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Review

What the BTBR/J mouse has taught us about diabetes and diabetic complications

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SUMMARY

Human and mouse genetics have delivered numerous diabetogenic loci, but it is mainly through the use of animal models that the pathophysiological basis for their contribution to diabetes has been investigated. More than 20 years ago, we serendipidously identified a mouse strain that could serve as a model of obesity-prone type 2 diabetes, the BTBR (Black and Tan Brachyury) mouse (BTBR T+ Itpr3tf/J, 2018) carrying the *Lep*^{ob} mutation. We went on to discover that the BTBR-*Lep*^{ob} mouse is an excellent model of diabetic nephropathy and is now widely used by nephrologists in academia and the pharmaceutical industry. In this review, we describe the motivation for developing this animal model, the many genes identified and the insights about diabetes and diabetes complications derived from >100 studies conducted in this remarkable animal model.

INTRODUCTION

Type 2 diabetes is a polygenic disorder where many gene loci make small contributions to diabetes risk. The penetrance of the risk alleles at these loci is greatly influenced by obesity. Obesity can be viewed as a "sensitizer", much like stressors have long been used in genetic screens in model organisms to enhance the penetrance and effect sizes of gene loci. It is fair to say that the diabetes epidemic is not because of a change in the frequency of diabetes risk alleles, but rather, a consequence of the obesity epidemic and its enhancement of the penetrance of existing diabetogenic risk alleles.

Human and mouse genetics have delivered numerous diabetogenic loci, but it is mainly through the use of animal models that the pathophysiological basis for their contribution to diabetes has been investigated. More than 20 years ago, we serendipidously identified a mouse strain that could serve as a model of obesity-prone type 2 diabetes, the BTBR/J (Black and Tan Brachyury) mouse[1] carrying the *Lep*^{ob} mutation.[2,3] We went on to discover that the BTBR/J *Lep*^{ob} mouse is an excellent model of diabetic nephropathy[4] and is now widely used by nephrologists in academia and the pharmaceutical industry.

In this review, we describe the motivation for developing this animal model, the many genes identified and the insights about diabetes and diabetes complications derived from more than 100 studies conducted in this remarkable animal model.

It should be noted that the BTBR/J mouse is also a widely used model of autism.[5] We will not review this literature because we are not aware of any evidence of any genetic or mechanistic relationship between autism and diabetes.

INSPIRATION FROM DOUGLAS COLEMAN

The classic studies of Douglas Coleman were the first to demonstrate that the relationship between genetic obesity and diabetes in mice depends on strain background. Although C57BL/6J mice with the *Lep*^{ob} mutation are mildly hyperglycemic, the same mutation in the C57BL/6Ks mice results in severe diabetes.[6] This study was a milestone in mouse genetics because it introduced in the context of diabetes, the concept of genetic modifiers as genes that influence the penetrance of a specific mutation. It immediately suggested that that experimental crosses could be used to discover those modifiers. Coleman went on to carry out an F2 intercross to map the modifier locus and identified the gene encoding malic enzyme as a modifier of the glucose phenotype.[7]

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Another way to frame the problem is that the *Lep*^{ob} mutation is a sensitizer in that it brings out phenotypes in a susceptible mouse strain that would otherwise be silent. This concept opens the door for other sensitizers like individual gene knockouts, diets, and drug treatments. At a population level, it provides a conceptual framework for interpreting complex trait genetics which is, essentially, polygenic traits and their interaction with the environment.

Coleman's work was broadly influential. For example, in the cancer field, Dove employed chemical mutagenesis to identify the *Min* mutation in the *Apc* gene as a cause of intestinal cancer.[8] In analogy to Coleman's experiments, Dove's team performed a genetic screen for modifiers of *Min* and discovered that the gene encoding a secretory phospholipase influences the penetrance of intestinal cancer caused by the *Min* mutation.[9]

Our earliest deployment of mouse genetics to discover genes affecting the susceptibility to type 2 diabetes involved an experimental cross with the BTBR/J mouse strain. Alexandra Shedlovsky, in Dove's laboratory, had been using the mice to search for modifiers of *Min*[10] and provided us with the mice. We made the serendipitous discovery that male F1 offspring from a cross between BTBR/J and C57BL/6J mice are hyperinsulinemic and insulin resistant.[3]

There has been a long debate over which event is causal in the relationship between hyperinsulinemia and insulin resistance.[11,12] In fact, there is strong evidence that in some instances the level of insulin secretion leads to a compensatory change in insulin sensitivity of peripheral tissues.[13] It has also been argued that insulin hypersecretion precedes β-cell exhaustion and the onset of diabetes.[11]

We used the BTBR/J mouse strain to study the basis for its hyperinsulinemia. A survey of several known insulin secretagogues revealed that BTBR/J islets have a dramatic response to α-ketoisocaproate (αKC).[14] α-KC can be converted to leucine, an allosteric activator of glutamate dehydrogenase (GDH). Gain-of-function mutations in GDH lead to hyperinsulinemia and hyperammonia.[15] Deamination of glutamate to form α-ketoglutarate provides a source of mitochondrial oxaloacetate, a substrate for mitochondrial PCK, encoded by *Pck2*. Deletion of *Pck2* leads to a profound deficiency in insulin secretion.[16] Recent work argues that this is a critical source of phosphenolpyruvate for the pyruvate kinase reaction, the latter being involved in inhibition of the K_{ATP} channel.[17] The mechanism behind the hyperresponsiveness of BTBR/J islets to αKG remains unclear, yet provides an example where the hyperinsulinemia is in response to a non-glucose secretagogue.

Inspired by Coleman's work, we introgressed the *Lep*^{ob} mutation into the BTBR/J mouse strain. The resulting congenic strain developed severe hyperglycemia and provided a new genetic background that could be used to screen for novel diabetes susceptibility loci.[2] As described below, the mice have also proven to be a valuable model of essentially all diabetes complications.

POSITIONAL CLONING OF DIABETES SUSCEPTIBILITY LOCI

To discover gene loci responsible for the difference in diabetes susceptibility between the C57BL/6J and BTBR/J mice, we carried out an F2 intercross. This involved breeding *Lep*^{ob} heterozygous C57BL/6J and BTBR/J mice for two generations to obtain F2 mice homozygous for the *Lep*^{ob} allele. The F2 mice displayed a very wide range of fasting serum glucose and insulin. Mice with the lowest insulin and highest glucose had a dramatic reduction in β-cell mass, consistent with the action of the diabetes susceptibility genes playing out in pancreatic β-cells.[2] Three loci achieved genome-wide statistical significance, one linked to glucose at chromosome 16 and two linked to insulin on chromosomes 2 and 19.

In genetic studies, the resolution of genetic mapping is not usually set by the density of genetic markers. Rather, it is defined by the boundaries set by the sites of meiotic recombination. In addition, studies on most inbred mouse strains do not interrogate the entire genome because many of these strains share as much as 25% of their genomes with one another; i.e., they are distant cousins. Thus, QTL mapping in an F2 intercross cannot by itself lead to gene identification.

One way to overcome this limitation is to carry out additional crosses and then screen for increasingly rare recombination events within the genomic interval identified with QTL mapping. With backcrossing, one can fix these recombinant chromosomes within a particular strain background. These mice are termed

"interval-specific congenic" strains.[18] Unlike F2 mice, one can breed multiple 'copies' of these mice and thus obtain biological replication of phenotypes, in addition to deeper phenotyping.

We derived 12 congenic strains with recombinations within a 7 Mb region on mouse chromosome 19.[19] Phenotyping of these strains for fasting and glucose-stimulated insulin secretion enabled us to narrow the QTL region to just 242 kb, a region harboring a segment of one large gene, *Sorcs1*. Soon afterward, a study in the Mexican-American coronary artery disease cohort found SNPs at the *SORCS1* locus associated with type 2 diabetes.[20] Surprisingly, in the most recent aggregated data available on the T2D Portal, there are no SNPs with a significant association with diabetes in the vicinity of the *SORCS1* locus. However, there is still the possibility that there is genetic variation in loci that control *SORCS1* expression and thus indirectly affect diabetes susceptibility.

Sorcs1 is one of five mammalian proteins descended from yeast vacuolar protein sorting 10 (Vps10).[21,22] In yeast, Vps10 is required for sorting vacuolar enzymes from the trans-Golgi network to the vacuole. In protozoans, the Vps10 homolog is involved in the biogenesis of dense core vesicles (in *Tetrahymena*),[23] and secretory organelles (rhoptries and micronemes in *Toxoplasma* gondii).[24] In mammals, proteins of the Vps10 family (Sortilin, SorLA, Sorcs1, Sorcs2, and Sorcs3) play diverse roles; e.g., intracellular trafficking of proteins, and multifunctional receptors, with involvement in a wide range of cellular functions and diseases, including dyslipidemia, atherosclerosis, cancer, and Alzheimer's Disease.[25]

Mice with a knockout of the *Sorcs1* gene, when made obese with the Lep^{ob} mutation, are severely diabetic.[26] Their pancreatic β-cells are severely deficient in insulin and in secretory granules. Lean $Sorcs1^{KO}$ mice were not diabetic but were slightly glucose-intolerant.

