Research Article

Animal Model Studies Reveal that Common Human-Centric Non-Coding Variants from Epidemiology are By-products of Primate Evolution Unrelated to Physiological Control of Blood Pressure

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Abstract

Background: Human genome-wide association studies (GWAS) on blood pressure (BP) have been undertaken by avoiding its physiology and mechanisms controlling BP. Consequently, the physiological significance of GWAS on BP remains undiscovered. A shared mechanistic foundation starts to untangle human physiological regulations of BP as primate versions of rodents and *vice versa*. Thus, understanding mechanisms in rodents is equivalent to unraveling the same in humans rooted in their common ancestors.

Methods: We used BP quantitative trait loci (QTLs) from hypertensive rats as functional proxies to seize human orthologs marked by GWAS.

Results: 6 BP QTLs correspond to 6 specific human genes. BP was altered by these QTL alleles, and yet, the human non-coding GWAS variants are absent in rodents. They cannot contribute to physiological modulations of BP by these QTLs, because depleting such a variant has no impact on BP. Thus, these variants mark QTLs nearby, are not QTLs *per se*, since they only emerged during primate evolution. When functioning together, these human QTLs physiologically attain the same magnitude of BP effect

as a single QTL alone. Mechanistically, these QTLs may function in a common pathway. Each is involved in a different pathway step leading to BP control, not by altering BP by merely affecting QTL expressions. One pathway is muscarinic cholinergic receptor 3 (M3R) signaling. A new M3R component is implicated from current work.

Conclusions: In spite of cognitive impedance from a human-centric dogma, the modularity/pathway concept is evolving into a paradigm physiologically applicable to mammalian polygenic and quantitative traits.

Keywords: Quantitative trait loci; Modularity; Common pathway; Epistasis; GWAS

1. Introduction

1.1. A high prevalence of chronically-elevated blood pressure, hypertension, is a compelling risk for cardiovascular, renal and infectious diseases [1]. This risk has been highlighted by a recent hospitalization rate of COVID-19 patients with underlying conditions (DOI: 10.15585/mmwr.mm6915e3). The most common among them is hypertension that needs to be treated with anti-hypertensive drugs. This alarming hazard urgently demands our actions in unraveling pathogeneses of hypertension, and in distinguishing mechanistic causes of pathophysiology from their outward effects found in epidemiology.

1.2. Thanks to genome-wide association studies (GWASs), detecting human quantitative trait loci (QTLs) for blood pressure (BP) has statistically marked the vicinity of more than 900 BP QTLs by more than 10000 single nucleotide polymorphisms (SNPs) [2]. So far, no human QTLs have been functionally identified to belong to a physiology system known to affect blood pressure. We are no closer in understanding a pathogenesis for human polygenic hypertension than before the advent of GWASs [3]. With due respect, >90% of these SNPs cannot be functional variants in spite of the equally-strong statistics associating them all with blood pressure. These SNPs are pure markers for potential QTLs close by. Thus, in identifying a human QTL, statistics are insufficient and physiological studies *in vivo* are needed.

1.3. A physiological distinction is unmistakable between locating a SNP marking a QTL nearby and identifying the QTL itself. By focusing on after effects of BP-regulating mechanisms, human GWASs have reached their limitations in finding causes of these mechanisms. A QTL refers to a locus residing in a chromosome segment when genetically defined, but a QTL is a single gene when molecularly identified [4]. For example, *C17QTL1* on rat Chromosome 17 is a single gene encoding *Chrm3* [muscarinic cholinergic receptor 3 (M3R)] [5,6]. No combination with another QTL/gene is necessary to physiologically affect blood pressure [7,8].

1.4. Functionally, an alteration in a physiological mechanism will cause variations in blood pressure, but not all BP variations are a result of mechanistic changes. Experimental advantages using rodent models allow causative mechanisms modulating blood pressure driven by physiology to be unveiled [9]. Because of conserved mechanisms, studying them in regulating rodent BP is equivalent to revealing the same mechanisms in humans originating from their common ancestors. Evidently, most land living mammals attain a similar range of blood pressures [10], despite differences in separate physiology characters (e.g. corporal bulk). The only way for this to materialize is that basic mechanisms modulating blood pressure must

have been formed and held constant in common ancestors of these mammals before 90 million years ago (www.timetree.org), before humans existed.

1.5. *In vivo* studies now unify animal model and human QTLs into a basic framework in physiological mechanisms of BP control. Rodent QTLs as proxies from inbred strains have functionally captured distinct human QTLs [11,12]. The intergenic GWAS SNP close to *CHRM3* [13] is only a marker for the human QTL, not the QTL itself [11]. Thus, a 'common' SNP from human GWAS merely marks a nearby physiological QTL that has a 'rare' functional variant. Replicating such a 'common' SNP by other GWAS seems an epidemiological exercise [2] that is irrelevant to its physiological impact on BP, because removing the SNP has no effect on BP [5,7].

1.6. These atypical and counter-intuitive results may appear confusing and disturbing, because they contradict the prevailing tenet in epidemiology that a quantitative and polygenic trait should be made by accumulating 'miniscule' effects from multiple QTLs. This is lately molded into an 'omnigenic' hypothesis [3], which has been believed to universally apply to all polygenic and quantitative traits in whatever organisms in both inbreds and oubreds. (Comparing inbreds to outbreds will be elaborated further in discussions). However, facts are facts. There is no *in vivo* evidence that any of human genetic architectures of GWAS in outbreds [2] could actually impact on blood pressure physiologically, and controlling gene expressions from a GWAS SNP might affect BP physiologically.

1.7. The shift of paradigm from 'omnigenicity' to QTL modularity [11,12,14] has become a part of the literature in polygenic research [15]. Invisible QTL modularity from human epidemiology reflects limitations of GWAS [2], because GWAS is done by ignoring BP-controlling physiology and mechanisms. Among mammals, modularity is a physiological reality [9] but hidden from GWAS. It is analogous to lacking evidence for black holes in Newton's mechanics, although they exist from Einstein's theory of gravity [9]. It seems that physiologically understanding quantitative and polygenic traits such as BP in biology mimics studying gravity in physics.

