reasons, including genetic background affecting metabolism differently, the use of high-fat diet in the mouse studies to elicit a phenotype, and the presence of 2 protein-coding Cfb transcripts in the mouse, whereas rats and humans have only one. On a high-fat diet, Ldlr−/−/Cfb−/− mice showed protection against atherosclerosis,35 which is distinct from the amelioration in metabolic and cardiovascular phenotypes that we observed here. However, the 2 studies combined strongly encourage further investigation of Cfb as a target for protection from the development of cardiovascular disease.

Rat Cfb resides in chromosome 20p12, a region previously found to be important in the regulation of blood pressure, glucose homeostasis, and adiposity in SHR.18–21 We propose that Cfb, at least in SHR, plays a major part in the development of key features of MetS that are linked to 20p12. However, given that the SHR.1N congenic that covers 20p12 has a reduction of 20 mm Hg, other genes in the region may also contribute.19

The location of human CFB and the syntenic region to the rat gene is on human 6p21.33,48 We located 18 SNPs with genome-wide significant associations to cardiometabolic traits ≤1 Mb from CFB. Several GWAS hits in the region were associated with type 2 diabetes mellitus and components of MetS. Two SNPs, rs13196329 and rs2247056, were correlated with visceral fat, triglycerides, and CFB expression. Further, 1 SNP, rs805303, was significantly positively correlated with visceral fat, triglycerides, and CFB expression associated with these SNPs may be causally linked to accumulation of visceral fat, circulating lipids, and development of hypertension in humans.

In addition to altering complement activity, Cfb ablation reduced proinflammatory cytokines Ifn-γ, II-6, and Tnftα whose elevated levels are associated with hypertension, obesity, and insulin resistance.36,37 Further, chronic low-grade inflammation and overactivation of the innate immune system are now recognized causes of type 2 diabetes mellitus,4,5 with clinical trials for therapeutic targets against inflammatory pathways for the treatment of diabetes mellitus and cardiovascular disease currently underway.38

| SNP identifier | Distance From TSS | Nominal P Value | P Value (FDR) | Slope† | Tissue | Proxy/GWAS Hit |
|---------------|------------------|----------------|--------------|--------|--------|----------------|
| rs805303      | 0.0020           | 0.0489         | 0.226        | heart left ventricle | GWAS hit |
| rs805301      | 0.0020           | 0.0489         | 0.226        | heart left ventricle | proxy to rs805303 |
| rs9264664     | 0.0012           | 0.0263         | −0.224       | artery sorda | proxy to rs2247056 |
| rs2858881     | 0.0028           | 0.0408         | 0.387        | adipose subcutaneous | proxy to rs13196329 |

Cfb indicates Complement factor b; FDR, false discovery rate; GWAS, genome-wide association study; QTL, quantitative trait locus; SNP, single-nucleotide polymorphisms; and TSS, transcription start site.

* P value (FDR). †P value after adjustment for false discovery rate.
Compounds that target CFB already exist, and taken together with the findings in our study, suggest that CFB has significant potential as a novel target for treatment of metabolic disease\(^9,40\).

This is the first study to report the widespread amelioration of metabolic and cardiovascular phenotypes through deletion of an alternative complement pathway gene in a model of MetS. Cfb deletion improves glucose homeostasis, adipose distribution and function, lowers blood pressure and reduces cardiac hypertrophy, protecting against LV stress. Together with our analysis of the human CFB region for cardiometabolic traits, we conclude that CFB expression and function may directly or indirectly regulate multiple metabolic and cardiovascular processes in health and disease in the rat and in humans.

**Perspectives**

CFB is elevated in human cohorts with type 2 diabetes mellitus and cardiovascular disease, although a causal relationship has yet to be established. We identified alterations in Cfb expression as a possible cause of hypertension and insulin resistance in the SHR. Cfb knockout rats have improved glucose homeostasis linked to favorable alterations in adipose tissue distribution and function and reduced blood pressure and LV mass suggesting new adipose tissue-intrinsic and blood pressure-independent mechanisms for SHR insulin resistance and cardiac hypertrophy. SNPs in human CFB are associated both with hypertension and visceral adiposity and with CFB gene expression, suggesting that genetic variation in CFB may, in part, explain the genetic associations at the human CFB locus. Further studies are required to establish whether overexpression of adipose tissue Cfb alone is the prime determinant of MetS traits. Clinical trials are presently being undertaken to test the therapeutic effects of CFB inhibitors and to investigate AP components as causal factors in human diseases related to overactivity of the innate immune system. Given the findings in this study, CFB may also be a valid therapeutic target to treat or prevent progression of human MetS.

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**Disclosures**

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Novelty and Significance

What Is New?

• Cfb—an innate immune component—is a determinant of adipose tissue distribution, glucose homeostasis, blood pressure, and LV mass in the SHR.

What Is Relevant?

• Cfb, directly or indirectly, drives novel adipose tissue-intrinsic and blood pressure-independent mechanisms for SHR insulin resistance, hypertension, and cardiac hypertrophy. SNPs associated with cardiometabolic traits and CFB gene expression, suggest variation in CFB may, in part, underlie these traits in humans.