Studies of insulin secretion in isolated islets from the $Sorcs1^{KO}$ mice initially failed to detect a defect that could explain their diabetes. However, with multiple glucose challenges, the islets indeed demonstrated a pronounced defect in insulin secretion.[26]

It is interesting that the diabetogenic allele of *Sorcs1* is the from the non-diabetes-prone C57BL/6J, not the diabetes-prone BTBR/J strain. Between the two strains there is a Thr/Ile polymorphism in the pro-domain of the Sorcs1 protein. This polymorphism affects the maturation of the protein and when expressed in a β-cell line, affects insulin content and insulin secretion, similar to the phenotypes of the knockout mice.[27]

Using the interval-specific congenic strain strategy, Bhatnagar et al. positionally cloned *Syntaxin binding protein 5-like (Stxbp5l) or Tomosyn-2* as the gene responsible for a glucose QTL on chromosome 16.[28] Several congenic mouse strains were generated by the introgression of regions of the BTBR/J chromosome 16 into the C57BL6/J mice. Metabolic phenotyping identified strains containing a non-synonymous coding polymorphism resulting in a change in a single amino acid, S912L The S912L mutation increases Tomosyn-2 protein abundance by decreasing proteasomal degradation. The work showed that Tomosyn-2 is an inhibitor of insulin secretion.[28]

Tomosyn-2 is a 130 kDa soluble cytoplasmic protein whose functions are attributed to the binding with the plasma membrane-bound syntaxin family of proteins; syntaxin is a key component of the complex that is required for the fusion of insulin granules to the plasma membrane in exocytosis. A paralog of Tomosyn-2, Tomosyn-1 is also expressed in β-cells and has been demonstrated to inhibit insulin secretion. However, these proteins differ in tissue expression profile,[29] gene regulation,[29] and molecular mechanism.[30,31] In addition, the Tomosyn-2 coding SNP (S912L) is responsible for β-cell phenotypes in the presence of the wild-type Tomosyn-1, suggesting that it has non-redundant functions.[28]

Decades of work by numerous laboratories have led to the identification and characterization of proteins involved that facilitate the exocytosis of insulin granules, whereas little is known about those that inhibit insulin secretion. Why do β-cells express an inhibitor of insulin secretion? Because hypoglycemia is a greater acute threat to survival than is hyperglycemia, it is conceivable that β-cells place brakes on the fusion of insulin granules to prevent insulin secretion when glucose is not elevated or decrease the spontaneous hypersecretion of insulin in response to stimuli, essentially, working as a rheostat to fine-tune insulin release from β-cells.

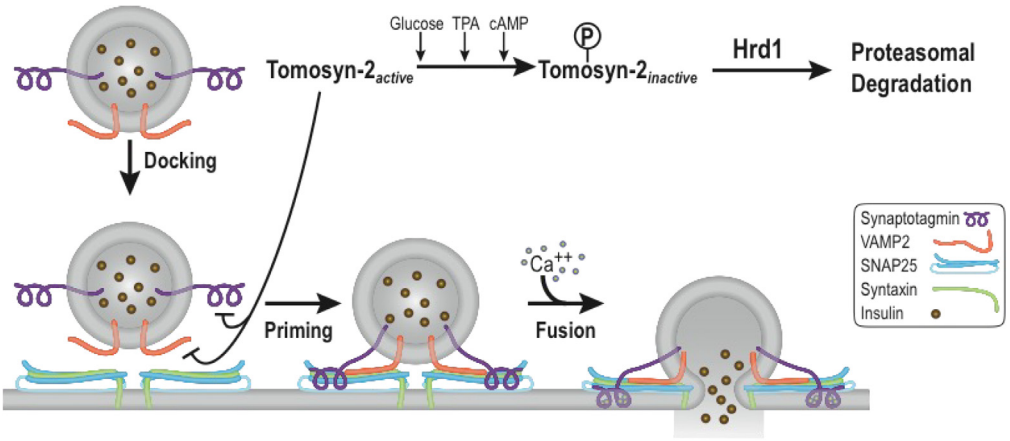

Figure 1. Tomosyn-2 is a glucose-regulated negative regulator of insulin secretion

Tomosyn-2 binds to syntaxin and blocks the formation of the SNARE complex, a key step in the fusion of dense core vesicles with the plasma membrane and thus places a brake on insulin secretion. This break is released when glucose stimulates the phosphorylation of Tomosyn-2. Phospho-tomosyn-2 is a substrate of ubiquination by the E3 ubiquitin ligase Hrd1 and is thus targeted for proteosomal degradation.

If β-cells block insulin secretion with Tomosyn-2, how do they release the brakes? When β-cells are exposed to the 15 mM glucose stimulation, the abundance of the Tomosyn-2 protein drops by 50% within 1 h.[32] The loss of Tomosyn-2 coincides with its phosphorylation and proteasomal degradation. Knockdown of the ER-localized E3 ubiquitin ligase Hrd1 rescues the abundance of Tomosyn-2, suggesting that phosphorylated Tomosyn-2 recruits Hrd1 and leads to de-repression of insulin secretion[32] (Figure 1).

THE ROLE OF TXNIP IN TYPE 2 DIABETES

Given the strong phenotypes of BTBR/J-*Lep*^{ob} mice, including obesity, insulin resistance and severe hyperglycemia, these mice provide an attractive model for severe human type 2 diabetes. In addition, although β-cell apoptosis is pronounced during progression of diabetes in BTBR/J-Lep^{ob} mice, their compensatory β-cell expansion is impaired compared to C57BL/6J-*Lep*^{ob} mice. This may also better reflect the limited β-cell proliferative capacity of human β-cells.

When looking for a stringent type 2 diabetes model, Shalev et al., therefore, chose the BTBR/J-Lep^{ob} mouse. Shalev had identified *TXNIP* as the top glucose induced gene in a human islet gene expression microarray study[33] and shown that it promotes β-cell apoptosis *in vitro*[34] suggesting that it may play a role in diabetic β-cell dysfunction and loss. Next, they wanted to test whether Txnip deficiency in turn could protect against (ideally severe) diabetes and therefore turned to BTBR/J-Lep^{ob} mice. They first confirmed that *Txnip* expression is significantly increased in diabetic BTBR/J-Lep^{ob} mice as compared to their lean littermates[35] and then started the lengthy backcrossing of the *Txnip*-deficient HcB-19 strain identified initially by Lusis et al.[36] from their original C3H genetic background into the BTBR/J background. By crossing the resulting BTBR/J-*Txnip*^{hcb} with BTBR/J-Lep^{ob} mice, Shalev generated a double mutant, congenic BTRB/J-*Lep*^{ob} *Txnip*^{hcb} line lacking Leptin and Txnip.

Of interest, these double-mutant mice maintained normal blood glucose and were completely protected from diabetes, while BTBR/J-Lep^{ob} became severely diabetic before reaching 10 weeks of age.[37] Of note, this protection was afforded in the face of severe obesity as double-mutant mice were equally or even more obese than BTBR/J-Lep^{ob} mice.[37] In addition, no increase in β-cell proliferation was observed and further analysis revealed that it was primarily an inhibition of β-cell apoptosis that conferred this improvement in glucose homeostasis.[37] In addition, the double-mutant mice also showed some improvement in insulin sensitivity,[37] consistent with the downregulation of ubiquitously expressed Txnip in extra-pancreatic tissues and the reported effects of Txnip knockdown promoting glucose uptake in muscle and adipose tissue.[38,39]

Based on these clear results indicating beneficial effects of Txnip deficiency even in the context of such a stringent model of type 2 diabetes, Shalev and others launched a number of follow-up studies. Not

surprisingly, Txnip deletion also protected against diabetes in other animal models, including those induced by high-fat diet,[40] endoplasmic reticulum stress (Akita, *Ins2*$^{+/Akita}$)[41] and streptozotocin.[37] So, when the time came to test the effects of pharmacological Txnip inhibition using the repurposed blood pressure medication, verapamil, Shalev again turned to BTBR/J-Lep^{ob} mice. Oral verapamil treatment replicated the effect of the Txnip deletion by improving glucose control in the severely diabetic BTBR/J-Lep^{ob} mice.