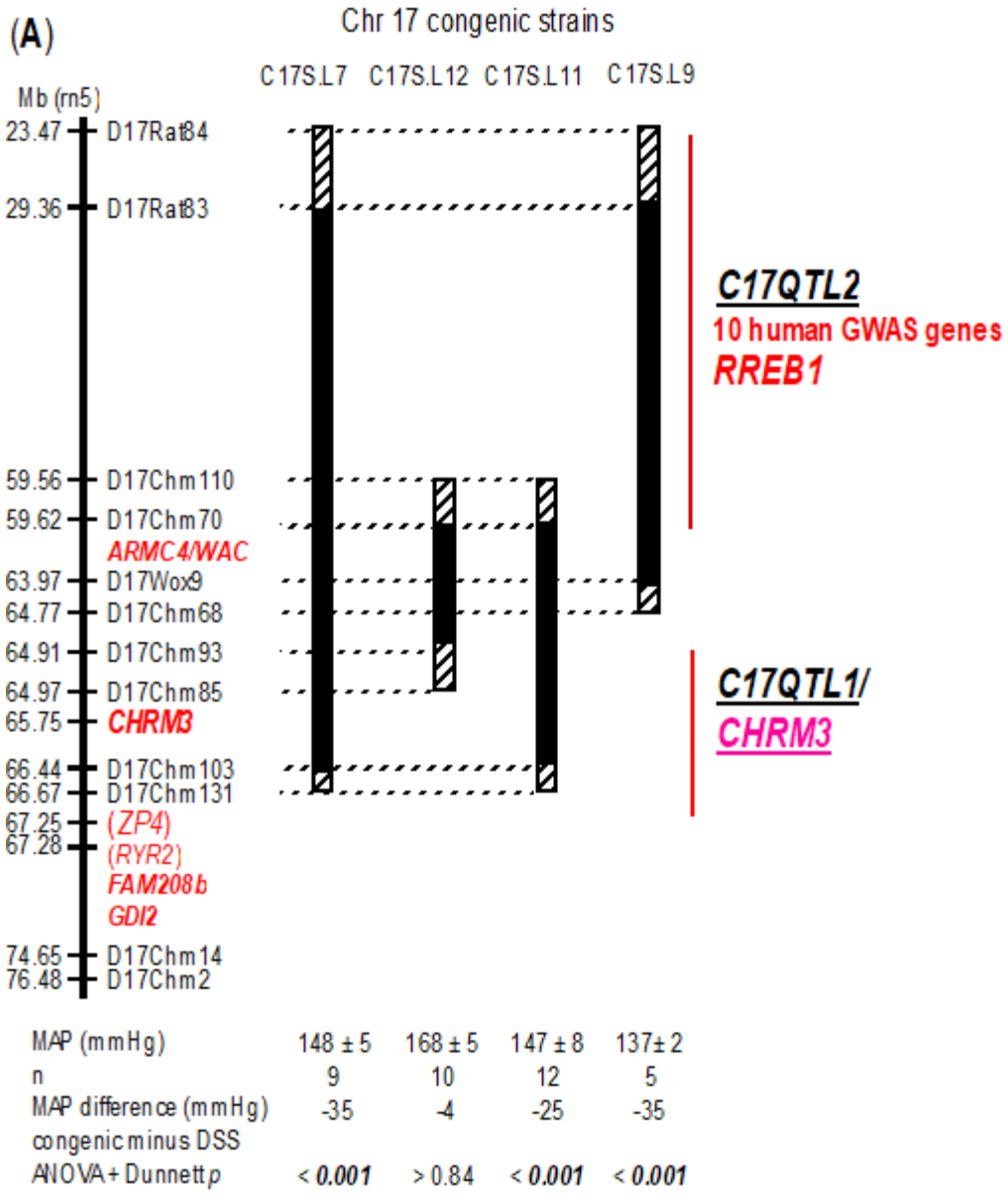
1.8. Only by accumulating evidence can we make this paradigm shift recognizable and accepted by the concerned scientific community. In retrospect, the inherent truth embodied in Mendelism became established and appreciated after a 35-year oblivion, only when Mendel's discoveries were reproduced by others. Hopefully, our previous work [11,12,14] and the current confirmation of them, will encourage different scientists to expand one-lab-based findings in animal models and humans to broader polygenic traits including BP. In this way, the validity of this developing paradigm can be further tested.

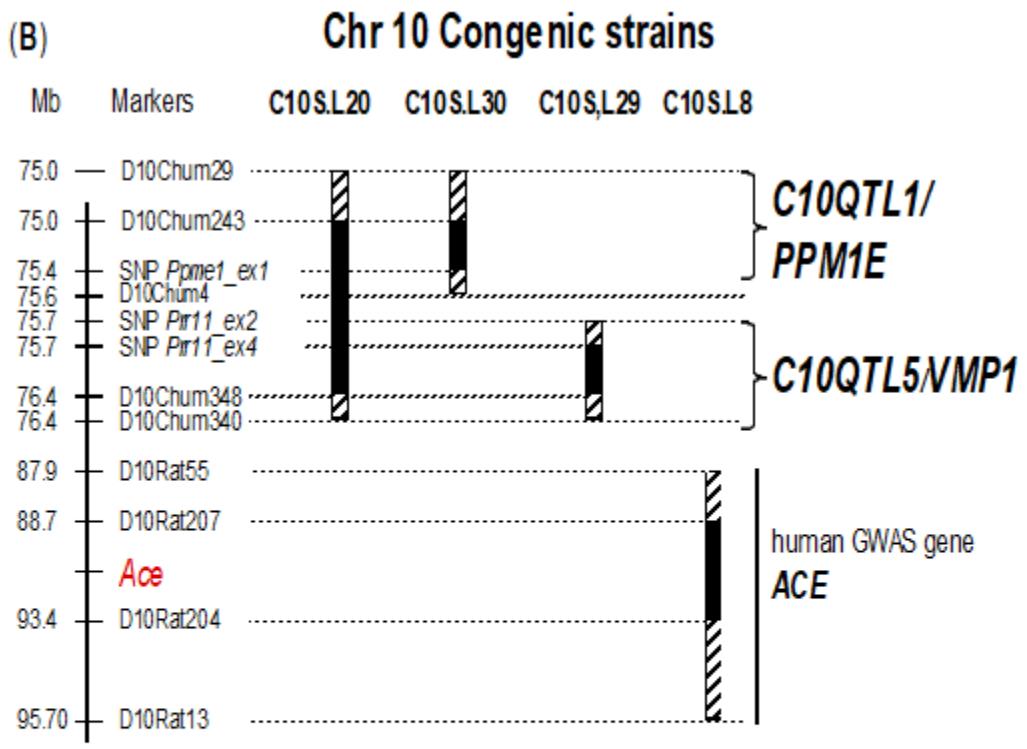
1.9. Arguably, a narrow range of work [11,12] might not represent a broad mechanistic reality. We have extended the rat QTL coverage to evaluate additional human GWAS genes in progressive stages, as a lone investigator-initiated lab can. Instead of replicating same QTLs in other populations as human GWASs do [3], we tested the reproducibility of mechanistic outcomes from analyzing previously-unexplored rat QTLs that respond to different human GWAS gene orthologs. In this process, our new data have validated and widened the paradigm of QTL modularity to both rodents and humans as pathogenic pathways to polygenic hypertension [9]. Previously-unsuspected components of these pathways have been implicated.

2. Materials and Methods

2.1. Animals Protocols for handling as well as maintaining animals were approved by our institutional animal committee (CIPA). Inbred hypertensive Dahl salt-sensitive rats (DSS) are our functional proxy of choice. In order to detect the physiological impact from a BP QTL, our work was done in the DSS genetic background that has lost its genome buffering capacity in impeding BP fluctuations [16] and in suppressing hypertension [17,18].

2.2. Experimental protocols and analyses Breeding procedure, dietary treatments, telemetry implantation, postoperative care and BP measurement durations were essentially the same as reported previously [14]. In brief, male rats were weaned at 21 days of age, kept on a low salt diet followed by a high salt diet starting from 35 days of age until the end of the experiment. Telemetry probes were implanted at 56 days of age (namely 3 weeks from the time of the high salt diet). In the BP presentation (Figure 1), averaged readings of mean arterial pressures (MAP) for the duration of measurement were given for each strain.


(A)
Chr 17 congenic strains
C17S.L7
C17S.L12
C17S.L11
C17S.L9
Mb (rn5)
23.47 D17Rat84
29.36 D17Rat83
59.56 D17Chm110
59.62 D17Chm70
ARMC4/WAC
63.97 D17Wox9
64.77 D17Chm68
64.91 D17Chm93
64.97 D17Chm85
65.75 CHRM3
66.44 D17Chm103
66.67 D17Chm131
67.25 (ZP4)
67.28 (RYR2)
FAM208b
GDI2
74.65 D17Chm14
76.48 D17Chm2
C17QTL2
10 human GWAS genes
RREB1
C17QTL1/
CHRM3
MAP (mmHg) 148 ± 5 168 ± 5 147 ± 8 137± 2
n 9 10 12 5
MAP difference (mmHg) congenic minus DSS -35 -4 -25 -35
ANOVA+Dunnett p < 0.001 > 0.84 < 0.001 < 0.001



MAP (mmHg)	144±5	128±8	131±3	164±7
n=	11	8	6	7
MAP difference DSS minus congenics (mmHg)	33	49	46	13
ANOVA + Dunnet *P*	***<0.001***	***<0.001***	***<0.001***	>0.60

