Molecular Genetics of Experimental Hypertension and the Metabolic Syndrome
From Gene Pathways to New Therapies
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Abstract—Genetic studies of human and experimental hypertension provide a means to identify key pathways that predispose individuals to increased blood pressure and associated risk factors for cardiovascular and metabolic diseases. The pathways so identified can then serve as targets for therapeutic intervention. This article discusses genetic studies in animal models of hypertension in which specific genes have been identified that regulate blood pressure and biochemical features of the metabolic syndrome. Consistent with studies in humans with monogenic disorders of blood pressure regulation, studies in rat models have demonstrated that naturally occurring genetic variation in pathways regulating sodium chloride transport can contribute to inherited variation in blood pressure. Such studies have also indicated that naturally occurring variation in genes, such as Cd36, that regulate fatty acid metabolism and ectopic accumulation of fat and fat metabolites can influence both biochemical and hemodynamic features of the metabolic syndrome and mediate the antidiabetic effects of drugs that activate the peroxisome proliferator-activated receptor-γ. Angiotensin II receptor blockers with the ability to selectively modulate activity of peroxisome proliferator-activated receptor-γ and expression of genes in these fat metabolism pathways may represent useful prototypes for a new class of transcription modulating drugs aimed at treating patients with hypertension and the metabolic syndrome. (Hypertension. 2007;49:941-952.)

Key Words: genetics ■ rats ■ inbred SHR ■ metabolic syndrome X ■ hypertension ■ angiotensin II type 1 receptor blockers ■ peroxisome proliferator-activated receptors

Genes involved in the pathogenesis of complex clinical disorders including essential hypertension and the metabolic syndrome are often referred to as quantitative trait loci (QTL). Identification of QTL at the molecular level is considered to be 1 of the major challenges in modern medicine, and it is hoped that QTL discovery in animal models and in humans will reveal mechanistic pathways that can ultimately serve as new targets for therapeutic intervention. The spontaneously hypertensive rat (SHR) and the Dahl salt-sensitive rat are the most widely studied animal models of spontaneous hypertension, and their value for investigating the pharmacology and pathophysiology of hypertension and cardiovascular disease has been recognized for many years.1-3 Until recently, however, it was unclear whether these spontaneous animal models of hypertension would prove useful for the identification of naturally occurring gene variants involved in the regulation of blood pressure (BP) or related cardiovascular and metabolic phenotypes. It was also unknown whether any such genes would prove relevant to the pathogenesis and treatment of any clinical disorders in humans. Major advances in methods for QTL mapping, together with the pioneering research of Rapp4 and other investigators, sparked multiple efforts to map genes influencing BP-related phenotypes in Dahl rats and in the SHR. These efforts resulted in the successful identification of many chromosome regions containing QTL-regulating BP or related cardiovascular and metabolic phenotypes in SHR and Dahl models (eg, see References 4–13). Although the discovery of chromosome regions harboring QTL for hypertension-related phenotypes turned out to be quite feasible, the task of moving beyond QTL mapping to pinpointing the identity of QTL at the molecular level has proven to be far more difficult. Although numerous chromosome regions have been linked to cardiovascular and other phenotypes in rat models of hypertension, rigorous criteria for establishing the identity of specific genes regulating complex traits have been difficult to satisfy.14 Although great progress has been made in the molecular identification of gene defects underlying monogenic forms of hypertension, efforts to identify specific DNA variants involved in polygenic forms of hypertension and related complex traits have proceeded at a far slower pace.15

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941
The search for specific genes that underlie variation in complex traits like BP is extremely challenging, because such phenotypes are determined by the interaction of multiple environmental and genetic factors. Because variation in a single QTL is neither necessary nor always sufficient to promote variation in a complex phenotype and because the effects of individual QTL on polygenic traits may be quite modest it is very difficult to identify the location of QTL at the molecular level. However, by careful application of a research paradigm originally proposed by Rapp and by using experimental strategies in congenic and transgenic strains that greatly reduce genetic and environmental complexity, studies in Dahl rats and in SHRs have established that, in animal models of spontaneous hypertension, QTL for BP and related complex traits can be distinctly identified at the molecular level, and QTL so identified can indeed be relevant to the pathogenesis and treatment of related human disorders. Several examples of QTL identified at the molecular level in the Dahl and SHR models are discussed further below. The studies of Bianchi et al in Milan hypertensive rats provide further examples of specific gene variants implicated in other animal models of hypertension that also have potential relevance to the pathogenesis and treatment of human hypertension.