These promising verapamil effects have now been translated into retrospective clinical studies demonstrating that verapamil use is associated with lower fasting blood glucose[42] and lower risk of developing type 2 diabetes, especially in subjects over 65 years of age.[43] Finally, Shalev recently also demonstrated the beneficial effects of Txnip inhibition with verapamil in a randomized, double-blind, placebo-controlled trial in subjects with type 1 diabetes.[44]

HEPATIC STEATOSIS

Diabetes is associated with hepatic steatosis. Thus, one of the surprising phenotype differences between the C57Bl/6J *Lep*^{ob} and the BTBR/J-*Lep*^{ob} mice is that the relatively non-diabetic strain, the C57Bl/6J *Lep*^{ob} mice, have severe steatosis while the diabetic strain, the BTBR/J-*Lep*^{ob} mice, are relatively protected from steatosis.[45] Conversely, the BTBR/J-*Lep*^{ob} mice are hypertriglyceridemic whereas the C57Bl/6J *Lep*^{ob} mice are not. We proposed that the strain difference could be a consequence of differences in fuel partitioning where the carbon flow in the BTBR/J-*Lep*^{ob} favors gluconeogenesis whereas in the C57Bl/6J *Lep*^{ob} mice, it favors lipogenesis. Indeed, the gene expression profiles of the livers of these mice supports this model.[45]

We carried out a QTL survey of liver triglyceride in the F2 mice derived from the cross between the C57Bl/6J-*Lep*^{ob} and the BTBR/J-*Lep*^{ob} mice.[46] We found a locus that mapped to chromosome 17 with an LOD (log of the odds ratio) of 15, seven log units above the threshold for genome-wide significance. Within a broad QTL region, we identified a strong candidate gene, *Tsc2*, which encodes Tuberin, a GTPase-activating protein that activates an inhibitor of mTorc1, Rheb. The homozygous deletion of *Tsc2* is embryonic lethal, but the hemizygous mice are viable. In these mice, the expression of lipogenesis genes is increased. The BTBR/J mice carry a protein polymorphism in Tuberin, M552V, which we showed is associated with a slower turnover of the protein.

A more recent study of BTBR/J-*Lep*^{ob} mice showed that at a much more advanced age, 22 weeks, the mice develop steatosis and many of the features that characterize the transition to steatohepatitis.[47] Perhaps this later-onset disease is a consequence of the pre-existing diabetes.

In another model of Leptin deficiency, the lipoatrophic mice carrying the A-ZIP mutation, there is a strain difference, between C57BL/6J and FVB mice, where the FVB A-ZIP mice have no steatosis and are diabetic whereas the C57BL/6J mice are steatotic and protected from diabetes. Similar to our strain comparison, the gene expression profiles showed increased expression of lipogenic genes in the non-diabetic mice.[48]

These results have therapeutic implications. Drugs that activate hepatic lipogenesis (e.g., activators of PPAR-γ or LXR decrease hepatic gluconeogenesis, consistent with the concept that there is a competition between carbon flow toward glucose versus fatty acids.[49] Unfortunately, some drugs are effective to lower glucose at the expense of excess lipogenesis and hepatic steatosis. An example is some glucokinase activators.[50]

A BODY WEIGHT LOCUS CONTROLLING FOOD INTAKE IN BTBR/J-LEP^{OB} MICE

In addition to the loci controlling glucose and insulin, the F2 intercross identified a very strong linkage of a locus on chromosome 2 with body weight. This was surprising because all of the F2 mice were already morbidly obese because of the *Lep*^{ob} mutation.[51] Nonetheless, the QTL affected adiposity with the BTBR/J allele increasing body weight. Another surprise is that this QTL acts by affecting food intake, even though the animals were all leptin-deficient.[52] Thus, this genetic linkage provides a lead to a powerful regulator of food intake that operates in a leptin-independent fashion. Susanne Clee (University of British Columbia) was investigating this locus when she unexpectedly passed away in 2020, leaving behind a fascinating, but still unsolved question.[53]

Table 1. Age, strain, and obesity converge to uncover tissue-specific gene signatures

	Unrelated to diabetes	Reactive to diabetes	Causal for diabetes	
Tissue	All groups	10 weeks BTBR/J only	4 & 10 weeks C57BL/6J only	4 & 10 weeks BTBR/J only
Islet	885	3548	386	768
Adipose	580	1177	671	171
Liver	372	3907	328	288
Soleus	14	1497	42	96
Gastrocnemius	12	2085	49	46
Hypothalamus	0	714	2	20

Number of transcripts differentially expressed in response to *Leptin*$^{ob/ob}$ in 4 and 10 weeks old C57BL/6J and BTBR/J mice

TRANSCRIPTION PROFILING OF A MOUSE DESTINED TO BECOME DIABETIC

To evaluate the convergence of age, strain and genetic obesity (*Lep*^{ob}) on the transcriptional signature of diabetes susceptibility, we profiled gene expression in multiple tissues (islet, adipose, liver, soleus and gastrocnemius skeletal muscles, and hypothalamus) from male C57BL/6J and BTBR/J mice, when either lean or obese (*Lep*^{ob}), at ages 4 and 10 weeks. Importantly, among these 8 groups of mice, only 10-week-old obese BTBR/J mice are diabetic (blood glucose ~600 mg/dL). All other groups maintained normal glucose levels (~150 mg/dL).

In every tissue, there were many transcripts that were differentially regulated in response to *Lep*^{ob} in diabetic but not non-diabetic mice regardless age. Liver and islet showed the greatest number of diabetes-related transcripts (~3,900 and ~3,500, respectively), whereas hypothalamus showed the least (714); Table 1. Gastrocnemius, soleus and adipose contained ~2,100, ~1,500, and ~1,200 transcripts respectively, that were differentially regulated only in diabetic mice. One possible interpretation is that these diabetes-related transcripts are regulated in response to diabetes; i.e., glucose reactive gene regulation.

To discriminate between these glucose *reactive* transcripts, and those that may play a *causal* role in diabetes, we exploited the trajectory of diabetes progression in BTBR/J-Lep^{ob} mice. For example, all male BTBR/J-Lep^{ob} mice are diabetic at 10 weeks of age; and non-diabetic at 4 weeks of age. Thus, BTBR/J-Lep^{ob} mice progress from pre-diabetes to diabetes over ~6 weeks. Because leptin deficiency is required to reveal the pro-diabetic alleles in BTBR/J mice, we asked if there are transcripts that are regulated in response to *Lep*^{ob} in one strain, but not the other at both 4 and 10 weeks of age. This proved to be a powerful filter, enabling us to focus on a relatively small number of transcripts in each tissue that may play a causal role in diabetes susceptibility, rather than reacting to diabetes. Finally, there were a number of transcripts in all tissues except hypothalamus that were differentially regulated in response to *Lep*^{ob} regardless of age or strain, ranging from ~900 transcripts in islets to 14 and 12 transcripts in soleus and gastrocnemius, respectively. These transcripts are likely unrelated to diabetes.

To identify groups of transcripts that discriminated C57BL/6J from BTBR/J for obesity and age-dependent regulation, we used the WGCNA method[54] to compute co-expression gene modules, allowing the transcripts to group in an unsupervised fashion based solely on their correlation structure within each tissue. This yielded 105 co-expression modules across six tissues. To evaluate the physiological significance of the modules, we asked if they were enriched with transcripts associated with specific biological processes. In all tissues except hypothalamus, we identified a module highly enriched with transcripts associated with control of the cell cycle.[55]

Transcripts within the cell cycle modules were differentially regulated depending on tissue. For example, in adipose tissue, the cell cycle module was strongly driven by age and obesity in both C57BL/6J and BTBR/J mice. A similar result was observed in islets from C57BL/6J mice; both age and obesity-dependent regulation. In striking contrast, the islet cell cycle module in BTBR/J was solely driven by age, suggesting that obesity-dependent regulation of this module was lost in the diabetes-prone strain.

We validated these two cell cycle modules with a direct measure of cellular proliferation. In islets, obesity resulted in increased cellular proliferation in C57BL/6J but not BTBR/J mice. In adipose tissue, obesity was equally effective to drive proliferation in C57BL/6J and BTBR/J mice. We confirmed our interpretation of the gene expression data by directly measuring the incorporation of $^{2}H_{2}0$ into DNA in adipose tissue and islets. This measurement aligned with the expression of the cell cycle module genes. These results demonstrate that by deconstructing the modules for the relative contribution of age, strain and obesity to drive the correlation structure of the transcripts, we were able to pinpoint a disconnect in the obesity-dependent regulation of cell cycle regulatory transcripts in islets of BTBR/J mice that is likely to play a causal role in their diabetes susceptibility.