Summary

Metabolic and cardiovascular components of MetS are improved by ablation of the CFB gene in SHR. At the human CFB locus, 3 SNPs are significantly associated with visceral adiposity, hypertension, and CFB gene expression.
Complement Factor B Is a Determinant of Both Metabolic and Cardiovascular Features of Metabolic Syndrome

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COMPLEMENT FACTOR B IS A DETERMINANT OF BOTH METABOLIC AND CARDIOVASCULAR FEATURES OF METABOLIC SYNDROME

Short title: complement factor b knockout rat

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Supplemental Methods

Rats

*Cfb*−/− rats were generated on an SHR/NCrl background (Charles River, Margate, UK), by microinjecting ZFN mRNA (Sigma), targeted to exon 6 of *Cfb* (target sequence: CCCCTCGGGCTCCATGaatatcTACATGGTGCTGGATG), into one-cell stage SHR/NCrl embryos that were implanted into pseudopregnant rats. Heterozygous progeny, from a founder harboring a 19 bp deletion in *Cfb*, were intercrossed to generate homozygous knockout rats. A search for off-target events was conducted by whole genome sequencing and analysed as described previously, confirmed the 19 bp deletion. Six additional putative variants, analysed by Sanger Sequencing, were determined to be false positives (Table S1). Rats were housed in open cages with free access to food and water. All procedures were carried out in accordance with UK Home Office regulations.

Serum analysis

Following an overnight fast, serum was extracted from whole blood exsanguinated under terminal isofluorane anaesthesia (n = 6 per group). Serum lipids were analysed by the Veterinary Pathology Laboratory, Edinburgh. In-house ELISAs were used to determine: serum Alternative complement (AP) activity (Hycult Biotech), leptin and total adiponectin (Merck Millipore), and high-molecular-weight (HMW) adiponectin and aldosterone (AMS Biotech). Serum Th1 cytokine concentrations were quantified using the LEGENDplex Rat Th1 Panel (6-plex) kit (BioLegend) and BD Accuri C6 Flow Cytometer (BD Biosciences). Those cytokines reported undetectable, were below the sensitivity of the assay.

Adipocyte morphometry

Epididymal fat pads were weighed, cut into five equal pieces, and processed for paraffin wax embedding (n = 6 rats per group). A random image was taken from one 4 μm thick H&E stained section per piece at 20x magnification to estimate mean adipocyte volume: a line grid was superimposed on to each image and point sampled intercept lengths (PSI) measured between two points on the cell membrane. One hundred PSI were measured per pad and adjusted for shrinkage. Fat pad weight was converted to volume according to Farvid et al, which was then divided by mean adipocyte volume to estimate volume-weighted adipocyte number.

Glucose homeostasis

Oral glucose tolerance (OGTT) (n = 10 per group) and intravenous insulin tolerance tests (IVITT) (n = 7 per group) were performed as described. Glucose clearance (KITT) was calculated as described.

Adipocyte metabolic rate

Isolated primary rat adipocytes (n = 6 rats per group) in Kreb’s buffer (118 mM NaCl, 1.2 mM MgSO₄, 15 mM NaPO₄, 1.265 mM CaCl₂, 5.56 mM Glucose, 1% BSA) were adhered to Matrigel (Corning) coated Seahorse plates (Agilent), washed with XF-DMEM (Agilent, supplemented with 1 mM Pyruvate and 10 mM Glucose, pH 7.4), and incubated (37°C, without CO₂, 15 min). A mitochondrial stress test was performed as described previously in an XFe24 Seahorse Bioanalyser (Agilent) and oxygen consumption rate data calculated according to the manufacturer’s instructions (Agilent Technologies LDA UK, Cheshire, UK).
Telemetry
Blood pressure transmitters were implanted, using isofluorane anaesthesia, according to manufacturer’s instructions (HD-S10, Data Sciences International). Following surgical recovery (>7 days), blood pressure, temperature and activity were recorded for 72 h (5 min/h) (n = 8-9 per group), before subcutaneous implantation of osmotic pumps, under brief isoflurane anaesthesia, (1003D, Azlet) containing either isoproterenol (1.2 mg/kg/h) or saline (n = 4-5 per group), and further data collected for 72 h.

Echocardiography
In vivo ultrasound echocardiography was performed by using a Vevo 770 ultrasound biomicroscope (Visualsonics) with a RMV710B 25 MHz center frequency transducer in 7 week-old male rats. Briefly, isoflurane anesthetized rats were placed on a thermostatically controlled ECG monitoring table and maintained at 37°C. Parasternal long axis (PLAX) ECG-Gated Kilohertz Visualisation (EKV) B mode and M-mode views of the left ventricle (LV) were acquired. LV end-systolic and end-diastolic areas were measured by tracing the endocardial border using Vevo Analysis Software (Visualsonics) in order to calculate ejection fraction (EF) from the PLAX EKV B mode view and fractional shortening from the M-mode view.

Cardiomyocyte diameter
Left ventricle mean cardiomyocyte diameter was determined as described previously using images taken by QImaging Micropublisher 3.3RTV camera (QImaging) attached to an Olympus BX51 microscope (Olympus) and measured using the STEPanizer program (n = 8 per group).