**Fulfilling Rapp’s Paradigm at the Molecular Level: Identification of Mutations in the Gene Encoding 11β-Hydroxylase in the Dahl Model**

More than 2 decades ago, Rapp published a paradigm for the identification of primary genetic causes of hypertension in rats. To establish that a specific genetic locus was involved in regulating BP, this paradigm required identification of variants that not only cosegregated with effects on BP but also were responsible for Mendelian inheritance effects on a biochemical or physiological trait that could be logically expected to influence BP. This latter criterion was included to minimize the possibility that the suspect locus of interest would turn out to be a marker for a closely linked gene regulating BP rather than a true BP regulatory locus itself. Although not without limitations, fulfillment of Rapp’s paradigm can establish a wealth of evidence that may be considered sufficient for purposes of QTL identification. To obtain conclusive proof of QTL identity at the single gene level, definitive studies can be conducted in which comparative phenotyping is performed in 2 strains that differ at only a single locus.

The first discovery of specific DNA sequence variants that fulfilled Rapp’s paradigm involved the identification of mutations in the coding sequence of the gene for 11β-hydroxylase. In studies in Dahl salt-sensitive (SS/Jr) and salt-resistant (SR/Jr) rats conducted in collaboration with George Cicila and John Rapp, we identified DNA variants encoding 5 amino acid substitutions in 11β-hydroxylase that cosegregated with Mendelian effects on the adrenal capacity to synthesize a mineralocorticoid hormone, 18-hydroxy-11-deoxycorticosterone, and with effects on BP. In contrast to the hypertensive Dahl SS/Jr strain, the normotensive Dahl SR/Jr strain carried a particular allele for 11β-hydroxylase that helped protect against salt-induced increases in BP. Although a variety of DNA polymorphisms had been identified previously in chromosome regions linked to the regulation of BP, none of them had fulfilled Rapp’s paradigm or had otherwise involved sequence variants of any known functional significance. The functional effects of the 11β-hydroxylase mutations on synthesis of 18-hydroxy-11-deoxycorticosterone and on BP regulation were later confirmed in transfection studies and in painstaking studies that involved the development of high-resolution congenic strains. Although 18-hydroxy-11-deoxycorticosterone does not bind to mineralocorticoid receptors as strongly as aldosterone, its circulating levels in the rat are several orders higher than those of aldosterone. Thus, despite the lower potency of 18-hydroxy-11-deoxycorticosterone, its greater circulating concentrations may enable it to effectively compete with aldosterone for binding to mineralocorticoid receptors and thereby influence renal sodium reabsorption.

The discovery of specific DNA variants in 11β-hydroxylase that influenced BP in genetic crosses derived from Dahl SS/Jr and SR/Jr rats was important in establishing that QTLs regulating BP in animal models of spontaneous hypertension could be pinpointed at the molecular level. Moreover, Lifton et al had discovered that mutations in 11β-hydroxylase were involved in causing glucocorticoid remediable aldosteronism, a monogenic form of human hypertension. Thus, the results in Dahl rats confirmed that at least some of the same genes affecting BP in animal models of spontaneous hypertension were also involved in BP regulation in humans. The genetic studies in Dahl rats, as well as those by Bianchi et al in Milan rats, are consistent with the research of Lifton and colleagues demonstrating that genetic variants affecting BP typically involve mechanisms that regulate renal sodium chloride transport. In the Milan rat model, the identification of sequence variants in adducin genes that increase sodium–potassium pump activity and BP has helped motivate clinical studies of an antihypertensive drug that can antagonize these effects of adducin mutations.

**Molecular Genetics of Hypertension and the Metabolic Syndrome: Extending Rapp’s Paradigm in the SHR**

Essential hypertension is often associated with the clustering of multiple risk factors for diabetes and cardiovascular disease, including insulin resistance and dyslipidemia, as well as increased BP. This clustering of multiple risk factors for heart disease and diabetes is frequently referred to as the metabolic syndrome and appears to affect 25% of the general population and as many as 50% of patients with hypertension. Although considerable emphasis has been placed on insulin resistance as the root cause of the metabolic syndrome, much of the evidence in support of this concept has been based on studies of a correlative nature that have limited ability to dissect cause and effect relationships.