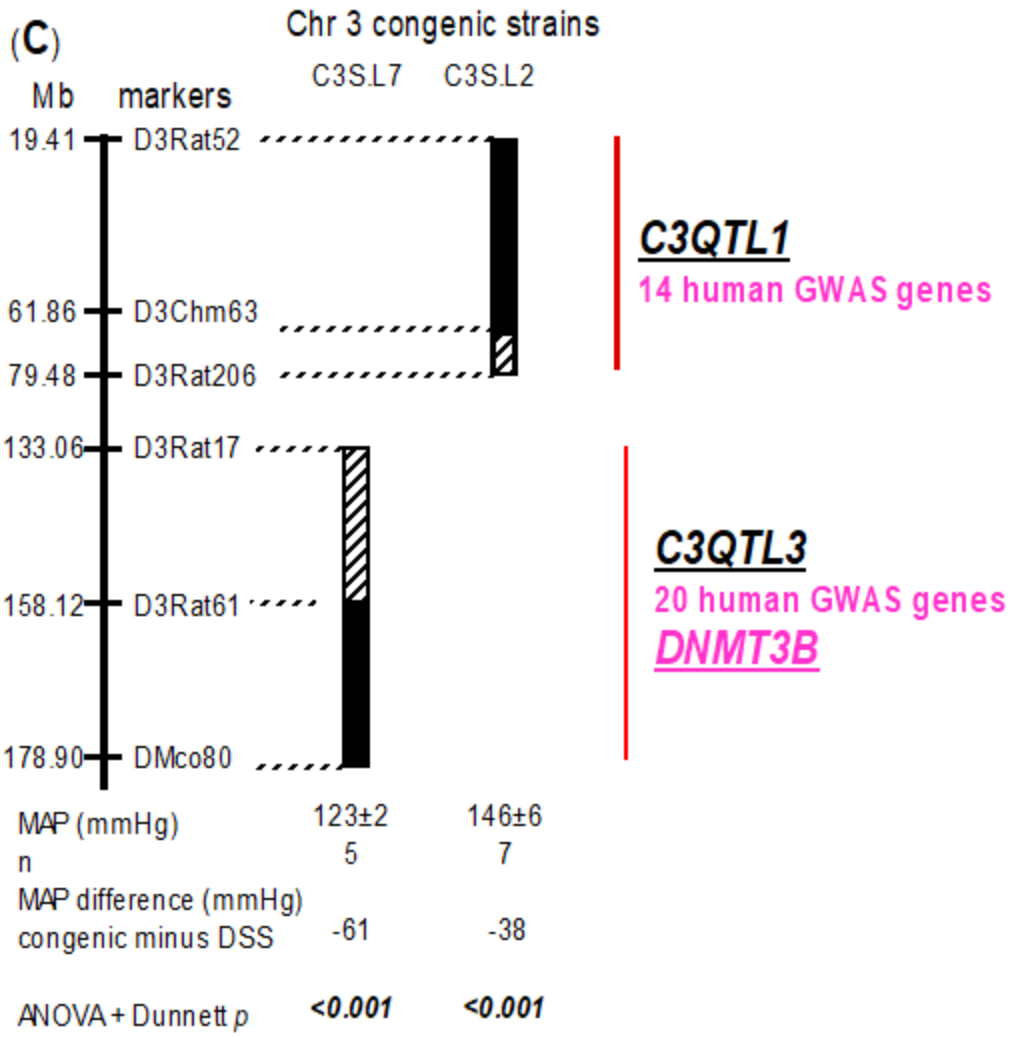


Figure 1: Congenic knock in genetics defining chromosome regions containing BP QTLs *in vivo*. Solid bars under congenic strains symbolize the Dahl salt-sensitive (DSS) chromosome fragments that have been replaced by those of Lewis. Hatched bars indicate ambiguous regions. The full gene names with abbreviations is given in the Table 1 legend. Mean arterial pressures (MAPs) for DSS and congenic strains are averaged for the period of measurement and are given at the bottom of the map. Significant *p* values are put in bold and italics. ± indicates SEM. (**A**) Chr 17; (**B**) Chr 10; (**C**) Chr 3.

2.3. Repeated measures' analysis of variance (ANOVA) followed by Dunnett's test, which corrects for multiple comparisons and unequal sample sizes, was used to compare a parameter in MAP between 2 groups as reported previously (14). The power and sample size calculations in the analysis are the same as given previously [11].

3. Results

3.1. Congenic knock in genetics is a proxy tool in physiologically catching human GWAS genes by causality. The congenic principle is similar to that of SNP 'knock-in' with a variation in a genome scale [4], and is employed as congenic knock in genetics [11,12].

Despite the DSS rats are known for their 'salt-sensitivity' for physiological studies, the human GWAS genes that have been captured by the DSS model commonly function in humans from general populations, with or without salt sensitivity [11,12]. Furthermore, the M3R signaling pathway found in DSS is pro-hypertensive even under low salt diet [5]. High salt diet merely accelerated hypertension and our studies of it using the model. Thus, BP-regulating mechanisms discovered in DSS rats are applicable to those of human essential hypertension in general populations, irrespective of the salt content. Among DSS chromosome segments known to contain BP QTLs [14], only those matching human QTL signals from GWAS [2] were investigated here (Supplementary Table 1).

3.2.1. The M3R signaling pathway in regulating BP existed in common ancestors of humans and rodents. Since they have similar blood pressures in a polygenic and quantitative context, we hypothesized that humans and rodents may use same pathways originating from their common ancestors and their similar BP states are not due to a convergent evolution event. We tested this hypothesis by focusing on M3R as *C17QTL1*, a QTL on rat Chromosome 17 and human CHROMOSOME 1, because M3R has been physiologically proven to be *C17QTL1* [5,7,11].

3.2.2. Despite no M3R sequence is available from extinct common ancestors of humans and rodents 90 million years ago, the M3R signaling already existed in them. This is because M3R of Tasmania Devil, a marsupial, shares 90% of homology conservation with humans and rats overall, and 95% in the M3R signaling domain to either humans or rats (Supplementary Table 2, http://genome.ucsc.edu). M3R's presence in them indicates that the M3R signaling pathway was present even in common ancestors of marsupial and placental mammals. Marsupials and placentals split 160 million years ago, prior to the divergence of rodent and human ancestors 90 million years ago, and before modern humans existed around 300 thousand years ago (www.timetree.org). Marsupials have similar BP as most placental land mammals [10].

3.2.3. This is a proof that, common ancestors of humans and rodents possessed a BP-regulating mechanism of M3R signaling pathway, in spite of the fact that M3R is pleiotropic in functions in addition to regulating BP. This evidence explains similar BPs between humans and rodents, for which a possible convergent evolution by different mechanisms to obtain similar blood pressures has no proof. This contrast will be dealt with further in discussions.

3.3.1. Defining physiological effect of each QTL/GWAS gene on blood pressure: GWAS SNPs were chosen solely by their elevated minor allele frequencies, not based on their influences on blood pressure. Total variance used in GWAS [3] gauges a spread of BP in heterogeneous populations as an epidemiology parameter, and thus is irrelevant to physiological mechanisms of BP control. Total variance is largely due to environmental effects, not due to mechanistic actions of QTLs [9]. Since environmental factors are not inherited and mostly unquantifiable, the often-termed 'missing' heritability is not equivalent to missing total variance. Regardless how many BP QTLs really exist in an individual organism, identifying the physiological impact of a particular QTL on BP is critical that can be untangled from the non-physiological total variance

3.3.2. The BP effect for a human GWAS SNP [2] and presumably from one QTL marked by it, seemed

'miniscule' when fractionated from total variance [3]. What then is the significance in identifying 6 'trifle' QTLs among 900 [2], each encoded by a single gene (Table 1, Figure 1)? An illuminating answer came from revealing the actual magnitude of physiological BP effect for each of these 6 QTLs. It turns out to be substantially larger when physiologically defined, alone in homogeneity, by causality, and under a uniform environment [details were given in Table 1 in reference] [19].