CDK1

Human diabetes is associated with β-cell compensation and decompensation can be captured by Starling's curve of pancreas, in which the mean plasma insulin response is plotted vs. fasting plasma glucose.[56] In patients with impaired glucose tolerance and mild diabetes mellitus, plasma insulin response to ingested glucose increases progressively until the fasting glucose concentration reaches 120 mg/dL. Further increases in fasting glucose are associated with a progressive decline in insulin secretion. In mice, β-cell compensation and decompensation modeled using leptin-deficient mice on the C57BL/6J and BTBR/J backgrounds. Within the islet cell cycle module,[55] the mRNA levels of *Cdk1* and its activators, *Ccnb1* (Cyclin B1) and *Cdc25c*, were increased in C57BL/6J-*Lep*^{ob} mice relative to their lean counterparts at 10 weeks of age. At the same age, the mRNA levels of *Cdk1* and *Ccnb1* were decreased in BTBR/J-*Lep*^{ob} mice relative to their lean counterparts. Although CDK1 has been found in multiple cellular compartments, Gregg et al.[57] observed signficiant amounts of Cyclin B1 and CDK1 were found a in a mitochondrial fraction of the mouse insulinoma β-cell line, MIN6. Further experiments revealed a strong upregulation of complex I activity in compensating C57BL/6J-*Lep*^{ob} islets relative to lean controls that was blocked by CDK1 inhibition. CDK1 blockade also slowed glucose-dependent citrate cycling and reduced Ca^{2+} influx in C57BL/6J-*Lep*^{ob} islets but not lean controls. These studies identified complex I as a critical mediator of β-cell compensation and implicate CDK1 signaling as the mechanism driving this effect.

FoxM1 AND TCF19

The islet cell cycle module identified a coordinately regulated set of genes that were upregulated in response to obesity in the C57BL/6J-*Lep*^{ob} mice, but not in the BTBR/J-*Lep*^{ob} mice. Pancreatic β-cells are known to proliferate in response to obesity as an adaptive response to increase β-cell mass and insulin secretion. As noted above, this failure to upregulate cell cycle genes in response to obesity was correlated with a failure to increase adaptive islet cell proliferation in response to obesity in the BTBR/J.[55] Therefore, the obese BTBR/J mouse is a model of failed adaptive β-cell proliferation and provided an opportunity to identify transcriptional regulators of proliferation in response to the metabolic stress of obesity.

A candidate regulator of this transcriptional response was the transcription factor, FoxM1, which is part of the cell cycle islet module and failed to upregulate in response to obesity in the BTBR/J islets. The Gannon lab had identified FoxM1 as necessary for adaptive β-cell proliferation in response to the stressors of pregnancy and partial pancreatectomy.[58–60] FoxM1 is involved in transcriptional regulation of all phases of the cell cycle, including directly inducing *Cdk1* expression.[61,62]

Using an F2 population of (B6xBTBR/J)-*Lep*^{ob} mice, we found that mice with the highest expression of *Foxm1* had improved metabolic phenotypes with a 3-fold higher fasting plasma insulin level in the "high expressors".[63] Again, high islet *Foxm1* expression correlated strongly with many other cell cycle genes. Overexpression of *Foxm1* in BTBR/J islets led to an increase in β-cell proliferation and increases in cell cycle gene expression, suggesting that a failure to upregulate *Foxm1* is one of the key defects in the BTBR/J islet. Connecting to other pathways involved in the BTBR/J islet, FoxM1 is involved in regulation of EP3 and EP4 in the islet, with additional evidence for reciprocal regulation of *Foxm1* expression by EP3 and EP4 in human islets from type 2 diabetes donors. *Foxm1* overexpression can also promote β-cell survival and this anti-apoptotic function requires EP4 and is inhibited by EP3 activity.[64,65] Therefore FoxM1 has been shown to be a key regulator of β-cell mass dynamics with critical insight coming from the BTBR/J model.

An additional candidate for transcriptional regulation of β-cell proliferation identified in the islet cell cycle module was the transcriptional regulator Tcf19. BTBR/J islets also fail to upregulate *Tcf19* in response to obesity.[55,66] *Tcf19* is expressed during the cell cycle and can bind to tri-methylated histone H3 to function as a chromatin reader and regulate transcription of cell cycle genes, including Cyclin D1.[67] Knockdown of *Tcf19* impairs β-cell proliferation whereas overexpression of *Tcf19* leads to upregulation of cell stress response pathways, including those important in DNA damage repair.[66,68] *Tcf19* expression, along with numerous other cell cycle genes, is induced by Nfatc2 in the islet.[69] DYRK1A inhibitors are an exciting new therapeutic target to promote β-cell proliferation, and a potential signaling pathway includes activation of NFATc2, leading to expression of *Tcf19* and promotion of cell cycle gene expression.[61,70,71] Future studies will uncover the exact role of Tcf19 and other transcriptional regulators in these pathways to promote β-cell proliferation.

DIABETES IS A MULTI-TISSUE DISORDER

In addition to the cell cycle module in pancreatic islets, we identified transcripts in other tissues that showed obesity dependence at both 4 and 10 weeks of age in one strain, but not the other. Like the islet cell cycle module, these additional gene sets may play a causal role in diabetes susceptibility (Table 1). For example, in adipose there were 671 transcripts that were obesity-dependent in C57BL/6J, but not BTBR/J, and another 171 transcripts that showed the opposite pattern; obesity-dependent in BTBR/J but not C57BL/6J. In islets, there were 768 transcripts that showed obesity regulation only in BTBR/J. Liver, the two muscle tissues sampled, and hypothalamus also contained these obesity and strain-specific gene sets. These results demonstrate that by imposing a severe metabolic challenge (*Lep*^{ob}) and exploiting the time course of diabetes progression in BTBR/J mice, we identified specific gene sets in islets and key insulin target tissues that may underlie obesity-evoked diabetes.

EXPRESSION QTLs. IDENTIFICATION OF DRIVERS OF GENE EXPRESSION; OATP1A6 AND NFATC2

The advent of microarrays in the late 1990s ushered in the era of whole-genome transcriptional profiling. We[72,73] and others[74] realized that in combination with a genetic cross, we could broadly map gene loci controlling gene expression. This involves treating the abundance of each mRNA, at that time obtained from microarrays, as a quantitative trait, a procedure that had been carried out in the 1990s but not on the scale now provided by microarray technology. In the context of a genotyped population, an experimental cross or a free-living outbred population, linkage or association mapping can identify gene loci controlling gene expression, termed expression quantitative trait loci (eQTL).

The expression traits can map to regions of the genome where the gene encoding the mRNA is physically located. These are termed "*cis*-eQTL" or "local eQTL".[75] When the eQTL is located far away or on a different chromosome from the gene encoding the mRNA, it is termed a "*trans*-eQTL" or a "distal eQTL". The *cis*-eQTL tend to be preserved across tissues and across experimental crosses, whereas *trans*-eQTL are not; they are far more context-dependent. Nonetheless, the *trans*-eQTL provide an indication of transcripts that may share a common genetic driver by virtue of co-mapping to a common genomic locus. Often, these co-mapping *trans*-eQTL are driven by the same alleles; i.e., they show *shared genetic architecture*.

In all tissues, we found striking clusters of *trans*-eQTL, where a large number of mRNA traits co-mapped to the same locus, where the genes encoding those mRNAs are not physically located. These spots can occur because there is a common locus that regulates the expression of these genes or because there are differences in the composition of the tissue. The most prominent eQTL cluster occurred in islets where several hundred mRNA traits co-mapped to the distal end of Chr 6 with LOD scores >100.[76] The strength of these linkages, which included several genes physically located within a small genomic interval, enabled us to apply linear discriminant analysis[77] to narrow the candidate genes to just three. Exon sequencing identified a mutation in the bile acid transporter OATP1A6 (encoded by *Slco1a6*) in BTBR/J mice that alters a highly conserved proline residue to serine (P564S), at a site that faces the central acqueous pore of the protein through which bile acids are transported. We further showed that OATP1A6 cloned from BTBR/J mice yielded enhanced transport of taurocholic acid (TCA) compared to C57BL/6J OATP1A6.[76] It is possible that the enhanced transport of TCA by OATP1A6 in BTBR/J mice results in the regulation of the islet mRNAs that map to the OATP1A6 locus on Chr 6.

In addition to the islet hotspot on Chr 6, we identified a locus on Chr 2 that not only was an eQTL hotspot, it showed strong linkage for plasma insulin levels. Just nine of the many genes that mapped to this locus mapped in *cis*. When we conditioned the linkage analysis on the mRNA abundance of each of the nine genes, just one, *Nfatc2*, proved to be required for the genetic signal of the *trans*-eQTL, including ~50 that are linked to genes associated with diabetes risk in human GWAS.[69] By performing functional studies with adenoviral-mediated overexpression of a constitutively active form of Nfatc2, we showed that this transcription factor regulates insulin secretion and β-cell proliferation in both mouse and human islets. Genes found to be regulated by Nfatc2 by RNA-seq analysis included many of the genes encoding mRNAs whose abundance traits mapped to the chromosome 2 locus, including the diabetes GWAS candidates.[78] These results suggest that although the GWAS gene candidates were originally identified because of their proximity to diabetes risk alleles, their coordinate regulation by Nfatc2 may translate into a more substantial contribution to diabetes susceptibility than each locus yields individually.