Gene expression
RNA was extracted from fat depots (subcutaneous (SAT), epididymal (EAT) and brown fat (BAT)) (n = 6 per group) and left ventricle (LV) (n = 4-5 per group) for qPCR, as described previously. Primer sequences are listed in Table S2. Actb was used as a reference gene for adipose transcripts and LV transcripts. LV transcripts from telemetric studies were normalised to Hprt, due to effects of isoproterenol on Actb expression. Ct values were compared using the 2−ΔΔCt method.

In silico analysis of the CFB locus
Single-nucleotide polymorphisms (SNPs) associated with cardio-metabolic traits related to type 2 diabetes and MetS residing ≤1 Mb from human CFB (Table S3) were identified by mining the NHGRI GWAS catalog. Proxy SNPs, based on linkage disequilibrium were determined using SNAP (https://archive.broadinstitute.org/mpg/snap/ldsearchpw.php) with the 1000 genomes Pilot 1 and HapMap (release 21 and 22) databases using default parameters (0.8 r² threshold, 500nt distance). GWAS and proxy SNP locations (280 in total) were converted to hg19 coordinates using dbSNP and the UCSC Liftover tool. Associations between SNPs and cis-regulated expression quantitative trait loci (cis-eQTLs) ≤1 Mb from CFB transcription start site (TSS) were determined from tissue data files (adipose subcutaneous, artery tibial, adipose visceral omentum, artery aorta, heart atrial appendage, heart left ventricle, pancreas, artery coronary, and liver) for SNP-gene association pairs downloaded from the GTex portal.
False discovery rate (FDR) was determined in R according to the Benjamini-Hochberg approach (https://www.r-project.org).
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## Supplementary Tables

### Table S1. Putative ZFN off-target events that were found to be false positives

| Gene name | Off-target position | Rnor_6.0 | SHR/NCrI (Illumina) | Cfb<sup>+/−</sup> (Illumina) | SHR/NCrI (Sanger) | Cfb<sup>+/−</sup> (Sanger) |
|-----------|---------------------|----------|---------------------|-----------------------------|------------------|-----------------------------|
| Grb2      | 1:98046688          | GCCC     | GC/GC               | G/GC                        | GCCC             | GCCC                        |
| Abhd17c   | 1:146289241         | C        | C/C                 | C/G                         | C                | C                           |
| AABR07051532.1 | 3:16440449-749 | C        | G/G                 | G/T                         | C                | C                           |
| AABR07065498.1 | 6:132175624    | A        | ACCCCC/             | ACCCCC/                     | A                | A                           |
| AABR07065768.3 | 6:140407070  | T        | T/C                 | G/C                         | T                | T                           |
| Ppidl1    | 9:121457023-68      | C        | C/C                 | C/T                         | C                | C                           |
| Gene      | Forward                  | Reverse                   |
|-----------|--------------------------|---------------------------|
| Aco1      | TCAGATAAAAGCTGGACACCCGGG | CCACTGGGGCCATCTTTCGGAT    |
| Actb      | ATGTACCCAGGGCATTTCTGAGA | GAGTACTTGGGCTTACAGGAGGA  |
| Actc1     | CAAAGCACGCCTACAGATCCCA  | GAAGACAGCCTCTGGGAGCATCA  |
| Adipoq    | CTGCCACCAAGGAAAACTTGCTG | TTAGGACAAAGAACAACCTGCAGT |
| Agt       | GCTGGGAGCTAAAGGACACACAG | AAAGGGGTGGATGTATACGCGG   |
| Camk2d    | AGTGAGGCTGATGCAAGTCTTTT | CAGGTCCCTGTGAACTATGCA    |
| Cfb       | AGTAGAGATCAAAGGCGGCTCC  | TCGAGTCTGACACGGGTATGG    |
| Cfb (ZFN) | AGGTTGAGGAGGAAGCTCACG  | AGGACTCGGACCCAGAGAAT     |
| Cpt1      | CTGAGACAGACTCACACCGCTT | GTTTTCCCTCCGTGTGGCTCAG   |
| Fasn      | TGGTGGCAGGGAGGTACACCC   | CCATGCTCTGAGCCAGAAAGAT   |
| Hprt1     | TCAGTCCCCAGCTGCTGATTAG  | TCGAGCAAGTCTTTCAGTCTGTG  |
| Lep       | CAGCAAGCTGCAAGGTCCAAGA  | TAGGACCAAAAGCCACAGGAACC  |
| Myh6      | ACACCAACCTGTCAAGTTCC    | ATCGTCATTTTCCTGGGCTCAG   |
| Myh7      | CAACCTGTCAAGTTCCGCAAGA | ACTCTTATTCAGGGCCTTGCC    |
| Nppa      | ATTTCAAGAACTCTGCTAGACC | GCACCTCCAGAGAGGAGCTAAG   |
| Nppb      | ACAATCCACAGATGCAAGTCTG | GAAGGCGCTGCTTGCAGACCTA   |
| Pgc1a     | TTGAACGCTGACCACCAAGGAG | CCAGGAGCAGCAACTCTATGG    |
| Renin     | GATCACTGCAAGGAGGCTCTCT | GATCACTGCAAGGAGGCTGGTA   |
| Slc2a4    | TTTGCACACCACCTCCGAAGGC | GGTTCCTTCATTCAGAGCAGCAT  |
| Ucp1      | ACATACTGGGCAAGTGACCTCCC | GCTGGGTACACTTGGGTACTGT   |
Table S3. Trait terms from the NHGRI-EBI GWAS catalog that were used to identify SNPs associated with cardiometabolic traits in the **CFB** locus