Rat QTL name	**Functional magnitude BP effect**	**Rat functional candidate gene**	**Human GWAS SNP**	**Rat GWAS SNP ortholog**	**Closest human functional gene**	**# Probable coding mutations**
C17QTL1	28%	*missensed *Chrm3*	Intergenic rs2820037	Non-existent	***CHRM3***	12
C17QTL2	42%	§missensed *Rreb1*	Multiple intronic SNPs	Non-existent	***RREB1***	10
C10QTL1	60%	§missensed *Ppm1e*	Multiple intergenic/ intronic SNPs	All non-existent	***PPM1E***	8
C10QTL5	56%	No missense mutation (*Vmp1*)	Intronic SNP rs264566	Non-existent	(***VMP1***)	1
C3QTL1	46%	none				
C3QTL3	73%	§missensed *Dnmt3b*	Intronic rs6141767	Non-existent	***DNMT3B***	7

Table 1: Selective rat QTLs with missense mutations functionally capturing human orthologs.

Footnote to Table: QTLs and their BP effects are given in Fig. 1. § shows confirmed mutations; * indicates the functional impact identified. Detailed data are presented in Supplemental tables. ***CHRM3***, *muscarinic cholinergic 3 receptor (M3R) gene*; ***DNMT3B,*** DNA methyltransferase 3 beta; ***PPM1E***, *protein phosphatase, Mg2+/Mn2+ dependent 1E*; ***RREB1,*** ras responsive element binding protein 1; ***VMP1***, vacuole membrane protein 1.

3.3.3. Here, a distinction is revealing between a statistical fractionation from total variance and a real physiological impact for each QTL. This is because a fractionated BP effect for every one of these 900 human QTLs in GWAS [2] was compounded by those from other genes in heterogeneity, and by environmental influences in study populations. Due to these interferences, an estimated effect for each QTL from total variance does not prove if the GWAS signal itself can have a physiological impact on BP, let alone its magnitude of BP effect. *C17QTL2* on DSS rat Chromosome 17 proves the point (Figure 1A).

3.3.4. Ten different human GWAS genes [2] fell into the congenic knock-in segment that defined *C17QTL2* (Figure 1A). One human GWAS signal close to *CHRM3* [13] was present in the segment containing *C17QTL1*. The 2 QTLs were detected as a single QTL statistically and explained 6.7% of total variance in a heterogeneous rat population [20]. In contrast, each of them was capable of physiologically and independently altering BP by 28-42% in the total difference by mmHg *in vivo* between 2 parental rat strains and under a uniform environment (Table 1). The calculation is as follows.

3.3.5. The congenic knock-in defining *C17QTL2* lowered blood pressure by 35 mmHg (Figure 1A). The total BP difference between DSS and Lewis strains is 83 mmHg, i.e. 178 mmHg for DSS minus 95 mmHg for Lewis. Thus, the physiological BP effect of *C17QTL2* is calculated as 35/83 = 42%. By the same calculation, each of 4 QTLs, *C10QTL1*, *C10QTL5*, *C3QTL1*, and *C3QTL3*, singularly possesses a physiological BP effect ranging from 46-76% (Figure 1, Table 1). This is contrary to the non-physiological estimate from fractionated total BP variance [2].

3.3.6. If the 'miniscule' hypothesis were physiologically valid [3], eliminating 1 QTL/GWAS gene among 900 should have a negligible consequence. This is not the case, since *Chrm3* is a single gene responsible for *C17QTL1*. Depleting *C17QTL1*/*Chrm3* alone lowered BP by at least 50% in the BP difference between $Chrm3^{+/+}$ and $Chrm3^{-/-}$ (5). Cumulatively, the 6 QTLs by themselves (Table 1) seem physiologically more than sufficient to explain the total BP difference between the 2 parental strains.

3.3.7. Therefore, the crucial issue to address is not how 'miniscule' could be the BP effect that each GWAS gene was supposed to have, but rather why there is such an over-abundance of GWAS genes that are more than physiologically necessary in regulating BP of an organism. Summing them up cannot be a valid physiological justification, whereas fractionating each from total variance is not physiological. Thus, a meaningful physiological solution is required on the collectivity of their functional impact on BP.

3.4.1. QTL modularity on BP is physiologically conserved between humans and rodents: We first combined *C17QTL1* and *C17QTL2* in a 'double' congenic strain as shown in C17S.L7. Their aggregated MAP (148±5, n=9) was similar to either of them alone (Figure 1A). A 2x2 ANOVA (14) showed epistasis (p <0.003) between them, i.e. their combined BP is non-cumulative and they belong to the same epistatic module, epistatic module 2. Epistasis means one QTL hiding the effect of another and occurs regardless the number of GWAS genes involved. The *C17QTL2*-residing segment bears 10 human GWAS genes and the *C17QTL1*-residing segment carries 1 human GWAS gene (Figure 1A).

3.4.2. The congenic strain C10S.L20 (Figure 1B) contains *C10QTL1* and *C10QTL5* and showed similar BP (144±5, n=11) as either of the 2 QTLs alone. There is epistasis (p <0.001) between the 2 QTL-residing intervals carrying 4 human GWAS genes together (Figure 1B, Supplementary Table 1). Either of *C3QTL1* and *C3QTL3* showed epistasis with *C10QTL1* [14]. Thus, these 4 QTLs belong to the same module, epistatic module 1. The chromosome segments lodging *C3QTL1* and *C3QTL3* lodge 34 human GWAS genes (Figure 1C, Supplementary Table 1).

3.4.3. In total, 49 human GWAS genes are contained in the chromosome segments harboring the 6 QTLs (Figure 1). On the basis of their known epistasis by

functional proxy, these 6 human GWAS genes may be classified physiologically into 2 epistatic modules, or 2 independent pathways in determining BP. BP is functionally additive between 2 members of 2 separate modules [14], and is the basic mechanism of QTL actions. Since M3R is a signaling pathway [5], epistatic module 2 to which *C17QTL1*/*CHRM3* and *C17QTL2* belong constitutes a pathway with multiple steps composed of different QTLs leading to BP control. *C17QTL2* most likely participates in one of these steps in the M3R signaling pathway.

3.4.4. In order to identify a specific step in a pathway, a molecular identification of a QTL is necessary. Identifying *CHRM3* as a causal gene to *C17QTL1* is the precedent for genetically discovering a component of a step in a pathway in a polygenic context [5,6]. *CHRM3* has been proven to be *C17QTL1* not only in DSS rats [5], but also a strong candidate for humans [11]. *Chrm3* carries a function-changing missense mutation [5]. Thus, missense mutations are priority, although not exclusive, targets for identifying candidate genes for the following QTLs.

3.5.1. *C17QTL2* of DSS rats may be a physiological ortholog of a human GWAS gene, *RREB1* (ras responsive element binding protein 1). 10 different human GWAS genes (2) fell into the large congenic knock-in segment that defined *C17QTL2* by changing BP *in vivo* (Figure 1A). It appears that at least 3 human QTLs among the 10 GWAS genes may exist in this interval, since they are located on 3 separate human CHROMOSOMEs (CHRs) (Supplementary Table 1). Of 6 GWAS genes on CHR 6, *RREB1* has become a functional candidate gene for *C17QTL2*, because it carries 2 missense mutations in DSS rats that may potentially alter the function of the Rreb1 protein (Supplementary Table 3).

3.5.2. The human *RREB1* gene was marked by 2 intronic SNPs and 1 missense mutation (Supplementary Tables 1&3). If any of them would affect BP *in vivo*, it should be present in rodents. However, no similar SNP sequence, nor homology in 1 kb non-coding sequence surrounding the 2 intronic SNPs, were detected in the rat genome (Table 2). These non-coding SNPs are present only among primates including humans (Table 3). Since BPs of rodents and primates are similar (10), these non-coding SNPs seem a by-product of primate evolution, rather than a requirement in physiologically controlling BP. Since BP changed *in vivo* without them (Fig. 1A), these SNPs themselves cannot be responsible for changing BP by *C17QTL2*, and appears solely as human-centered markers for the physiological QTL nearby.