Given the potential for genetic strategies to identify QTL at the molecular level and uncover primary mechanisms driving complex phenotypes, we began genetic studies in the SHR, a widely used animal model of hypertension and the metabolic syndrome. It was known that, under the appropriate experimental conditions and depending on the types of experimental control strains that were studied, systemic and cellular
alterations in both carbohydrate and lipid metabolism could be demonstrated in this hypertensive rat model. For example, 

20 years ago, Mondon and Reaven reported finding differences in insulin and glucose metabolism between SHRs and Wistar–Kyoto rats.

Developing New Tools and Strategies for Genetic Dissection of Hypertension and the Metabolic Syndrome in SHRs

In the early 1990s, experimental resources for mapping QTL in the SHR and resolving them at the molecular level were not available. Thus, new tools were required to conduct detailed mapping studies in hypertensive rat models. Although it was possible to perform molecular and functional studies of particular candidate genes as with 11β-hydroxylase in the Dahl model, resources for high-resolution, genome-wide mapping studies in the rat were not readily available. Thus, it was necessary to create improved genetic maps of the rat and to characterize large genetic crosses derived from the SHR for QTL mapping studies. The efforts of Howard Jacob and many others resulted in the generation of linkage maps and other genetic resources based on crosses between the SHR and the normotensive Brown Norway (BN) rat and between other strains as well. The SHR–BN model was emphasized because the 2 progenitor strains constituted a highly polymorphic genetic system that afforded an abundance of contrasting DNA markers, as well as a variety of contrasting phenotypes relevant to hypertension and related metabolic disorders. Ultimately, to confirm the results of QTL mapping studies and establish proof of QTL identity at the molecular level, it would be necessary to develop congenic and transgenic strains of SHR.

By capitalizing on comprehensive strategies that coordinate the use of specialized genetic strains and advanced molecular tools, it has become possible to definitively identify specific DNA variants that influence complex cardiovascular and metabolic traits in the SHR and other models. One such strategy involves QTL mapping studies followed by sequential use of congenic strains, cDNA microarrays, DNA sequence analysis, gene and protein function studies, and transgenic strains to ultimately establish the identity of specific genes that influence the pathogenesis of complex clinical disorders (Figure 1). This particular experimental approach leading from QTL mapping to establishing QTL identity at the molecular level has been applied to studies of hypertension and the metabolic syndrome in SHRs as discussed further below.

Mapping QTL for Intermediate Phenotypes Related to the Metabolic Syndrome

A popular, although controversial, approach to the initial genetic dissection of complex clinical disorders involves linkage analysis of so-called “intermediate phenotypes,” that is, cellular, biochemical, or physiological traits that are presumed to be involved in pathogenesis of the clinical disorder of interest. The advantage of this strategy is that intermediate phenotypes are genetically less complex than clinical disorders such as the metabolic syndrome and are, thus, more likely to be amenable to linkage analysis. However, the Achilles heel of this approach is that it depends on the correct a priori understanding of intermediate steps truly involved in pathogenesis of the clinical disorder of interest. Unfortunately, for most complex clinical disorders, a vast number of intermediate phenotypes can be imagined, and the fundamental problem is a lack of clear knowledge of the primary pathways that actually initiate disease pathogenesis. Thus, some investigators refer to such traits as “candidate” or “likely” intermediate phenotypes to convey the uncertainty associated with use of this terminology.

To begin searching for QTL that promote elements of the metabolic syndrome in SHR, Atman et al mapped chromosome regions linked to the regulation of adipocyte insulin sensitivity and catecholamine-induced lipolysis, 2 intermediate phenotypes thought to be relevant to the pathogenesis of the metabolic syndrome. Given that impaired insulin sensitivity and disordered fatty acid metabolism represent characteristic features of the metabolic syndrome, it was presumed that linkage analysis of these cellular phenotypes might lead to the identification of gene variants involved in the primary pathogenesis of at least some of the clinical components of the syndrome.