| NHGRI-EBI genome-wide association cardio-metabolic trait |
|---------------------------------------------------------|
| Basal_mетabolic_rate                                      |
| Blood_pressure                                            |
| Blood_pressure_(age_interaction)                          |
| Blood_pressure_(anthropometric_measures_interaction)     |
| Blood_pressure_(smoking_interaction)                      |
| Cardiac_hypertrophy                                       |
| Cardiovascular_disease_in_hypertension_(ACE_inhibitor_interaction) |
| Cardiovascular_disease_in_hypertension_(calcium_channel_blocker_interaction) |
| Cardiovascular_disease_risk_factors                       |
| Cardiovascular_heart_disease_in_diabetics                 |
| Cholesterol                                              |
| Cholesterol_and_Triglycerides                             |
| Cholesterol_total                                        |
| Coronary_heart_disease                                    |
| Coronary_heart_disease_event_reduction_in_response_to_statin_therapy_(interaction) |
| Diabetes_related_insulin_traits                           |
| Diastolic_blood_pressure                                  |
| Diastolic_blood_pressure_(alcohol_consumption_interaction) |
| Fasting_glucose-related_traits                           |
| Fasting_glucose-related_traits_(interaction_with_BMI)    |
| Fasting_insulin_(interaction)                             |
| Fasting_insulin-related_traits                           |
| Fasting_insulin-related_traits_(interaction_with_BMI)    |
| Fasting_plasma_glucose                                   |
| Fasting_plasma_glucose_(childhood)                       |
| Glucose_homeostasis_traits                               |
| Glycemic_traits                                           |
| HDL_cholesterol                                           |
| HDL_Cholesterol_-_Triglycerides_(HDLC-TG)                 |
| Hypertension                                              |
| Insulin_resistance/response                               |
| LDL_cholesterol                                           |
| Lipoprotein_(a)_-_cholesterol_levels                      |
| Lipoprotein_(a)_levels                                   |
| Metabolic_syndrome                                       |
| Metabolic_traits                                         |
| Systolic_blood_pressure                                   |
| Systolic_blood_pressure_(alcohol_consumption_interaction) |
| Systolic_blood_pressure_in_sickle_cell_anemia            |
| Triglycerides                                             |
Triglycerides-Blood Pressure (TG-BP)
Two-hour glucose challenge
Type 2 diabetes
Type 2 diabetes (dietary heme iron intake interaction)
Type 2 diabetes (young onset) and obesity
Type 2 diabetes and gout
Type 2 diabetes and other traits
Type 2 diabetes nephropathy
Visceral adipose tissue
Visceral adipose tissue adjusted for BMI
Visceral adipose tissue/subcutaneous adipose tissue ratio
Visceral fat
Table S4. Serum analytes

| Analyte                        | SHR       | Cfb<sup>−/−</sup> |
|--------------------------------|-----------|------------------|
| Cholesterol (mM)               | 1.62 ± 0.05 | 1.26 ± 0.06<sup>***</sup> |
| Triglyceride (mM)              | 0.28 ± 0.01 | 0.24 ± 0.02<sup>**</sup> |
| Adiponectin (total) (ng/mL)    | 38.3 ± 2.8  | 43.4 ± 2.6      |
| Adiponectin (HMW<sup>+</sup>) (ng/mL) | 3.81 ± 0.14 | 2.36 ± 0.05<sup>***</sup> |
| Leptin (ng/mL)                 | 0.95 ± 0.08 | 0.95 ± 0.05      |
| Aldosterone (ng/mL)            | 272 ± 14   | 150 ± 6<sup>***</sup> |
| IL-2 (pg/mL)                   | undetected | undetected       |
| IL-6 (pg/mL)                   | 108.7 ± 6.4| undetected       |
| IL-10 (pg/mL)                  | 182.2 ± 24.6| 45.9 ± 17.9<sup>*</sup> |
| GM-CSF<sup>†</sup> (pg/mL)     | 19.25 ± 4.9 | 10.5 ± 2.2      |
| IFN-γ (pg/mL)                  | 18.2 ± 1.1  | 7.12 ± 0.3<sup>***</sup> |
| TNFα (pg/mL)                   | 8.05 ± 1.98 | undetected       |

Results are mean ± SEM; *P < 0.05, **P < 0.005, ***P < 0.0001.