Human SNP/ Marked gene	Rat Homology	Note
rs4960295/*RREB1* (intron)	No	No hits in 1Kb sequence used for blast on Chr17
rs2151942/*RREB1* (intron)	No	Haphazard hits in 1 Kb sequence used for blast; 2 mini regions of homology randomly distributed, but not in the right region.
rs1334576/*RREB1* (missense Gly195Arg)	SNP is absent	91 bases hit (93.4% homology) in 1 Kb sequence used for blast on Chr17
rs6141767/*DNMT3B* (intron)	No	Similar to rs2151942/*RREB1*
rs304295/*PPM1E* (intergenic)	No	Similar to above
rs304298/*PPM1E* (beginning of intron1)	No	Similar to above
rs12942969/*PPM1E* (middle of intron1)	No	No hits in 0.6Kb sequence used for blast on Chr10
rs35082135/*PPM1E* (end of intron1)	No	No hits in 1Kb sequence used for blast on Chr10
rs2645466/*VMP1* (intron3)	No	Similar to rs2151942/*RREB1*
rs2820037/*CHRM3* (intergenic)	No	No hits in 4Kb sequence used for blast on Chr17

Table 2: A survey of sequence homologies between humans and the rat for GWAS SNPs

Footnote: Gene names are given in legends of Table 1. Appropriate sequence surrounding a SNP in question was blasted into the rat genome at: https://genome.ucsc.edu/cgi-bin/hgGateway.

More recent ← more ancient

Human SNP/ Marked gene	**Chimpanzee**	**Bonobo**	**Gorilla**	**Orangu-tan**	**Gibbons**	**Old World monkeys**	**New World monkeys**
rs4960295/*RREB1* (intronic)	homology	homology	homology	homology	homology	homology	homology
rs1334576/*RREB1* (missense Table 4)	homology	homology	homology	homology	homology	homology	homology/
rs2151942/*RREB1* (intronic)	homology	homology	homology	homology	homology	homology	homology
rs304295/*PPM1E* (intergenic)	homology	homology	homology	homology	homology	No homology	No homology
rs6141767/*DNMT3B* (intronic)	homology	homology	homology	homology	homology	homology	Homology; surrounding sequence is not
rs304298/*PPM1E* (intron1)	homology	homology	homology	homology	homology	homology	Similar to above
rs12942969/*PPM1E* (intron1)	homology	homology	homology	homology	homology	homology	No homology/ Squirrel Monkey
rs35082135/*PPM1E* (intron1)	homology	homology	homology	homology	homology	homology	surrounding sequence less conserved.
rs2645466/*VMP1* (intron3)	homology	homology	homology	homology	homology	homology	No homology/ Squirrel Monkey
rs2820037*CHRM3* (intergenic)	homology	homology	homology	homology	homology	No homology	No homology

Table 3: Survey of conservation/homology for non-coding GWAS SNPs and 1 missense mutation during primate evolution

Footnote: Gene names are given in the legend for Table 1. Homology indicates that the SNP and/or surrounding sequences are conserved. Old World monkeys are represented by Rhesus macaque, baboon; New World monkeys are represented by marmoset, squirrel monkey. Searches were done at https://genome.ucsc.edu/cgi-bin/hgGateway.

3.5.3. The physiological *C17QTL2* has to be both conserved between the rat and human as well as capable of potentially altering BP by function. The coding domain of f *RREB1* fulfils these 2 criteria. First, missense mutations modifying its protein structure may have a function impact. They are the human rs1334576 missense mutation changing Gly195Arg, and 2 missense mutations in DSS rats (Supplementary Table 3). Additional missense mutations [21] are found in the human *RREB1* (Table 4). In contrast to the 2 intronic SNPs (Table 2), *RREB1* coding regions are highly conserved between rodents and humans. Although the coding mutations in humans are not the same as in inbred DSS rats, they may affect the function of RREB1 from different positions of amino acids. This shared feature and presence of coding mutations support the candidacy of the RREB1 protein for *C17QTL2* for both humans and DSS rats.

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Human_RREB1 MTSSSPAGLEGSDLSSINTMMSAVMSVGKVTENGGSPQGIKSPSKPPGPNRIGRRNQETK  60
DSS_Rat_Rreb1 MTSNPPTGLEGSDLSSVNTMMSAVMSISSVTENGGSPQGIKSPMKPPGPNRIGRRNQETK 60
 ***. *:**********:**********:..********************** ******************

Human_RREB1 EEKSSYNCPLCEKICTTQHQLTMHIRQHNTDTGGADHSCSICGKSLSSASSLDRHMLVHS  120
DSS_Rat_Rreb1 EEKSSYNCPLCEKVCTTQHQLTMHIRQHNTDTGGADHSCSICGKSLSSASSLDRHMLVHS 120
 ************:**************************************************

Human_RREB1 GERPYKCTVCGQSFTTNGNMHRHMKIHEKDPNSATATAPPSPLKRRRLSSKRKLSHDAES  180
DSS_Rat_Rreb1 GERPYKCTVCGQSFTTNGNMHRHMKIHEKDTNSTTAAAPPSPLKRRRLSSKRKLSHDAES  180
 ****************************** **:**:************************

Human_RREB1 EREDPAPAKKMVEDGQSGDLEKKADEVFHCPVCFKEFVCKYGLETHMETHSDNPLRCDIC 240
DSS_Rat_Rreb1 EREDPGPAKKTVEDGQSSGLDKMADETFHCPVCFKEFVCKYGLETHMETHSDNPLRCDIC  240
 *****.**** *******..*:* ***.**********************************

Human_RREB1 CVTFRTHRGLLRHNALVHKQLPRDAMGRPFIQNNPSIPAGFHDLGFTDFSCRKFPRISQA  300
DSS_Rat_Rreb1 CVTFRTHRGLLRHNALVHKQLPRDAMGRPFIQNNPSIPAGFHDLGFTDFSCRKFPRISQA 300
 ************************************************************

Human_RREB1 WCETNLRRCISEQHRFVCDTCDKAFPMLCSLALHKQTHVAADQGQEKPQATPLPGDALDQ 360
DSS_Rat_Rreb1 WCETNLRRCISEQHRFVCDTCDKAFPMLSSLILHRQTHIPADQGREKLQTKTLAADTLDQ  360
 ****************************.** **:***: *****:** *:. * .*:***

Human_RREB1 KGFLALLGLQHTKDVRPAPAEEPLPDDNQAIQLQTLKCQLPQDPGCTNLLSLSPFEAASL 420
DSS_Rat_Rreb1 KVFLAFLGLQHTKDVKPAPAEELLPDDSHIIQLQTLKCQLPQDPGCTNVLSLSPFEAASL 420
 * ***:**********:******* *****.::*******************:************

Human_RREB1 GGSLTVLPATKDSIKHLSLQPFQKGFIIQPDSSIVVKPISGESAIELADIQQILKMAASA  480
DSS_Rat_Rreb1 GGSLTVLPATKENMKHLSLQPFQKGFIIQPDSSIVVKPISGESAIELADIQQILKMAASA  480
 ***********.::**************************************************

Human_RREB1 PPQISLPPFSKAPAAPLQAIFKHMPPLKPKPLVTPRTVVATSTPPPLINAQQASPGCISP  540
DSS_Rat_Rreb1 PPQISLPPLSKAPATPLQAIFKHMPPLKPKPLVAPRTVVAASTPPPLINAQQASPGCISP  540
 *********:******:*******************:*******:********************

Human_RREB1 SLPPPPLKLLKGSVEAASNAHLLQSKSGTQPHAATRLSLQQPRAELPGQPEMKTQLEQDS 600
DSS_Rat_Rreb1 SLPPQSLKFLKGSVEAVSSAHLLQSKSGIQPNTSTQLFLQQPGVELPGQAEMKAQLEQDS  600
 **** **:********.*.********** **:::*:* ***** .****** ****:*******

Human_RREB1 IIEALLPLSMEAKIKQEITEGELKAFMTAPGGKKTPAMRKVLYPCRFCNQVFAFSGVLRA  660
DSS_Rat_Rreb1 IIEALLPLNMEAKIKQEITEGDLKAIMTGPSGKKTPAMRKVLYPCRFCNQVFAFSGVLRA  660
 *********.*************:****:**.*.*****************************