In parallel with our studies on Nfatc2, Crabtree et al. demonstrated that Dyrk1A is the protein kinase that phosphorylates Nfatc2, causing it to be excluded from the nucleus.[79] Conversely, Kim, Crabtree et al. showed that the protein phosphatase calcineurin dephosphorylates NFAT proteins, promoting their transfer to the nucleus and thus explaining how the inhibitor of calcineurin cyclosporin, a widely used immunosuppressant, causes diabetes.[80] Dyrk1A is being actively pursued as a therapeutic target for treatment of diabetes.[70,71,81–83]

Several other eQTL hotspots were identified in extra-islet tissues, including loci in liver (chromosomes 2 and 8), and hypothalamus (chromosomes 1 and 11). Although *trans*-eQTL tend to be tissue-specific, we did identify a locus on Chr 17 at ~30 Mb to which a large number of transcripts co-mapped in all tissues. Future work is required to understand the physiological significance of these additional hotspots, as well as identification and validation of potential drivers like we did for Nfatc2.

THE ROLE OF THE EP3 PROSTAGLANDIN RECEPTOR IN β-CELL FUNCTION

By 10 weeks of age, the BTBR/J-Lep^{ob} phenotype is fully penetrant.[55,84] Islets from BTBR/J mice secrete less insulin as a percent of cellular insulin content than those isolated from C57BL/6J mice. Gene expression analyses revealed that *Ptger3* is highly up-regulated in islets of T2D BTBR/J-Lep^{ob} mice relative to non-diabetic 4- or 10-week-old C57BL/6J, C57BL/6J- *Lep*^{ob}, lean BTBR/J mice, and 10-week-old C57BL/6J- *Lep*^{ob} mice.[55,85,86] *Ptger3* encodes for prostaglandin EP3 receptor (EP3): a G protein-coupled receptor for the arachidonic acid (AA) metabolite, prostaglandin E_2 (PGE_2).

The mRNA abundance of all three mouse EP3 splice variants (EP3α, EP3β, and EP3γ) is > 100-fold higher in islets from T2D BTBR/J-Lep^{ob} mice than in non-diabetic control islets.[85,86] Genes encoding enzymes in the PGE_2 synthetic pathway are also upregulated in BTBR/J-Lep^{ob} islets, including cyclooxygenase (COX) 1 and 2, which catalyze the rate-limiting step in prostanoid synthesis.[85–87] Enhanced expression of PGE_2 synthetic genes is of biological consequence, as it directly correlates with increased secretion of PGE_2.[85,86]

Increased receptor expression is also functionally relevant; acute treatment of BTBR/J-Lep^{ob} islets with an EP3-selective (PGE_1) or a specific agonist (sulprostone) diminshes insulin secretion while having no effect on islets isolated from non-diabetic mice, likely because the latter islets do not induce the expression of the EP3 receptor. Treating BTBR/J-Lep^{ob} islets with an EP3-specific antagonist, restores their insulin response to that of BTBR/J lean controls, again, with no effect on islets from non-diabetic mice.[85] As an antagonist does not have biological activity on its own, these studies provide strong support for the important role of PGE_2-mediated EP3 signaling on the reduced insulin response of BTBR/J-Lep^{ob} islets. As an alternative way to target endogenous EP3 signaling, Neuman et al. incubated BTBR/J-Lep^{ob} islets with eicosapentaenoic acid (EPA), reducing plasma membrane AA abundance and limiting PGE_2 production (Neuman 2017). EPA-incubated BTBR/J-Lep^{ob} islets had a significantly improved GSIS response as compared to controls, further confirming the negative impact of elevated EP3 signaling on T2D beta-cell dysfunction.

EP3 acts in part by reducing cyclic adenosine monophosphate (cAMP) production. BTBR/J-Lep^{ob} islets have constitutively lower cAMP production than those from lean controls.[85] One of the most well-characterized β-cell targets for T2D therapeutics is the cAMP-stimulatory glucagon-like peptide 1 receptor (GLP1R). Stable GLP1R agonists, such as exenatide and liraglutide, have a number of systemic effects, but one of their primary mechanisms of action is to restore the dysfunctional GLP1R signaling: a known

contributor to the pathophysiology of T2D.[88] When *Lep*^{ob} islets are acutely treated with an EP3 antagonist, GLP1 is better able to promote cAMP production.[85] Furthermore, while GLP1R agonists do augment BTBR/J-Lep^{ob} islet GSIS in a dose-dependent manner, sulprostone significantly reduces their maximal potentiating effect, without affecting the EC_{50},[85] suggesting that EP3 agonists function as non-competitive antagonists of the GLP1R.

One possible mechanism behind the increased islet expression of EP3 and PGE_2 synthetic enzymes is the inflammatory state of T2D itself. Insulin resistance promotes adipose meta-inflammation and dysregulated lipolysis. BTBR/J-*Lep*^{ob} mice are more insulin resistant and have higher plasma levels of circulating insulin and triglycerides than C57BL/6J-*Lep*^{ob}.[84] Higher plasma levels of free fatty acids (including AA), the inflammation-related factors Resistin and PAI-1, and the PGE_2 metabolite itself are all elevated in BTBR/J-*Lep*^{ob} mice versus non-diabetic controls.[87,89] These changes correlate with increased islet gene expression of the pro-inflammatory cytokine, interleukin 1β (IL-1β), EP3, and PGE_2 synthetic enzymes, negatively impacting downstream GSIS.[86,87,90] Studies comparing islets from a cohort of BTBR/J-*Lep*^{ob} mice spontaneously remaining euglycemic to genetically identical T2D controls reveal euglycemic BTBR/J-*Lep*^{ob} islets have no response to sulprostone to reduce insulin secretion.[87] The above changes are partially associated with diet composition, but require the development of a T2D phenotype itself, further supporting the link between up-regulated EP3 signaling to T2D β-cell dysfunction.[87,90]

Systemic inflammation, as measured by clinical tests for C reactive protein (CRP) and erythrocyte sedimentation rate (ESR), plasma PGE_2 metabolite levels, islet PGE_2 production, and islet EP3 expression are all elevated in clinical biosamples from individuals with T2D.[64,85,91] Combined with the information gleaned from studies of the BTBR/J-*Lep*^{ob} mouse, these findings suggest EP3 could be a potent therapeutic target to improve or restore β-cell function in T2D individuals.

Surprisingly, BTBR/J-*Lep*^{ob} mice lacking EP3 are not protected from T2D, with fasting blood glucose levels essentially identical to wild-type BTBR/J-*Lep*^{ob} controls.[92] This phenotype might be because of the important role EP3 has in the adipose tissue in regulating lipid homeostasis, with its loss causing unregulated lipolysis, insulin resistance, and lipotoxicity to β-cells.[93]

A β-cell-specific EP3 KO BTBR/J mouse could be used to tease apart the β-cell-autonomous effects of EP3 loss from the deleterious effects of EP3 loss in extra-pancreatic tissues. Wisinski et al. attempted to create such a mouse using the RIP-Cre^{Herr} driver line, but germline recombination of the deleted allele created a full-body EP3 *Lep*^{ob} instead.[92] In addition to the lack of protection, EP3 KO BTBR/J-*Lep*^{ob} mice from T2D described above, the authors found a significantly poorer metabolic phenotype of lean EP3 KO BTBR/J mice as compared to wild-type controls. EP3 KO BTBR/J mice exhibit significantly worse glucose tolerance and insulin sensitivity than wild-type controls, even though they are lean, with normal fasting blood glucose levels.[92] Increased insulin resistance on its own could certainly contribute to glucose intolerance, but a direct effect of EP3 loss on β-cell function is highlighted by an extreme insulin hypersecretion phenotype, exacerbating the underlying insulin resistance of the BTBR/J strain.[84,92,94] A clue to a potential molecular mechanism is higher EP3 mRNA expression in islets from BTBR/J mice as compared to those from C57BL/6J, a strain where several studies with young, lean mice have shown no detrimental effect of full-body EP3 KO.[95–97] Notably, the 10- to 30-fold increase in BTBR/J islet EP3 mRNA is limited to the EP3α and EP3γ splice variants, which have partial constitutive activity.[90,98–100] Neither expression of the agonist-dependent EP3β splice variant nor PGE_2 production itself, though, are increased in BTBR/J vs. C57BL/6J islets. These results support a model in which constitutive EP3 activity is a functional, required check on insulin secretion in situations where β-cell stress is elevated. This model would include the BTBR/J strain, where underlying defects in peripheral insulin sensitivity and insulin granule exocytosis mean that their β-cells have to secrete more insulin than normal to maintain euglycemia.[28,32,84–86,92,94]