*HMW, high molecular weight.
†GM-CSF, granulocyte macrophage colony-stimulating factor.
| Parameter                                      | SHR      | Cfb<sup>−/−</sup> |
|-----------------------------------------------|----------|-------------------|
| LV<sup>−</sup> Mass; d (mg)                    | 646 ± 29 | 542 ± 43          |
| LV (mg/kg)                                    | 4500 ± 154 | 3649 ± 268*      |
| Endocardial Volume; d<sup>†</sup> (μL)         | 251 ± 14 | 248 ± 10          |
| Endocardial Volume; s<sup>‡</sup> (μL)         | 85 ± 9   | 64 ± 4            |
| Endocardial Area Change (mm<sup>2</sup>)       | 29.1 ± 1.5 | 33.6 ± 1.8      |
| LV wall thickness; d (mm)                     | 1.24 ± 0.05 | 1.08 ± 0.06      |
| Heart Rate (beats/min)                        | 324 ± 6  | 315 ± 7           |
| Endocardial Stroke Volume (μL)                | 165 ± 10 | 183 ± 9           |
| Ejection fraction (%)                         | 66.2 ± 2.3 | 73.9 ± 1.7*      |
| Fractional area change (%)                    | 47.0 ± 1.8 | 54.8 ± 1.9**     |
| Fractional shortening (%)                     | 36.1 ± 0.6 | 43.4 ± 1.0**     |
| Cardiac output (mL/min)                       | 53.9 ± 3.5 | 57.6 ± 2.5       |

Results are mean ± SEM; *P < 0.05, **P < 0.005, ***P < 0.0001.

<sup>†</sup>left ventricle.
<sup>‡</sup>d, diastole.
<sup>‡</sup>s, systole.
| Disease/trait                  | Strongest SNP/ risk allele | Chromosome position | Distance from Cfb (Mb) |
|-------------------------------|---------------------------|---------------------|----------------------|
| Type 2 diabetes               | rs3132524-G               | 31168937            | 0.775                |
| Coronary heart disease        | rs3869109-G              | 31216419            | 0.728                |
| LDL cholesterol, total cholesterol | rs9357121               | 31272702            | 0.671                |
| Triglycerides                 | rs2247056-T              | 31297713            | 0.646                |
| SBP, DBP                      | rs9266359-C              | 31364962            | 0.579                |
| Type 2 diabetes               | rs2244020-G              | 31379674            | 0.564                |
| Visceral fat adjusted for BMI | rs12175489-A             | 31409810            | 0.534                |
| Metabolic syndrome            | rs3099844-A              | 31481199            | 0.463                |
| SBP, DBP, Hypertension        | rs805303-G               | 31648589            | 0.296                |
| SBP, DBP, Hypertension        | rs2021783-C              | 32077074            | 0.126                |
| Triglycerides                 | rs419132-G               | 32243022            | 0.292                |
| Visceral fat                  | rs13196329-C             | 32357594            | 0.407                |
| Coronary heart disease        | rs9268402-G              | 32373576            | 0.423                |
| Cholesterol, total            | rs3177928-A              | 32444658            | 0.494                |
| Cholesterol, total            | rs114067101-G            | 32490183            | 0.539                |
| HDL cholesterol               | rs116569761              | 32680379            | 0.729                |
| Coronary heart disease        | rs11752643-T             | 32701596            | 0.751                |
| Type 2 diabetes               | rs3916765-A              | 32717773            | 0.767                |
| SNP Id          | P-value     | Effect size | Tissue                     | Chromosome position (Hg38) | Distance from TSS |
|-----------------|-------------|-------------|---------------------------|---------------------------|------------------|
| rs115056371     | 0.000084    | 0.17        | Adipose_Subcutaneous      | 31238942                  | -706731          |
| chr6_32630981_D | 0.000051    | 0.18        | Adipose_Subcutaneous      | 32663204                  | 717531           |
| rs9274179       | 0.000054    | 0.18        | Adipose_Subcutaneous      | 32662687                  | 717014           |
| rs28746813      | 0.000065    | 0.18        | Adipose_Subcutaneous      | 32665453                  | 719780           |
| chr6_32656068_I | 0.000072    | 0.18        | Adipose_Subcutaneous      | 32688291                  | 742618           |
| rs28746811      | 0.000076    | 0.18        | Adipose_Subcutaneous      | 32665420                  | 719747           |