Human_RREB1 HVRSHLGISPYQCNICDYIAADKAALIRHLRTHSGERPYICKICHYPFTVKANCERHLRK  720
DSS_Rat_Rreb1 HVRSHLGISPYQCNICDYIAADKAALIRHLRTHSGERPYICKICHYPFTVKANCERHLRK  720
 ************************************************************

Human_RREB1 KHLKATRKDIEKNIEYVSSSAAELVDAFCAPDTVCRLCGEDLKHYRALRIHMRTHCGRGL  780
DSS_Rat_Rreb1 KHLKATRKDIEKNIQYVSSSTAELVDALCSPETVCRLCGEDLKHYRALRIHMRTHCSRGL  780
 ***************:******:*******:*:*:*************************.***

Human_RREB1 GGGHKGRKPFECKECSAAFAAKRNCIHHILKQHLHVPEQDIESYVLAAD-GLGPAEAPAA  839
DSS_Rat_Rreb1 GGCHKGRKPFECKECNAAFAAKRNCIHHILKQHLHVPEKDIESYVLATDAGLGPADTAA-  839
 ** ************.************************:*********:* ******:: *

Human_RREB1 EASGRGEDSGCAALGDCKPLTAFLEPQNGFLHRGPTQPPPPHVSIKLEPASSFAVDFNEP  899
DSS_Rat_Rreb1 -EASSREESSCVTFAECKPLTTFLEPQNGFLHSSPTQPLPSHISVKLEPASSFATDFNEP  898
 :. *:*.*.::.:******:********** .**** * *:*:********** .*****

Human_RREB1 LDFSQKGLALVQVKQENISFLS--PSSLVPYDCSMEPIDLSIPKNFRKGDKDLATPSEAK  957
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DSS_Rat_Rreb1 LDFSQKGLALVQVKQENMSSLLNSSSSSALYDCSMEPIDLSIPKSGKRGDKDTVVSSDAK  958
 ******************:* * ** . ***************. .::**** .. *:**

Human_RREB1 KPEEEAGSSEQPSPCPAPGPSLPVTLGPSGILESPMAPA----PAATPEPPAQPLQGPVQ      1013
DSS_Rat_Rreb1 KPEPEAGKAEQLSPHPPPCPTLSVTVEPKGSLDNPAGTVVAVTTAAKLEPHTQPLQGSVQ  1018
 *** ***.:** ** * * *:* **: *.* *:.* . . **. ** :***** **

Human_RREB1 LAVPIYSSALVSSPPLVGSSALLSGTALLRPLRPKPPLLLPKPPVTEELPPLASIAQIIS      1073
DSS_Rat_Rreb1 LAVPIYSSALISNAPLLGSSALLNSASLLRPLRPKPPLLLPKPAMTEELPPLASIAQIIS  1078
 **********:*. **:*******.::*****************  :****************

Human_RREB1 SVSSAPTLLKTKVADPGPASTGSNTTASDSLGGSVPKAATTATPAATTSPKESSEPPAPA      1133
DSS_Rat_Rreb1 SVSSAPTLLKTKAADPGPSSTSSNAVATDSPGGSIPKASPSPT--DIPSSREPTDPAPTA  1136
 ************.*****:**.**:.*:** ***:***: : * * :* ::* *

Human_RREB1 SSPEAASPTEQGPAGTSKKRGRKRGMRSRPRANSGGVDLDSSGEFASIEKMLATTDTNKF  1193
DSS_Rat_Rreb1 SSPEEASPPEQGPAASSRKRGRKRGMRNRPLPNSSAVDLDSSGEFASIEKMLATTDTNKF  1196
 **** *** ******.:*:*********.** **.*************************

Human_RREB1 SPFLQTAEDNTQDEVAGAPADHHGPSDEEQGSPPEDKLLRAKRNSYTNCLQKITCPHCPR  1253
DSS_Rat_Rreb1 SPFLQTAED-TQEEVAGTPADHRGPTEEEQGSSAEDRLLRAKRNSYANCLQKINCPHCPR  1255
 ********* **:****:****:**::***** **:*********:******.******

Human_RREB1 VFPWASSLQRHMLTHTGQKPFPCQKCDAFFSTKSNCERHQLRKHGVTTCSLRRNGLIPQS  1313
DSS_Rat_Rreb1 VFPWASSLQRHMLTHTDS---------------------------------------  1273
 *****************..

Human_RREB1 KESDVGSHDSTDSQSDAETAAAAGEVLDLTSRDREQPSE-GATELRQVAGDAPVEQATAE  1372
DSS_Rat_Rreb1 ----------QSDTDTLTTPGEVLDLTARDKEQPTSEGASELSPATQDLTETEAKAE  1320
 ***::* :: ********:**:***:. **:** .: * :*:**

Human_RREB1 TASPV-HREEHGRGESHEPEEEHGTEESTGDADGAEEDASSNQSLDLDFATKLMDFKLAE  1431
DSS_Rat_Rreb1 AAPPEEEEEEKGTEENPEPELECRVEESSGDADAPEEDTASNQSLDLDFATKMMDFKLAE  1380
 :* * ..**:* *. *** * .***:****. ***::*************:*******

Human_RREB1 GDG-EAGAGGAASQEQKLACDTCGKSFKFLGTLSRHRKAHGRQEPKDEKGD------GAS  1484
DSS_Rat_Rreb1 SEAGAVDSQGPAQQEPKHACNTCGKNFKFLSTLSRHRKAHGCQEPKEEEEAPSLENESLG 1440
 .:. ..: * *.** * **:****.****.********** *****:*:. .

Human_RREB1 TAEEGPQPAPEQEEKPPETPAEVVESAPGAGEAPAEKLAEETEGPSDGESAAEKRSSEKS  1544
DSS_Rat_Rreb1 RAVEGPSPSPEPEETPAESSA--TDPTAGTGEASGERQNEDTESPSDGEGTAEKR----G  1494
 * ***.*:** **.* *: * .: : *:*** .*: *:**.*****.:**** .

Human_RREB1 DDDKKPKTDSPKSVASKADKRKKVCSVCNKRFWSLQDLTRHMRSHTGERPYKCQTCERTF  1604
DSS_Rat_Rreb1 DSDRRPKTDSPKSMASKADKRKKVCSVCNKRFWSLQDLTRHMRSHTGERPYKCQTCERTF  1554
 *.*::*********:**********************************************

Human_RREB1 TLKHSLVRHQRIHQKARHAKHHGKDSDKEERGEEDSENESTHSGNNAVSENEAELAPNAS  1664
DSS_Rat_Rreb1 TLKHSLVRHQRIHQKARHSKHHGKDSDKDERAEEGSEDESTHSANNPASENEAESAPSTS  1614
 ******************:**********:**.**.**:******.** .******* **.:*

Human_RREB1 NHMAVTRSRKEGLASATKDCSHREEKVTA-----GWPSEPG-QGDLNPESPAALGQDLLE  1718
DSS_Rat_Rreb1 NHVAITRSRKESLANSGKECGREEKAIAEQATEPSAPKEPGSPGETDPQSPAAIVQDLLE  1674
 **:*:*******.**.: *:*.:.*: :: . *.*** *: :*:*****: *****

Human_RREB1 PRSKRPAHPILATADGASQLVGME  1742
DSS_Rat_Rreb1 LCGTRPA-PILAATDGASQLLGME  1697
 ..*** *****.::*******:****
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Table 4: Amino acid alignment and missense mutations in codons for ras responsive element binding protein 1 (RREB1) in humans and rats

Footnote for table: * indicates amino acid identity (86%). Probable human missense mutations, (21) are shaded. Amino acids in blue indicate that the minor allele has been observed more than 10 times. The human Gly195Arg is a GWAS coding SNP. The 2 rat missense mutations are detected in both our database, (36) and rat genome database, (37) and shaded in green. DSS, Dahl salt-sensitive rats.