In linkage studies in recombinant inbred strains and in F2 and backcross populations derived from SHRs, it was possible to map QTL-regulating adipocyte insulin sensitivity and catecholamine-induced fatty acid release to the telomeric region of rat chromosome 4 in the vicinity of the genes encoding the RT8 alloantigen and interleukin-6. In linkage studies in recombinant inbred strains derived from the SHR, Bottger et al had also linked the same region of chromosome 4 to the regulation of BP and high-density lipoprotein phospholipid levels. Taken together, these observations raised the possibility that naturally occurring variation in a gene or genes located near the telomere of rat chromosome 4...
might be influencing multiple hemodynamic and biochemical features of the metabolic syndrome in the SHR.

**Developing SHR Congenic Strains to Isolate QTL for the Metabolic Syndrome**

To confirm the linkage results and begin to isolate candidate genes for the metabolic syndrome within the QTL target region of chromosome 4, Pravenec and colleagues derived a congenic strain that carried the target segment of chromosome 4 from the normotensive BN rat on the SHR background. Transfer of this region of BN chromosome 4 onto the genetic background of the SHR partially improved the SHR defect in insulin-stimulated glucose uptake in adipocytes and completely corrected the impaired catecholamine-induced release of fatty acids in adipocytes. Moreover, the SHR congenic strain harboring this target segment of BN chromosome 4 showed significant decreases in BP; reduced circulating levels of insulin, fatty acids, and triglycerides; and improved glucose tolerance compared with the SHR progenitor strain. The extent to which variation in the target segment of chromosome 4 affected these systemic phenotypes ranged on the order of 10% to 50%. Thus, the region of chromosome 4 trapped in the congenic strain appeared to be regulating 1 of the intermediate candidate phenotypes (catecholamine-induced release of fatty acids from adipocytes) in a Mendelian fashion while influencing key systemic features of the metabolic syndrome in a more complex, quantitative fashion. The ability of this region of chromosome 4 to influence glucose tolerance and circulating fatty acid levels was observed in rats fed a high-fructose diet but not in rats fed normal chow. Hence, the findings also revealed a role for environment– genotype interaction in the pathogenesis of metabolic disturbances in the SHR model. Finally, the isolation of a QTL for the metabolic syndrome on the uniform genetic background of a congenic strain set the stage for identifying high-priority candidate genes within the target chromosome region worthy of further study.

**Use of Expression Profiling in Congenic Strains to Identify Defective CD36 as a High-Priority Candidate Gene for the Metabolic Syndrome**

Having confirmed that QTL regulating multiple cellular and systemic features of the metabolic syndrome could be isolated within a defined segment of rat chromosome 4, the next challenge was to identify candidate genes within the target chromosome region that could be subjected to further molecular and physiological analysis. Although several strategies exist for narrowing the focus on specific genes within a QTL region, and all are subject to various limitations, Aitman and colleagues chose to use an approach that combines the power of genetics with genome-wide expression profiling. The combined genetic analysis of gene expression levels and cardiovascular and metabolic phenotypes can be accomplished in segregating populations and in specialized animal models, including congenic strains. With respect to the chromosome 4 QTL linked to features of the metabolic syndrome, the approach involved the use of cDNA microarray analysis to search for genes that were differentially expressed in fat tissue of the SHR progenitor strain versus the SHR congenic strain that harbored the QTL target region of chromosome 4 from the BN strain. The comparison of 2 strains of SHRs that are genetically identical except for a single segment of chromosome 4 helped to reduce the complexity of the gene expression profiles and guide the focus on high-priority candidate genes for follow-up studies. In contrast to comparisons between conventional hypertensive and normotensive strains, the use of congenic strains in this study reduced the number of differentially expressed gene targets by 80%. Moreover, by concentrating on genes that were not only differentially expressed but that also physically mapped within the congenic chromosome segment, it was possible to narrow the focus of the gene profiling results even further.