An unexpected finding by Wisinski et al. is the penetrance of germline recombination of floxed alleles with the RIP-Cre^{Herr} driver directly correlates with the underlying insulin resistance of the background strain. Using a different floxed gene, deleted alleles were detected in 5.4% of BTBR/J progeny, 3.9% of C57BL/6J progeny, and 0.65% of NOD progeny: the latter strain being extremely insulin sensitive.[92] Dynamic expression of the insulin gene during spermatocyte maturation and function is a likely

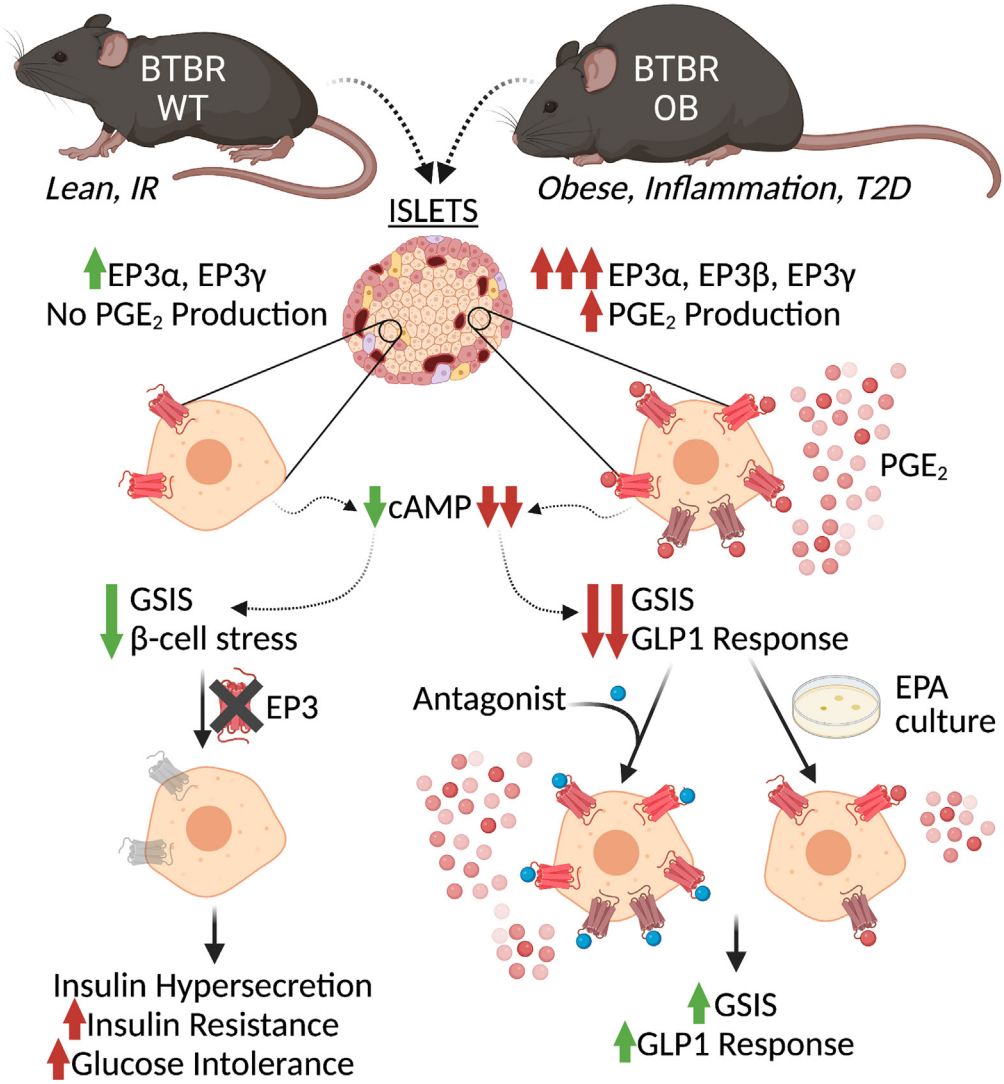

Figure 2. The role of agonist-independent and PGE2-mediated EP3 signaling in β-cell function and dysfunction as revealed by studies using the BTBR/J mouse strain

Islets from lean but insulin resistant BTBR/J mice have higher expression of the partially constitutively active EP3α and EP3γ splice variants as compared to other mouse strains, but negligible PGE2 production. Agonist-independent EP3 activity is important in reducing β-cell stress, as loss of receptor expression causes insulin hypersecretion and exacerbates the underlying insulin resistance of the BTBR/J strain, causing glucose intolerance. In contrast, islets from BTBR/J-*Lep*^{ob} mice, in which the T2D phenotype is fully-penetrant, have dramatically up-regulated expression of all three EP3 splice variants (including EP3β, which requires agonist binding for activity). Combined with significantly elevated PGE2 production, agonist-dependent EP3 signaling is a key contributor to the defective glucose-stimulated and GLP-1-potentiated insulin secretion of BTBR/J Ob β-cells. Treating BTBR/J-*Lep*^{ob} islets with an EP3 antagonist to prevent PGE2 binding or culturing islets in eicosapentaenoic acid (EPA) to reduce EP3 expression and PGE2 production improves their response to both glucose and GLP-1.

explanation for this finding.[101–103] These results call for caution when re-generating BTBR/J RIP-Cre lines using *in vitro* fertilization, which has a poor success rate in obtaining live, potential founder pups,[104] or in designing strategies for RIP-Cre-floxed BTBR/J colony maintenance. It is recommended that the RIP-Cre transgene be expressed only in female breeders, with a three-primer genotyping strategy to specifically detect the intact vs. deleted floxed allele in ear or tail tissue.[92] Using the C57BL/6J line, Spinelli et al. confirmed the presence of a deleted allele in genotyping tissue from RIP-Cre^{Herr} mice is confirmatory of a full-body KO (Figure 2).[105]

THE BTBR/J MOUSE AS A MODEL FOR DIABETES COMPLICATIONS

There is a growing global pandemic of diabetes. It was estimated that the global prevalence of diabetes in adults in 2021 was 536.6 million people and this number is projected to rise to 783.2 million people in 2045.[106] 90% of those affected have Type 2 diabetes.[107] Most patients with type 2 diabetes have at least one complication involving organ pathology.[107] Kidney disease is a leading complication that is present in approximately 40% of individuals with type 2 diabetes.[108] Kidney disease is the predominant risk factor for increased mortality in type 2 diabetes.[108] Diabetic nephropathy is the most common cause of both chronic kidney disease and end-stage renal disease in western countries. One way to address this major clinical problem is the use of animal models to identify pathophysiologic mechanisms and to provide a platform for pre-clinical testing of therapeutic interventions.[109]

The BTBR/J-*Lep*^{ob} mouse has proven to be an exceptional model of many of the organ-specific complications of diabetes including nephropathy, retinopathy, neuropathy, and, with additional interventions, atherosclerosis.[110] To the best of our knowledge, no other murine model of type 2 diabetes has been shown to develop such a wide constellation of diabetic complications. Hence, the BTBR/J-*Lep*^{ob} mouse offers unique opportunities to identify pathophysiologic mechanisms common to all injuries in different organ systems and to consider how systemic interventions may differentially affect the development and evolution of diabetes-related processes in susceptible organ systems. Importantly, as Pichaiwong et al. have demonstrated in the case of nephropathy, the provision of leptin to the leptin-deficient BTBR/J-*Lep*^{ob} mouse with normalization of the systemic metabolic milieu including insulin resistance and hyperglycemia, has enabled identification and characterization of previously unrecognized reparative responses in injured organs.[111]

THE BTBR/J-*LEP*^{OB} MOUSE IS A GOOD MODEL OF DIABETIC NEPHROPATHY

The principal features of diabetic nephropathy in humans include glomerular alterations of diffuse mesangial expansion because of accumulations of matrix, nodular mesangial sclerosis in advanced nephropathy, mesangiolysis, loss of glomerular podocytes, and basement membrane thickening. In particular, diabetic nephropathy involves major alterations of the glomerular filtration barrier, including damage to and loss of podocytes, thickening of basement membranes with alteration in proteoglycan composition, leading to alterations in net charge of the filtration barrier, which then lead to abnormally increased excretion of circulating proteins (proteinuria) ([112]. The BTBR/J-*Lep*^{ob} mouse reliably develops diffuse mesangial expansion because of matrix accumulation, and this process is detectable as early as age 10 weeks and is progressive. These mice have an altered glomerular filter with progressive thickening of the capillary basement membranes and a detectable loss of podocytes as early as ages 6 to 8 weeks, which persists in the absence of therapeutic interventions.[4] As occurs in humans with diabetes, the alterations in glomerular capillary basement membranes and the loss of podocytes correlates with proteinuria, which is well-established by 8 weeks of age.