3.6.1. *C3QTL3* may be a physiological ortholog of human GWAS gene DNA methyltransferase 3B (*DNMT3B*). The knock-in segment defining *C3QTL3* is large, and contains 20 human GWAS genes located on human CHR 20 (Supplementary Table 1). Among them, 5 rat orthologs for 5 GWAS genes carry synonymous mutations. 1 rat ortholog for *DNMT3B* carries a missense mutation changing Met80Val (Supplementary Tables 3&4). This function-altering potential made the DNMT3B protein a physiological candidate for *C3QTL3* conserved in basic mechanisms of BP physiology between rats and humans. The human *DNMT3B* coding region carries several missense mutations (Supplementary Table 4).

3.6.2. In contrast, the intronic human GWAS SNP, rs6141767, marked *DNMT3B*, is absent in the rats (Table 2), but is present in primates (Table 3). 'Knocking it out' in rodents has no impact on blood pressure (Figure 1C). Consequently, rs6141767 itself is a byproduct of primate evolution, and a human-centered marker for the functional *C3QTL3* nearby, not the QTL *per se*.

3.7. Closely-linked *C10QTL1* and *C10QTL5* of DSS rats, (22) may be a physiological human ortholog of *PPM1E* (*protein phosphatase, Mg2+/Mn2+ dependent 1E*) and a positional human ortholog of *VMP1* (*vacuole membrane protein 1*) [2].

3.7.1. Chr 10 of DSS rats carries several QTLs by changing BP [23]. Among them (Figure 1B), distinct *C10QTL1* and *C10QTL5* have become relevant to humans because of the following.

In the *C10QTL1*-residing region of 600 kb, 3 closely-linked genes within 329 kb are *Ppm1e*, *Rad51c* (*Rad51 homolog c*) and *Tex14* (*testis expressed 14*) as a genome block. Among multiple intronic GWAS SNPs, no conservation was found in the rat (Table 2). These SNPs are by products of primate evolution (Table 3). They alone or collectively do not change BP by *C10QTL1 in vivo* (Figure 1C), since humans and rodents achieve similar BP with or without them [10].

3.7.2. The level of *C10QTL1* expression does not have a physiological impact in changing BP, since 1 copy of the normotensive *C10QTL1* allele lowered BP to the same extent as 2 copies *in vivo* [24]. Among the 3 genes, *Ppm1e* possesses a missense mutation and appeared to be the principal function candidate for *C10QTL1* [22]. No other structural variants [25] were found in the remaining 2 genes. It is unclear if each of *PPM1E*, *RAD51C* and *TEX14* would represent a single QTL or they constitute a genome block with 1 QTL residing in it. Human data bases showed 8 probable *PPM1E* missense mutations (Supplementary Table 5).

3.7.3. Unlike *C10QTL1*/ *PPM1E*, *Vmp1* does not carry structural mutations (Supplementary Table 6), yet an intronic SNP, rs2645466, in *VMP1* was found to be associated with BP. Rs2645466 is a by-product of primate evolution (Table 3), is not conserved in the rat

(Table 2) and thus does not impact on the functionality of *C10QTL5*/*VMP1* on BP.

3.7.4. The functional candidate for *C10QTL5* from the DSS rat, proline rich 11 (*Prr11*) bears 2 missense mutations [22]. No GWAS signal appeared near the human *PRR11* (Figure 1C), which is about 400kb away from *VMP1*. Thus, it is likely that 2 separate QTLs may exist in the *C10QTL5*-residing interval.

3.7.5. In contrast to the functional correspondence of the 4 human GWAS genes to *C10QTL1* and *C10QTL5* (Figure 1B), there was no BP effect by knocking in the rat ortholog of a human GWAS gene, *ACE*, in C10S.L8. Thus, the relevance of *ACE* as a human GWAS gene in BP regulation needs to be tested in animal models other than DSS.

4. Discussion

Principal findings from this study are (**a**) *in vivo* studies and human GWAS have revealed shared mechanisms of BP control as a physiological framework originating from common ancestors of humans and rodents. The M3R signaling pathway is one of them. (**b**) Specifically, 6 distinct QTLs from inbred DSS rats have unraveled mechanistic causes in BP regulation for at least 6 new human GWAS genes. Each of them has a major physiological impact on BP, and they may collectively function in 2 pathways. (**c**) Previously-unsuspected components of these pathways have been implicated from the candidate genes for the QTLs. (**d**) The non-coding SNPs marking these 6 QTLs/human GWAS genes are offshoots of primate evolution irrelevant to BP regulation. These SNPs are human-centered and mark potential QTLs nearby, rather than being QTLs per se.

4.1.1. QTL Modularity/pathway is the physiological framework of QTLs regulating blood pressure invented in mammalian ancestors: QTL Modularity is the genetic framework in physiologically modulating BP embedded in their ancestral genomes [9]. The 6 QTLs and their corresponding human GWAS genes may function via only 2 modules in physiologically controlling BP and implicates 2 pathways of hypertension pathogeneses. One of them is the M3R signaling pathway [5,6]. This conservation of BP-regulating pathways such as the M3R signaling supports similarity in blood pressures between differing orders of mammals [10]. As a result, the fundamental framework of BP-controlling mechanisms in pathways with multiple steps must have been established in common ancestors of mammals before they started to diverge [9].

4.1.2. Humans and rodents along with most land placental and marsupial mammals diverged at various times during the past 160 million years (www.timetree.org), yet they all have similar blood pressures [10]. Environments have changed during the evolution of these mammals, and present-day humans and rodents live in very different surroundings. Convergent evolution from ancestors via different mechanisms cannot produce similar blood pressures by accident for all these orders of mammals. The only course for this to take is that key mechanisms regulating BP must have been established in common ancestors of mammals, before they evolutionarily diverged.

4.1.3. When we probe mechanisms regulating the current physiology in humans, we actually dig into the mechanistic past before humans existed. This means that, similar to those in rodents, physiological mechanisms controlling human blood pressure have already been given by ancestral QTLs in polygenic forms to virtually 100%. Nevertheless, this conservation

does not exclude a later emergence of new BP-modulating mechanisms during mammalian evolution. For example, elephants and giraffes have blood pressures different from humans and rodents [10], despite sharing ancestral genomes. Humans could have evolved new mechanisms regulating BP, but all these possible developments combined and unique to humans seems to have contributed to close to 0% in the total human physiology regulating blood pressure.

4.1.4. In this context, non-coding SNPs used in GWAS are unique to humans, as a result of a coincidence in primate evolution unrelated to blood pressure control. Such a human-centered SNP fortuitously marks a physiological QTL nearby, similar to a rodent polymorphism [26] marking *C17QTL1*/*Chrm3* next to it [5]. The paradox of a 'common' SNP/marker with no effect on BP identifying a 'rare' BP-impacting variant nearby has been previously addressed in Discussion in reference [11], and will not be reiterated here.

4.1.5. More than 10,000 human SNPs are found to be associated with over 900 genes [2]. Apparently, not all of these SNPs can be functional variants, in spite of their comparably-strong statistical significance in GWAS. Thus, genetic architectures from GWAS are no equivalent to physiological functions. Although <10% of these human-centric non-coding GWAS SNPs might potentially have functions in modulating cellular gene expressions, epigenetics and/or even be eQTLs [2], they contribute little to the primary mammalian physiology of BP controls including for humans.

4.2.1. Uncoupling between systemic BP and cell/tissue activities necessitates an *in vivo* physiological proof of a BP QTL: Even though a QTL is a genetic term covering a chromosome region marked by a GWAS SNP, a QTL is molecularly one gene, physiologically capable of altering BP [5,11]. Statistics is inadequate to establish this fact. Indirectly, GWAS results lead to certain studies that analyze *in vitro* functions of human non-coding SNPs in cells implicating in blood pressure control. However, a separation between systemic BP and cell activities casts doubt on this *in vitro* approach.