In the expression profiling studies, 1 particular gene was observed to show a dramatic difference in expression between the SHR progenitor strain and SHR chromosome 4 congenic strain; the gene was highly expressed in adipose tissue of the SHR chromosome 4 congenic strain but showed little or no expression in adipose tissue of the SHR progenitor strain. No other genes showed this major degree of differential expression, and, therefore, attention was immediately focused on this gene. The gene was found to encode the CD36 fatty acid transporter and mapped directly back within the differential segment on chromosome 4 linked to the hypertension metabolic syndrome. Thus, the segment of chromosome 4 trapped in the congenic strain was found to influence multiple cellular and systemic phenotypes involved in the metabolic syndrome, to regulate expression of the gene encoding the CD36 fatty acid transporter, and also to physically contain the gene for CD36 (Figure 2). Molecular studies demonstrated that the SHR progenitor strain (the SHR/ National Institutes of Health variety) carries a major deletion in the gene for CD36 that abolishes normal expression of the encoded protein. In addition, the potential impact of the...
mutation on cellular fatty acid transport was indirectly supported by studies demonstrating impaired uptake of long chain fatty acids by cardiomyocytes and adipocytes derived from the SHR progenitor strain harboring mutant CD36 compared with those derived from the SHR chromosome 4 congenic strain harboring wild-type CD36. These findings strongly suggested that the gene for CD36 was responsible for the chromosome 4 QTL linked to features of the metabolic syndrome. However, definitive proof that the gene for CD36 was acting as a QTL for the metabolic syndrome remained to be established, and its specific influence on clinically related features of the syndrome was unclear.

Establishing Proof of QTL Identity at the Molecular Level

Many chromosome regions have been identified that contain QTLS linked to the regulation of BP and other complex traits. In addition, numerous candidate genes have been proposed to be responsible for QTL mapped in linkage studies and in congenic strains. However, linkage of high-priority candidate genes to the regulation of complex phenotypes even in congenic strains does not provide definitive proof of QTL identity at the molecular level. Because many genes are present within QTL regions mapped in segregating populations and in congenic strains, further studies are required to definitively confirm a role for specific gene defects in the pathogenesis of a complex trait. This can be accomplished by deriving and comparing 2 strains that are genetically identical except that 1 carries the gene defect of interest and the other carries the wild-type form of the gene.

Derivation of Transgenic SHR to Establish That the Genetic Defect in CD36 Constitutes a QTL Promoting Multiple Features of the Metabolic Syndrome

To establish whether the mutant gene for CD36 was a bona fide QTL affecting biochemical and or hemodynamic features of the metabolic syndrome, Pravenec et al used transgenic techniques to create 2 strains of SHRs that are genetically identical except that 1 harbors mutant CD36 and the other expresses wild-type CD36. Thus, any phenotypic differences between these strains could then be directly attributed to their differences in CD36. National Institutes of Health–derived strains of SHR appeared to carry a loss of function mutation in the gene for CD36, and, therefore, we determined whether transgenic expression of wild-type CD36 on the SHR background could ameliorate features of the metabolic syndrome. However, definitive proof that the gene for CD36 was acting as a QTL for the metabolic syndrome remained to be established, and its specific influence on clinically related features of the syndrome was unclear.

Development of SHR With Coisogenic Kidneys to Establish That a Genetic Defect in CD36 Constitutes a BP QTL in the SHR/National Institutes of Health Strain

Although expression of wild-type CD36 on the SHR background significantly improved glucose and lipid metabolism in several transgenic lines, only a single transgenic line showed a reduction in BP. Initially, this observation suggested that whereas the defective gene for CD36 constituted a QTL regulating biochemical features of the metabolic syndrome, it did not constitute a QTL for BP. However, subsequent studies revealed that the 1 SHR transgenic line exhibiting a reduction in BP was also the only line in which the transgene for wild-type CD36 was clearly expressed in the kidney. This observation raised the possibility that genetically determined variation in the renal expression of CD36 might influence BP in the SHR. If so, this would mean that the gene defect in CD36 could be influencing both biochemical and hemodynamic features of the metabolic syndrome and also constitute 1 of the first BP QTL identified at the molecular level in SHRs.