| rs28746814      | 0.000085    | 0.18        | Adipose_Subcutaneous      | 32665470                  | 719797           |
| rs116066079     | 0.0001      | 0.18        | Adipose_Subcutaneous      | 32712646                  | 766973           |
| rs114682366     | 0.0001      | 0.18        | Adipose_Subcutaneous      | 32712664                  | 766991           |
| rs28724263      | 0.000023    | 0.19        | Adipose_Subcutaneous      | 32664152                  | 718479           |
| rs114830099     | 0.000028    | 0.19        | Adipose_Subcutaneous      | 32742444                  | 796771           |
| rs114515571     | 0.000041    | 0.19        | Adipose_Subcutaneous      | 32713384                  | 767711           |
| rs114227315     | 0.000041    | 0.19        | Adipose_Subcutaneous      | 32712602                  | 766929           |
| rs9274657       | 0.0000045   | 0.2         | Adipose_Subcutaneous      | 32668587                  | 722914           |
| rs9274659       | 0.0000045   | 0.2         | Adipose_Subcutaneous      | 32668608                  | 722935           |
| chr6_32656067_I | 0.000021    | 0.2         | Adipose_Subcutaneous      | 32688290                  | 742617           |
| rs9274209       | 0.000038    | 0.2         | Adipose_Subcutaneous      | 32663043                  | 717370           |
| rs28746806      | 0.000043    | 0.2         | Adipose_Subcutaneous      | 32665288                  | 719615           |
| rs28746832      | 0.00005     | 0.21        | Adipose_Subcutaneous      | 32666039                  | 720366           |
| chr6_32632717   | 0.000049    | 0.22        | Adipose_Subcutaneous      | 32664940                  | 719267           |
| rs9274227       | 0.000059    | 0.22        | Adipose_Subcutaneous      | 32663365                  | 717692           |
| rs191863247     | 0.0000039   | 0.27        | Adipose_Subcutaneous      | 32487582                  | 541909           |
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| chr6_32632878_I  | 0.000042 | 0.28 | Adipose_Subcutaneous | 32665101          | 719428          |
| chr6_32627913_D  | 0.000056 | 0.39 | Adipose_Subcutaneous | 32660136          | 714463          |
| rs60302302       | 0.0000064| 0.41 | Adipose_Subcutaneous | 32515926          | 570253          |
| rs181165562      | 0.000075 | 0.41 | Adipose_Subcutaneous | 32386129          | 440456          |
| rs76846904       | 0.000015 | 0.78 | Adipose_Subcutaneous | 32532140          | 586467          |
| rs76415507       | 0.000009 | -0.4 | Artery_Aorta         | 32524812          | 579139          |
| rs143726520      | 0.000044 | -0.36| Artery_Aorta         | 32520080          | 574407          |
| rs114624824      | 0.000013 | -0.34| Artery_Aorta         | 32524743          | 579070          |
| rs74655967       | 0.000013 | -0.34| Artery_Aorta         | 32524691          | 579018          |
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| rs80237386       | 0.000027 | -0.32| Artery_Aorta         | 32524716          | 579043          |
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| rs72492345       | 0.000049 | -0.32| Artery_Aorta         | 32564838          | 619165          |
| rs146763062      | 0.000027 | -0.31| Artery_Aorta         | 32523894          | 578221          |
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| chr6_32490131_D  | 0.000065 | -0.31| Artery_Aorta         | 32522354          | 576681          |
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| rs115918114      | 0.00005  | -0.3 | Artery_Aorta         | 32524524          | 578851          |
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| rs142399500      | 0.000059 | -0.29| Artery_Aorta         | 32521691          | 576018          |
| rs141142229      | 0.000082 | -0.29| Artery_Aorta         | 32524028          | 578355          |
| rs        | p-value   | MAF | Tissue/Location          | SNP_1   | SNP_2   |
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| rs114980010 | 0.0000041 | -0.33 | Artery_Tibial            | 31604704 | -340969 |
| rs1048709  | 0.000049  | -0.25 | Artery_Tibial            | 31947158 | 1485    |
| rs115804811 | 0.000022   | -0.81 | Lower_leg                | 32570025 | 624352  |
| rs74216018  | 0.000089   | -0.47 | Lower_leg                | 32524667 | 578994  |
| rs34382076  | 0.00001    | -0.44 | Lower_leg                | 32581548 | 635875  |
| rs79606458  | 0.000045   | -0.28 | Lower_leg                | 32522036 | 576363  |