The mice develop mesangiolysis, which is increasingly evident as the mice age. There is evidence from both humans and animal models that the loss of activity of vascular endothelial growth factor (VEGF), normally produced in podocytes where it is a trophic factor engaged in crosstalk with other glomerular cell populations, produces mesangiolysis.[113] These features compare favorably with other murine models of diabetic nephropathy. The degree of mesangial thickening, the presence of mesangiolysis, and the degree of basement membrane thickening place it among the best models of morphologically advanced diabetic glomerular injury with *db/db*/eNOS deficient and streptozotocin (STZ) treated eNOS knockout mice on a C57BL/6J background. It has the advantage over *db/db* based models resulting from leptin receptor deficiency by virtue of its being a model based on leptin deficiency which allows studies of the consequences of normalization of the metabolic milieu following leptin replacement.

The time to develop morphologically advanced lesions is faster in the BTBR/J mouse compared to both the *db/db* and STZ treated eNOS$^{-/-}$ C57BL/6J mice, allowing more timely and less costly efforts to complete a specific study. Unlike Akita and NOD mice, which are both models of Type 1 diabetes that develop nephropathy but in the case of the NOD mouse, also has immune disturbances resulting in mesangial deposition of immune complex deposits, the BTBR/J-*Lep*^{ob} mouse develops nephropathy without the confounding element of a concurrent secondary glomerular injury such as immune complex deposition.[110]

The model is not without significant limitations. These mice are not particularly hardy, and we have found it necessary to complete studies by 24 weeks of age lest study cohorts be compromised by excess mortality. The mice are subject to skin wound infections (urine scalds in particular), which may also model the susceptibility of humans with poorly controlled diabetes to wounds and ulcerations that heal poorly. As a consequence of leptin deficiency, the mice are hypotensive, in contrast to the great majority of human type 2 diabetics who have the co-morbid condition of hypertension. A deficiency common to virtually all mouse models of diabetic kidney injury, including the BTBR/J-*Lep*^{ob} mouse, is the lack of significant chronic tubulointerstitial injury including interstitial fibrosis and tubular atrophy and the lack of characteristic arteriolar hyalinosis that is a common feature of human diabetic nephropathy.[110,114] Because of the clinical and pathologic importance of tubulointerstitial injury and microvascular injury in the evolution of human diabetic nephropathy, there will be continued interest in developing more complete models of this condition that are suitable for preclinical testing of therapeutic interventions. Nonetheless, at present, the attributes of the BTBR/J-*Lep*^{ob} mouse model place it among the very best for such preclinical studies, and this mouse is widely used by major pharmaceutical companies worldwide for this purpose.

A KEY PATHOPHYSIOLOGIC INSIGHT: DIABETIC NEPHROPATHY IS REVERSIBLE

A major biological insight obtained from the BTBR/J-*Lep*^{ob} mouse came from the administration of leptin via implanted minipumps. Administration of leptin resulted in almost immediate cessation of hyperphagia, with consequent weight loss, loss of insulin resistance, and marked reduction of hyperglycemia. These functional improvements, which restored a metabolic milieu equivalent to BTBR/J wild-type mice, were accompanied by concurrent resolution of the nephropathy. Proteinuria was reversed and the morphologic lesions of diabetic nephropathy also were reversed.[111] These included resolution of mesangiolysis, reduction of basement membrane thickening, and mesangial remodeling with loss of the excess accumulated matrix acquired as part of the diabetic state (Figure 3). Particularly noteworthy was the finding that podocyte number and density, the key component of the glomerular filtration apparatus that regulates protein excretion, could be returned to normal (Figure 3). Podocytes are a terminally differentiated non-replicating cell and it had long been considered that podocyte loss was irreversible and leads to irreversible glomerulosclerosis. Yet, it had already been observed in a landmark study of type I diabetics with biopsy-proven morphologically advanced diabetic glomerulosclerosis that pancreas transplantation alone with resultant normalization of the systemic metabolic milieu could reverse the lesions of diabetic nephropathy after a period of 10 years.[115] Implicit with this finding, but not widely appreciated, was that the return to a normal glomerular structure and function must also include a return to normal podocyte number and function. There was no preclinical animal model or other model system that allowed study of the mechanisms leading to reversal of glomerular injury that was demonstrated in this pancreas transplantation study, and this observation lingered without significant clinical follow-up or development of further therapeutics.

The treatment of BTBR/J-*Lep*^{ob} mice, with leptin with the return to normal glomerular structure and function, allowed us to demonstrate for the first time in a mouse model that podocytes could be restored and provide a working clinical model to study mechanisms underlying this process. We were able to provide evidence for a progenitor cell niche in the parietal epithelial cells lining glomerular Bowman's capsule as a source for restoration of podocyte number.[111] We followed this study with clinical studies on renal biopsies of patients with diabetes to show the potential for a similar progenitor cell niche to be present in these patients that might be potentially mobilized to ameliorate clinically developed diabetic nephropathy.[116] Podocyte loss had been considered an insurmountable obstacle to the reversal of diabetic nephropathy. The studies with the BTBR/J-*Lep*^{ob} mouse point to the possibility for therapeutic interventions that might successfully reverse diabetic nephropathy in humans that might be less daunting than transplantation followed by 10 years of immunosuppression to prevent rejection of the transplanted organ.

PRECLINICAL STUDIES OF RECENTLY DEVELOPED THERAPEUTICS FOR DIABETIC NEPHROPATHY IN THE BTBR/J-*LEP*^{OB} MOUSE

The past 5 years has seen a revolution in our ability to successfully ameliorate diabetic nephropathy with new additions to our therapeutic armamentarium. Notably, dramatic improvements in renal function have been demonstrated with classes of drugs inhibiting the sodium glucose transporter 2 (SGLT2i),[117]