To test whether deficient expression of CD36 inside the kidney might be promoting increased BP, Pravenec et al performed a correlation analysis between renal expression levels of CD36 mRNA and BP in recombinant inbred strains derived from the SHR and BN progenitors. In these studies, BP correlated inversely with the renal expression of wild-type CD36 (r = −0.63; P = 0.0002; unpublished observations). This motivated us to perform kidney transplant experiments in which we created 2 groups of genetically identical SHRs that differed only in renal expression of CD36 (Figure 3). This was accomplished by transplanting kidneys from SHRs with mutant CD36 or from transgenic SHRs with abundant renal expression of wild-type CD36 into bilaterally nephrectomized SHR congenic rats that expressed wild-type CD36 in extrarenal tissues. This enabled us to determine the BP effects of selective deficiency of CD36 inside the kidney. The systolic BP of recipients with mutant CD36 in the kidney and, thus, selective renal deficiency of CD36 was significantly greater than in rats that expressed wild-type CD36 inside the kidney. Similar results were observed in transplantation studies in which we compared the BP effects of
Three of the main mechanisms that influence ectopic accumulation of fat and fat metabolites are depicted in Figure 4. A common cause of ectopic accumulation of fat and fat metabolites involves situations in which the fat storage capacity of subcutaneous adipose tissue is exceeded or impaired, thereby resulting in the diversion of fat to other sites. Ectopic accumulation of fat and fat metabolites may also occur in association with defects in a tissue’s ability to metabolize fat or in situations associated with increased lipogenesis in visceral organs. By causing impaired fatty acid uptake into adipose tissue, mutations in CD36 can promote increases in circulating levels of fatty acids that, depending on the dietary circumstances, may lead to the ectopic accumulation of fat or fat metabolites that disturb glucose metabolism. For example, in SHRs with mutant CD36 or in mice with targeted deletion of CD36, hepatic triglyceride levels are significantly increased compared with those in controls with wild-type CD36.53,54 Based on recent studies suggesting a role for CD36 in mitochondrial fatty acid metabolism, it is possible that defective CD36 might further promote ectopic accumulation of fat and lipid metabolites by imperiling fat oxidation within skeletal muscle and other tissues.55 In preliminary studies using a novel strain of SHR that carries the mitochondrial genome of the BN rat, we have found that a unique variant in the gene encoding mitochondrial cytochrome C oxidase subunit 1 is also linked to reductions in mitochondrial cytochrome C oxidase subunit 1 protein levels and biochemical features of the metabolic syndrome.56 Reductions in mitochondrial cytochrome C oxidase subunit 1 protein levels have been associated with increases in intramyocellular lipids and impaired skeletal muscle glucose metabolism in insulin-resistant offspring of diabetic parents.57 These observations raise the possibility that variants in the mitochondrial genome and in the nuclear genome could interact to impair fat metabolism and further increase the risk for the metabolic syndrome. Finally, studies in recombinant inbred and transgenic strains of SHRs have indicated that genetic variation in sterol regulatory element binding protein, a transcription factor regulating hepatic lipogenesis, may affect features of the metabolic syndrome by influencing susceptibility to ectopic fat accumulation in the liver.58–60
The mechanisms whereby CD36 deficiency may promote increased BP remain to be determined. Systemic deficiency of CD36 might promote fat accumulation affecting the renal parenchyma or induce alterations in adipocytokine levels that could affect BP. In addition, Zhu and Smart have shown that CD36 colocalizes with endothelial NO synthase in caveolae of endothelial cells and that CD36 is a determinant of endothelial NO synthase activation by fatty acids. Given that deficient renal NO synthesis has been implicated in the pathogenesis of hypertension, these findings raise the possibility that impaired renal CD36 expression might be affecting BP in SHRs by modulating effects of fatty acids on NO-related pathways. In CD36 knockout mice maintained on a normal or high-fat diet, short-term measurements of BP obtained in the anesthetized state were found recently to be similar to those obtained in control mice. However, the effects of CD36 deficiency on chronic BP levels of conscious, unrestrained mice have not been reported. It is possible that effects of CD36 deficiency on BP in mice might be observed in telemetry studies or in studies in which CD36 deficiency is tested on an assortment of different genetic and dietary backgrounds.