*TSS, transcription start site*
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| ENSG00000204544 | 30983718        | 30989903      | MUC21               |
| ENSG00000261272 | 31010474        | 31035402      | MUC22               |
| ENSG00000228789 | 31053450        | 31059890      | HCG22               |
| ENSG00000222895 | 31083010        | 31083109      | RN6-1133P           |
| ENSG00000204542 | 31111223        | 31112559      | C6orf15             |
| ENSG00000204540 | 31114750        | 31140092      | PSORS1C1            |
| ENSG00000204539 | 31115090        | 31120446      | CDSN                |
| ENSG00000204538 | 31137536        | 31139350      | PSORS1C2            |
| ENSG00000238211 | 31140727        | 31140913      | POLR2LP1            |
| ENSG00000204536 | 31142439        | 31158238      | CCHCR1              |
| ENSG00000137310 | 31158542        | 31167159      | TCF19               |
| ENSG00000204531 | 31164337        | 31180731      | POU5F1              |
| ENSG00000204528 | 31173735        | 31177899      | PSORS1C3            |
| ENSG00000272501 | 31195200        | 31198037      | XXbac-BPG299F13.17  |
| ENSG00000206344 | 31197760        | 31203968      | HCG27               |
| ENSG00000271821 | 31200165        | 31201918      | XXbac-BPG299F13.14  |
| ENSG00000255726 | 31222913        | 31223093      | XXbac-BPG299F13.15  |
| ENSG00000255899 | 31224342        | 31225058      | XXbac-BPG299F13.16  |
| ENSG00000204525 | 31268749        | 31272130      | HLA-C               |
| ENSG00000234745 | 31269491        | 31357188      | HLA-B               |
| ENSG00000214892 | 31275572        | 31278754      | USP8P1              |
| ENSG00000227939 | 31280317        | 31281519      | RPL3P2              |
| ENSG00000231402 | 31287510        | 31288964      | WASF5P              |
| ENSG00000256166 | 31293900        | 31301642      | XXbac-BPG248L24.13  |
| ENSG00000222895 | 31307815        | 31308549      | XXbac-BPG248L24.10  |
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| ZDHHC20P2    | 31380411         | 31380839       |                 |
| HLA-S        | 31382074         | 31382288       |                 |
| XXbac-BPG181B23.7 | 31394289 | 31395495       |                 |
| MICA         | 31399784         | 31415315       |                 |
| HCP5         | 31400702         | 31477506       |                 |
| Y_RNA        | 31402152         | 31402250       |                 |
| LINC01149    | 31441667         | 31446973       |                 |
| XXbac-BPG181B23.6 | 31462728 | 31463336       |                 |
| MICB         | 31494881         | 31511124       |                 |
| Y_RNA        | 31496689         | 31496790       |                 |
| XXbac-BPG16N22.5 | 31515979 | 31516211       |                 |
| PPIAP9       | 31519480         | 31520291       |                 |
| RPL15P4      | 31528114         | 31528693       |                 |
| MCCD1        | 31528717         | 31530232       |                 |
| DDX39B       | 31530219         | 31542448       |                 |
| ATP6V1G2-DDX39B | 31530219 | 31546608       |                 |
| SNORD117     | 31536374         | 31536449       |                 |
| SNORD84      | 31541101         | 31541178       |                 |
| DDX39B-AS1   | 31542304         | 31543138       |                 |
| ATP6V1G2     | 31544462         | 31548427       |                 |
| NFKBIL1      | 31546870         | 31558829       |                 |
| LTA          | 31572054         | 31574324       |                 |
| TNF          | 31575567         | 31578336       |                 |
| LTB          | 31580525         | 31582522       |                 |
| LST1         | 31586124         | 31588909       |                 |
| NCR3         | 31588895         | 31592985       |                 |
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| ENSG00000204463 | 31639028   | 31652705 | BAG6          |
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| ENSG00000204439 | 31658298   | 31660772 | C6orf47       |
| ENSG00000227198 | 31658329   | 31660721 | C6orf47-AS1   |
| ENSG00000204438 | 31661229   | 3166283 | GPANK1        |
| ENSG00000201207 | 31663288   | 31663401 | Y_RNA         |
| ENSG00000204435 | 31665236   | 31670343 | CSNK2B        |
| ENSG00000263020 | 31666102   | 31673546 | XXbac-BPG32J3.22 |
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| ENSG00000266776 | 31701029   | 31701091 | MIR4464       |
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| ENSG00000255552 | 31711771   | 31714065 | LY6G6E        |
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| ENSG00000213722 | 31727038   | 31730617 | DDAH2         |
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| ENSG00000204410 | 31739948   | 31762834 | MSH5          |
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| ENSG00000204387 | 31834608       | 31839766     | C6orf48     |
| ENSG00000201823 | 31835263       | 31835326     | SNORD48     |
| ENSG00000201754 | 31835263       | 31835326     | SNORD52     |
| ENSG00000204386 | 31857659       | 31862906     | NEU1        |
| ENSG00000204385 | 31863192       | 31879046     | SLC44A4     |
| ENSG00000204371 | 31879759       | 31897687     | EHMT2       |
| ENSG00000237080 | 31883761       | 31884204     | EHMT2-AS1   |
| ENSG00000166278 | 31897785       | 31945672     | C2          |
| ENSG00000204366 | 31899607       | 31901992     | ZBTB12      |
| ENSG00000244255 | 31927698       | 31952048     | XXbac-BPG116M5.17 |
| ENSG00000281756 | 31934474       | 31941724     | C2-AS1      |
| ENSG00000243649 | 31945650       | 31952084     | CFB         |
| ENSG000002024356 | 31952087       | 31959110     | NELFE       |