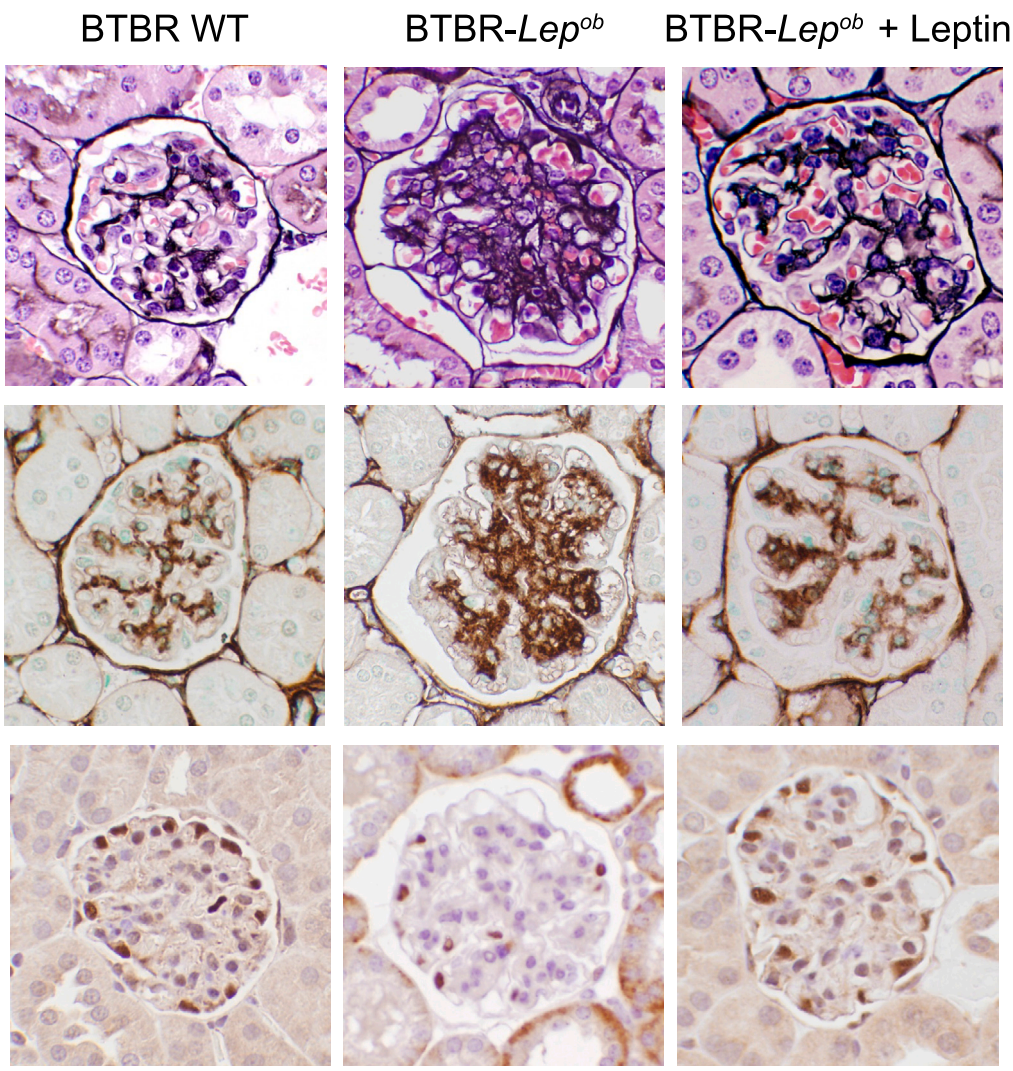

Figure 3. Diabetic nephropathy in the BTBR/J-*Lep*^{ob} mouse

Top row: Left column, Normal glomerulus in a non-diabetic BTBR/J wild type mouse; Middle column, representative glomerulus from a diabetic BTBR/J-*Lep*^{ob} mouse at 24 weeks, with marked expansion of the mesangium by silver (black) staining matrix; Right column, regression of the glomerular lesions of diabetic nephropathy after 6 weeks of Leptin restoration. Silver methenamine stain with hematoxylin and eosin counterstain. **Middle row** shows the expanded mesangial matrix contains copious amounts of collagen IV, which is diminished with regresssion of nephropathy. Bottom row: The staining of cell nuclei by WT-1 in glomeruli is highly specific for podocytes. Left column, Normal complement of podocytes at the periphery of a glomerulus from a non-diabetic BTBR/J wild type mouse stain for WT-1; Middle column, WT-1 staining podocytes are lost in the glomeruli of diabetic BTBR/J-Lepob mice, in this case from a 24 week old mouse; Right column, there is restoration of podocytes in a glomerulus from a 24 week old BTBR/J-Lepob mouse after 6 weeks of Leptin administration.

selective blockade of endothelin receptors,[118] and with finerenone, a nonsteroidal, selective mineralocorticoid receptor antagonist.[119] These studies employed extensive clinical phenotyping and sophisticated statistical modeling for patient enrollment and monitoring, but did not utilize renal biopsies for these purposes and so the structural changes affected by these therapeutics is largely unknown.

The Alpers Laboratory has studied two of these classes of drugs in the BTBR/J-Lep^{ob} mouse. Treatment with the SGLT2i empagliflozin resulted in a significant benefit in clinical parameters, in the

mice as it did in humans, but also demonstrated structural improvement in nephropathy and the ability of this drug to elicit podocyte restoration, similar to what was demonstrated with leptin replacement.[120] These findings provide a clue that there may be additional long-term benefits in kidney structure conferred by treatment with this class of drugs beyond the more immediate improvement in clinical parameters, documented in recent clinical trials. The group of Hugo et al. was the first to show a benefit of SGLT2i in amelioration of the nephropathy in BTBR/J-*Lep*^{ob} mouse.[121] Studies by Tanaka et al. also showed a benefit of treatment with SGLT2i in this model, which was associated with reduction of oxidative stress.[122] Another group, also using empagliflozin, demonstrated protection of the glomerular endothelium with preservation of fenestrae.[123] Similarly, treatment of BTBR/J-*Lep*^{ob} mice with a regimen of the selective endothelin receptor blocker Atrasentan in conjunction with renin-angiotensin inhibition, similar to the regimen used in patients in the successful SONAR trial,[118] also showed similar improvement in clinical and structural parameters, including partial restoration of podocyte density and number in the mouse model.[124]

Not all therapeutic trials in this mouse model have met with equal success. Because the BTBR/J-*Lep*^{ob} mouse develops both diabetes and hyperlipidemia akin to that seen in human type 2 diabetics, a potential therapeutic intervention might be directed to ameliorate the hyperlipidemia alone. We tested this approach by administering an antisense oligonucleotide directed against ApoC3, which resulted in reduction of circulating ApoC3 protein levels and resulted in substantial lowering of triglycerides to near-normal levels in the diabetic mice. We learned that successful lipid-lowering by this therapeutic regimen does not ameliorate the functional or structural manifestations of diabetic nephropathy,[125] and expect this result will help re-direct approaches to therapeutic development in diabetic nephropathy to alternate strategies. For example, Fornoni et al. who used cyclodextrin, an inducer of cholesterol efflux in cells, to reduce cholesterol metabolic features of diabetes in the renal cortex and concurrently demonstrate improvement in metabolic features of diabetes and improvement in nephropathy.[126]

The BTBR/J-*Lep*^{ob} mouse has also been adopted by others in the renal research community for studies relevant to diabetic nephropathy. Bulk transcriptomic profiling of glomeruli isolated from these mice by the group of Banas identified pathways linked to neoangiogenesis, semaphorin signaling, oxidation and reduction of oxidation, and complement activation.[127] This last finding is of interest in view of the studies of Morigi et al. who found that blockade of the activity of complement component C3a by treatment with a C3a receptor antagonist enhanced podocyte density and preserved their phenotype, thereby limiting proteinuria and glomerular injury in this model.[128]

Lavos et al. built on data showing that blocking a pro-inflammatory cytokine, interleukin-17A, can be beneficial in some chronic inflammatory diseases and demonstrated treatment with neutralizing interleukin-17A antibodies resulted in an improved clinical phenotype; it reduced infiltration of the kidney parenchyma by inflammatory cells and promoted podocyte restoration.[129] Studies by the group of Nangaku also highlighted a potential role for inflammation by first demonstrating elevated expression of the chemokine CCL2, also known as monocyte chemoattractant protein-1, in these mice and demonstrating reduced glomerular macrophage infiltration after treatment with a small molecule inhibitor was associated with improvement in nephropathy.[130] The studies of Anderberg et al. also indicated a role for chronic inflammation in diabetic renal injury by the identification of elevated levels of Serum Amyloid A (SAA), a potent pro-inflammatory protein, in the plasma of patients with diabetic kidney disease and proteinuria and finding elevated levels of SAA protein in the glomeruli and tubulointerstitium of BTBR/J *ob/ob* mice.[131] These studies and others point to a complex interaction between metabolic disturbances, inflammation in general and monocyte/macrophage infiltration in particular, which contribute to the evolution of diabetic nephropathy and offer potential clinical therapeutic targets if supported by further studies.

THE BTBR/J-*LEP*^{OB} MOUSE IS ALSO A GOOD MODEL FOR DIABETIC COMPLICATIONS IN ORGANS OTHER THAN THE KIDNEY

As noted earlier, patients with diabetes suffer from a multiplicity of organ complications. Although animal models have been developed for virtually all of the complications of diabetes, very few of these models mimic the situation in humans in which patients may be simultaneously suffering from complications in multiple organs. BTBR/J-*Lep*^{ob} mice have been shown to reliably develop complications of neuropathy,[132]

retinopathy (with thinning of the inner and outer plexiform layers,[133] and microvascular degeneration and increased vascular permeability[134] and cardiomyopathy (with interstitial fibrosis and a restrictive phenotype characteristic of human diabetic cardiomyopathy,[135] all of which are major contributors to morbidity in patients with diabetes.

A deficit of mouse models of type 2 diabetes is that none are a good model of atherosclerosis. Kanter et al. have rectified the situation by modifying BTBR/J-*Lep*^{ob} mice by delivery to the liver via a viral vector of a construct allowing overexpression of an inducible degrader of the LDL receptor (IDOL). Mice so treated developed atherosclerotic lesions while also demonstrating a diabetic nephropathy phenotype. These mice offer a unique opportunity to study the atherosclerotic cardiovascular disease that is the principal cause of mortality in patients with type 2 diabetes.[136]

CONCLUDING COMMENTS

We hope this review will encourage other researchers to carry out additional studies of the BTBR/J mouse in relation to diabetes, diabetes complications, and metabolic diseases in general. As mentioned earlier, BTBR/J mice are a widely-used model of autism, but we do not have any evidence that the autism has any relation to the diabetes susceptibility of this mouse strain. The studies by Shalev on *Txnip* and by Alpers on diabetic nephropathy demonstrate the value of this model for translational research. Finally, the BTBR/J mouse may be a very useful model for nutritional studies aimed at discovering strain effects on diet responsiveness.

DECLARATION OF INTERESTS

The authors declare no competing interests

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