Relevance of CD36 Deficiency to Hypertension and the Metabolic Syndrome in Humans

In humans, variants causing CD36 deficiency are relatively rare but have been reported to be associated with insulin resistance, dyslipidemia, and hypertension. More common variants in CD36 have also been associated with increased risk for type 2 diabetes and cardiovascular disease and with effects on serum lipid levels. For example, a common CD36 haplotype with a frequency of approximately 25% to 30% in nondiabetic white populations has been associated with increased serum levels of free fatty acids and triglycerides, and the same haplotype has also been associated with increased risk of cardiovascular disease in patients with type 2 diabetes. These observations indicate that in at least some cases, genetic defects associated with certain cardiovascular and metabolic risk factors in the SHR can be associated with similar cardiovascular and metabolic risk factors in humans. The fact that a primary genetic abnormality in a fatty acid transporter can promote multiple features of the metabolic syndrome in both rats and humans suggests that other kinds of genetic disturbances in lipid metabolism might also be involved in pathogenesis of the metabolic syndrome.

Implications for Treatment of the Metabolic Syndrome

The discovery that defective CD36 can contribute to the pathogenesis of disordered carbohydrate and lipid metabolism in animal models and in humans has implications for developing improved approaches to the treatment of the metabolic syndrome. The gene for CD36 is a known target for peroxisome proliferator-activated receptor-γ (PPARγ), a ligand-activated transcription factor that regulates the expression of multiple genes involved in carbohydrate and lipid metabolism. Thiazolidinedione ligands of PPARγ are clinically approved drugs for the treatment of type 2 diabetes and have been shown to increase expression of CD36, improve insulin sensitivity, decrease fatty acid levels, and reduce BP. These agents have also been shown to provide strong protection against the development of diabetes in patients with impaired glucose metabolism. In addition, patients with dominant-negative mutations in PPARγ, like patients with CD36 deficiency, have been reported to have insulin resistance, dyslipidemia, and hypertension.

The availability of SHR harboring mutant CD36 or wild-type CD36 enabled the use of a pharmacogenetic approach to test the importance of CD36 in the insulin-sensitizing actions of thiazolidinediones. SHRs with mutant CD36 were relatively resistant to the beneficial effects of pioglitazone on glucose and lipid metabolism compared with SHRs expressing wild-type CD36. Specifically, defective CD36 blunted the ability of pioglitazone to reduce circulating levels of fatty acids, triglycerides, and insulin and to decrease hepatic steatosis and visceral fat accumulation. These findings
indicate that CD36 can be a key determinant of the metabolic actions of a thiazolidinedione ligand of PPAR\(\gamma\) and suggest that other agents that increase the expression of CD36, like thiazolidinediones, might also be useful in patients with insulin resistance and the metabolic syndrome.

Although thiazolidinedione ligands of PPAR\(\gamma\) are valuable transcription-modulating drugs for treating insulin resistance and can greatly reduce the risk for new-onset diabetes, these agents are associated with certain adverse effects that may limit their use in clinical practice. Specifically, thiazolidinediones can promote fluid retention, weight gain, and heart failure and have been shown to increase the incidence of congestive heart failure by as much as 500%, even in patients at relatively low risk for cardiovascular disease.76,79 Thus, the use of thiazolidinediones to prevent diabetes in patients with hypertension and the metabolic syndrome, many of whom have pre-existing cardiovascular disease, could increase the risk for congestive heart failure even further. The identification of ligands of PPAR\(\gamma\) that increase CD36 expression and ameliorate the metabolic syndrome without causing fluid retention, weight gain, and increased risk of congestive heart failure could be of considerable clinical value.

**New Opportunities for Treating Hypertension and the Metabolic Syndrome: Angiotensin Receptor Blockers That Selectively Modulate PPAR\(\gamma\)**