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| ENSG00000204351 | 31959080       | 31969755     | SKIV2L      |
| ENSG00000204348 | 31969810       | 31972292     | DXO         |
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| ENSG00000233627 | 31999976       | 32003521     | C4A-AS1     |
| ENSG00000204338 | 32005636       | 32008451     | CYP21A1P    |
| ENSG00000248290 | 32008614       | 32012472     | TNXA        |
| ENSG00000250535 | 32013270       | 32013787     | STK19B      |
| ENSG00000224389 | 32014762       | 32035418     | C4B         |
| ENSG00000229776 | 32032713       | 32036258     | C4B-AS1     |
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| ENSG00000204314 | 32148359 | 32154373 | PRRT1 |
| ENSG00000258388 | 32153441 | 32163680 | PPT2 |
| ENSG00000241404 | 32164583 | 32168281 | EGFL8 |
| ENSG00000204310 | 32168212 | 32178096 | AGPAT1 |
| ENSG00000204308 | 32178354 | 32180793 | RNF5 |
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| ENSG00000204305 | 32184733 | 32185822 | XXbac-BPG300A18.13 |
| ENSG00000204304 | 32184741 | 32190186 | PBX2 |
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| ENSG00000204301 | 32194843 | 32224067 | NOTCH4 |
| ENSG00000277427 | 32255284 | 32300239 | XXbac-BPG154L12.5 |
| ENSG00000225914 | 32255711 | 32265838 | XXbac-BPG154L12.4 |
| ENSG00000204296 | 32288526 | 32371912 | C6orf10 |
| ENSG00000237285 | 32325219 | 32326178 | HNRNPA1P2 |
| ENSG00000223335 | 32352877 | 32352983 | RNU6-603P |
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| ENSG00000204287 | 32439842 | 32445046 | HLA-DRA |
| ENSG00000196301 | 32459821 | 32473500 | HLA-DRB9 |
| ENSG00000198502 | 32517343 | 32530287 | HLA-DRB5 |
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| ENSG00000229391 | 32552713 | 32560022 | HLA-DRB6 |
| ENSG00000196126 | 32578769 | 32589848 | HLA-DRB1 |
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| ENSG00000235040    | 32706124| 32706955| MTCO3P1             |
| ENSG00000232080    | 32718005| 32719170| XXbac-BPG254F23.7   |
| ENSG00000226030    | 32730758| 32731695| HLA-DQB3            |
| ENSG00000237541    | 32741342| 32747215| HLA-DQA2            |
| ENSG00000263649    | 32749912| 32749979| MIR3135B            |
| ENSG00000232629    | 32756098| 32763534| HLA-DQB2            |
| ENSG00000241106    | 32812763| 32817048| HLA-DOB             |
| ENSG00000250264    | 32813767| 32838822| XXbac-BPG246D15.9  |
| ENSG00000204267    | 32821833| 32838780| TAP2                |
| ENSG00000204264    | 32840717| 32844703| PSMB8               |
| ENSG00000204261    | 32844086| 32846495| PSMB8-AS1           |
| ENSG00000240065    | 32844136| 32859585| PSMB9               |
| ENSG00000168394    | 32845209| 32853978| TAP1                |
| ENSG00000234515    | 32879171| 32879848| PPP1R2P1            |
| ENSG00000235301    | 32896416| 32896490| HLA-Z               |
| ENSG00000242574    | 32934629| 32941070| HLA-DMB             |
Figure S1. Generation of a complement factor b knockout rat on an SHR background. (A) qPCR analysis of Cfb expression epididymal (Fat), left ventricle (LV) and liver from SHR (filled bars) and WKY (striped bars). (B) Serum alternative complement (AP) activity in SHR compared to WKY (+, positive control, -, negative control). (C) Graphical representation of Cfb detailing unique variants in SHR (red-circled) compared to BN and WKY. (D) Diagram of the exon-intron structure of the rat Cfb gene indicating the 19 bp deletion generated by zinc-finger nucleases in exon 6. (E) qPCR analysis of Cfb and immunoblot of Cfb protein expression in epididymal adipose tissue (Fat), left ventricle (LV) and liver, showing protein and transcript ablation in Cfb⁻/⁻ tissues, SHR (black-filled bars) and Cfb⁻/⁻ (white-filled bars). (F) Serum AP complement activity in Cfb⁻/⁻ (open bar) compared to SHR (filled bar) (+, positive control, -, negative control). (n = 5-6 per group). *P <0.05, **P <0.01, ***P <0.001.
Figure S2. Oxygen consumption rate (OCR) and CoxIV abundance in isolated adipocytes from SHR and Cfb⁻/⁻ rats. (A) basal respiratory rate, (B) reserve capacity (RC), (C) leak respiration, (D) ATP-linked respiration, and (E) ATP efficiency non-respiratory oxygen consumption rate in isolated epididymal adipocytes. (F) expression of CoxIV protein abundance in epididymal fat (n = 6 per group). *P <0.05.
Figure S3. Telemetric measurements of (A) mean core body temperature and (B) activity (n = 8-9 per group). 
*Cflb/−* (open bars) compared to SHR (filled bars). Significant differences between light and dark periods ***P < 0.001.
Figure S4. Baseline cardiovascular measurements. (A) Relative heart wet mass (n = 15 per group). (B) Light micrographs of representative H&E stained left ventricle sections (scale bar 10 µm). (C) Systolic and (D) diastolic blood pressure hourly plots during 72 h (n = 8-9 per group). (E) Mean diastolic blood pressure and (G) Heart rate. *P<0.05.
Figure S5. Gene expression of (H) renal renin and (I) hepatic angiotensinogen (n = 6 per group). **P <0.01, ***P <0.001
Figure S6. Wet cardiac masses taken from rats treated with isoproterenol and saline for 72 h. (A) Heart rate, (B) relative heart and (C) left ventricle wet masses (n = 4-5 per group). Black-filled bars, SHR, saline-treated; Stripe-filled bars, SHR, isoproterenol-treated; White-filled bars, Cfb^+/−, saline-treated; Hatch-filled bars, Cfb^−/−, isoproterenol-treated. Differences in genotype **P <0.01 or treatment †P <0.05, ††P <0.01, †††P <0.001 .
Figure S7. Myh6 expression levels in left ventricles following 72h isoproterenol or saline treatment. Black-filled bars, SHR, saline-treated; Stripe-filled bars, SHR, isoproterenol-treated; White-filled bars, Cfb\(^{-/-}\), saline-treated; Hatch-filled bars, Cfb\(^{-/-}\), isoproterenol-treated.