As part of a research effort to identify PPAR\(\gamma\) ligands that do not cause fluid retention and weight gain, we observed that a clinically approved angiotensin receptor blocker (ARB), telmisartan, not only blocks binding of angiotensin II to the angiotensin II type 1 receptor but can robustly activate PPAR\(\gamma\) even when tested at concentrations that might be achieved in plasma with conventional oral dosing.80 Other investigators have made similar observations and have found that telmisartan’s ability to activate PPAR\(\gamma\) does not depend on the presence of the angiotensin II type 1 receptor.81 Although several other ARBs can cause some activation of PPAR\(\gamma\) when tested at high concentrations (\(\approx 10 \mu\text{mol/L}\)), telmisartan seems to be the only commercially available ARB to clearly cause cellular activation of PPAR\(\gamma\) at concentrations of \(\approx 5 \mu\text{mol/L}\) (Figure 5).80,82 In addition, Janke et al83 have demonstrated that telmisartan in concentrations as low as 1 \(\mu\text{mol/L}\) can activate PPAR\(\gamma\) target gene sequences in human fat cells. Erbe et al84 reported that a variety of ARBs can promote physical binding of the transcription cofactor PGC-1\(\alpha\) to PPAR\(\gamma\), but telmisartan appeared to be the only ARB to cause substantial activation of PPAR\(\gamma\) when tested in a cell-based system. We and others have found that telmisartan can increase the expression of the gene for CD36 as well as additional downstream target genes in both the nuclear genome and mitochondrial genome that may contribute to the insulin-sensitizing effects of PPAR\(\gamma\) activators.80,85,86 Consistent with our observations of a role for CD36 in the insulin-sensitizing effects of thiazolidinedione activators of PPAR\(\gamma\), Li et al87 have reported that telmisartan can improve glucose metabolism to a greater extent in SHRs that express wild-type CD36 compared with SHRs with mutant CD36.

In contrast to the thiazolidinedione ligands of PPAR\(\gamma\), telmisartan is a partial agonist of PPAR\(\gamma\) and belongs to a class of molecules known as selective PPAR modulators (SPPARMs) that may improve glucose and lipid metabolism without promoting fluid retention and weight gain.80,85 In animal models of dietary-induced insulin resistance, we and others have found that administration of telmisartan can improve glucose metabolism without causing weight gain and may even have the ability to attenuate the accumulation of visceral fat.83,85,86 Although the clinical relevance of these animal studies remains to be determined, the results of preliminary studies in patients with features of the metabolic syndrome support the possibility that telmisartan may improve glucose metabolism and limit the accumulation of visceral fat.88–94 Clinical studies have also demonstrated that telmisartan is a well-tolerated molecule that is not associated
with fluid retention and weight gain.\textsuperscript{95} The differences in adverse-effect profiles between selective PPAR\(\gamma\) modulators like telmisartan and conventional PPAR\(\gamma\) agonists like the thiazolidinediones could be related to the fact that SPPARMs do not stimulate PPAR\(\gamma\) as much as the glitazones and also have more selective effects on the recruitment of key transcription cofactors that influence PPAR\(\gamma\) target gene expression profiles.\textsuperscript{85,96} Molecular modeling studies have suggested that telmisartan may interact with the ligand-binding domain of PPAR\(\gamma\) differently than the glitazones, thereby resulting in different conformational changes in the receptor.\textsuperscript{80} This could lead to different effects of telmisartan than the glitazones on the recruitment of key transcription cofactors that, in turn, modulate the effects of PPAR\(\gamma\) on target gene expression patterns.\textsuperscript{85}

Given that inhibitors of the renin–angiotensin system may improve glucose metabolism and that PPAR\(\gamma\) activators can decrease target gene expression profiles,\textsuperscript{85,96} it is well recognized that many of the mechanisms involved in the pathogenesis of atherosclerosis can also be modulated by PPAR\(\gamma\), angiotensin II, or both.\textsuperscript{97–99} Moreover, PPAR\(\gamma\) activators can decrease expression of the angiotensin II type 1 receptor gene, inhibit the effects of angiotensin II on intracellular signaling pathways, and may have additional beneficial vascular effects that go beyond their actions on glucose and lipid metabolism.\textsuperscript{100–104} Thus, multifunctional compounds that simultaneously block the angiotensin II type 1 receptor and selectively modulate the activity of PPAR\(\gamma\) might also provide improved opportunities for preventing atherosclerosis and cardiovascular disease (Figure 6). The cardioprotective and antidiabetic effects of a dual ARB/SPPARM are being evaluated in the Ongoing Telmisartan Alone in Combination With Ramipril Global Endpoint Trial and the Telmisartan Randomized Assessment Study in ACE-I Intolerant Subjects With Cardiovascular Disease.\textsuperscript{105} The results of these and other large-scale clinical trials will ultimately be required to assess the clinical impact of dual ARB/SPPARM molecules, as well as future therapeutic concepts that evolve from studies in animal models of spontaneous hypertension and related cardiovascular and metabolic disorders.

**Figure 6.** Potential antiatherosclerotic mechanisms of molecules that function both as ARBs and SPPARMs.

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