4.2.2. Depleting M3R diminishes vaso-relaxation that is supposed to increase BP, but contrarily decreases blood pressure [5,7]. Thus, viewing functions of these cell/tissue structures *in vitro* and in isolation cannot predict the actual physiological BP *in vivo* for a QTL. M3R is mostly produced in the brain, less in adrenals and not detectable in heart, kidneys [5,7]. We are still puzzled [6] as to how M3R promotes hypertension, and from the brain and adrenals, modulates concordant cardiac/renal functions, but discordant vaso-relaxation in vasculature. Partially inferring BP mechanisms from cells could be misleading. The systemic BP physiology *in vivo* is not random and detached cellular events, but integrated and offsetting interactions among various organs. No *in vitro* substitutes can replace the *in vivo* physiology in authenticating a QTL for blood pressure.

4.3.1 Genetic architectures of human GWAS designate probable locations of QTLs for functional proxies to follow *in vivo*. Results presented in the current manuscript are based on physiological studies *in vivo* from inbred rodent strains, and seem perplexing and unsettling from the perspective of the quantitative genetics principle predicating on GWAS from outbred populations [3]. However, exhibiting disparate genetic architectures between inbreds and outbreds does not imply their mechanistic differences in modulating their blood pressure physiology.

4.3.2. In outbred human populations, persons with high and low blood pressures exist, but their hypertensive and normotensive phenotypes do not become distinguishable and heritable traits until singled out and fixed as strains as by inbreeding. Inbreeding in rodents captures, but does not change, the machinery controlling BP physiological mechanisms for an individual from an outbred population. In this way, inbreedings have achieved in building hypertensive and normotensive strains the same way as Mendel was naturally given in contrasting sizes in peas from outbred populations [27]. For example, Mendel picked peas from outbred populations with explicit size differences that are heritable and contrasting. His choices were purely due to their clear and unambiguous distinctions to facilitate his phenotypings. In hindsight, if he would have analyzed continuous and ambiguous phenotypes in outbred populations, he might not have gained insights into fundamental laws of heredity.

4.3.3. By exploring inbred rat strains with heritable, contrasting and distinguishable BP features, we have unraveled mechanisms and the physiology of BP regulations from QTLs in an individual [9]. An outbred population of 100 is equivalent to 100 different inbreds in mechanisms of BP regulations. Studying all 100 in a mixture of continuous and equivocal variations is not mechanistically informative. Further in GWAS, a probable blood pressure effect from a single SNP is fractionated by total variance in phenotypic variations according to Fisher [3], not its actual physiological effect on blood pressure in mmHg.

4.4.1. Insights into mechanistic and physiological causes of blood pressure regulation from QTL modularity: The genetic modularity of QTLs [14,19] has broadened the scope of Mendelism to cover polygenic traits, and revealed causes and a mechanistic frame work of BP physiology [9]. Polygenicity of blood pressure is composed of individual Mendelian 'monogenic' components that are organized into modules. Mendelism is the fundamental basis for BP as a polygenic trait, and is in principle, equivalent to carbon being the basic chemical element in forming poly-carbon graphite and diamonds.

4.4.2. Recently, an 'omnigenic' hypothesis has been proposed to explain GWAS results on generic quantitative phenotypes [3]. It can be described as an anthropocentric (or human-centered) theory, because non-coding GWAS SNPs only exist in humans, and not in rodents, as our previous [11] and current findings have shown. It basically proposes that regulations at gene expressions at cellular level would determine the GWAS SNPs' roles in human phenotypes including BP. This is contrary to the modularity idea and physiological proofs underlying it [9].

4.4.3. The basic distinction between the two is that modularity is predicated on the physiological causes of pathogenic mechanisms of hypertension versus statistical epidemiology that focuses on after effects of these mechanisms and physiology. This is because mechanisms and physiology determining a polygenic trait are the prime mover and starting point driving the formation of the modularity paradigm. In contrast, mechanisms and physiology are only an afterthought that the omnigenic model injects.

4.4.4. These 2 opposing hypotheses generate contrasting predictions that can be experimentally tested for their physiological relevance to BP. On the basis of functional physiology in BP regulation, modularity has been validated, but omnigenicity has not, because of the following functional results.

4.4.5. Central to the 'omnigenic' hypothesis is the cellular gene dose, and by inference, the level of gene expressions that would lead to a phenotype. Contrary to this prediction, several lines of experimental evidence have proven that a gene dose is irrelevant to the physiological outcome on BP control. For example, blood pressures are the same between *Chrm3*$^{+/-}$ with one functional copy of *Chrm3* and *Chrm3*$^{+/+}$ with 2 copies [5,7]. Most QTLs function in similar gene-dose independence in the physiology of BP control [24], in that one dose of a normotensive QTL allele has the same BP impact as 2 doses. There was no evidence that the level of *Chrm3* expression was different in the organs tested when blood pressure changed [5].

4.4.6. The 'miniscule' effect from a single QTL fractionated from total variance is another prediction from the 'omnigenic' hypothesis. If this prediction were physiologically pertinent, removing one such QTL should have an inconsequential effect on BP. This is not the case, as introduced in Result (3.3) from gene targeting and congenic knock in experiments.

4.4.7. The modularity paradigm can, whereas the 'omnigenic' hypothesis cannot, explain the evolutionary conservation in pathways controlling BP rooted in common ancestors of humans and rodents. The anthropocentric non-coding GWAS SNPs that gave rise to 'omnigenicity' cannot affect these pathways, because they only began to appear in primates, but do not exist in rodents (Tables 2 and 3). Conversely, rodents' non-coding *Chrm3* SNPs are not conserved in humans. Inactivating the M3R signaling pathway did not touch any of them, yet BP changed [5]. Thus, the functional M3R signaling coexists in humans and rodents, and should be shared in determining the hypertension pathogenesis, to which the non-coding rodent *Chrm3* SNPs and the human-centered GWAS SNPs are irrelevant.

4.4.8. Certain QTLs starts to function at embryogenesis [5], before the onset of adult BP physiology. Modularity can [9], whereas the 'omnigenicity' cannot, explain that a pathway involved in BP control can temporally begin at embryogenesis and continue into adulthood [5].

4.4.9. In conclusion, modularity is supported by reproducible lines of physiological evidence as a signaling pathway, and is mechanistic in our understandings of causes for BP physiology [9]. The intuitive 'omnigenic' hypothesis [3] has little functional support for a physiological role on BP, despite explaining epidemiological exhibitions from human GWAS. It's analogous to Newtonian mechanics describing effects of gravity as a force pulling on objects. When it comes to the cause of gravity, we have to switch our mindset to Einstein's theory of a curvature in space time for explanation (https://www.britannica.com/science/general-relativity).

4.5.1. Pathogenic pathways of hypertension inferred from the molecular bases of QTLs: Since the 3 following QTLs (Figure 1, Table 1) have not been molecularly identified like *C17QTL1*/*Chrm3*, their roles in BP physiology are tentatively inferred from the functional candidate genes representing them.

4.5.2. In epistatic module 2/M3R signaling pathway [5,7], *RREB1* is ras responsive element binding protein 1, a transcription factor involved various molecular processes and implicated in certain diseases [28]. Its probable mechanistic step in the M3R signaling pathway remains to be investigated.

4.5.3. In epistatic module 1/pathway 1, 2 new components are suggested, i.e. *DNMT3B* and *PPM1E*

(Table 1). Their functions begin during embryogenesis [29]. *DNMT3B* encodes the DNA methyltransferase 3B whose defects cause human Immunodeficiency, Centromeric instability and Facial anomalies (ICF) (30). The DNMT3B enzyme methylates distinct CpG islands *de novo* in embryonic stem cells [29]. 2 *Dnmt3b* missense mutations, at A609T and D823G amino acids, seem to hypo-methylate repetitive DNA sequences and resemble the human ICF syndrome in certain phenotypes [31].

4.5.4. PPM1E is a protein phosphatase, Mg2+/Mn2+ dependent 1E [32] principally expressed in the brain [33], and belongs to a family of serine/threonine-protein phosphatases. Very little is known of its function *in vivo*, since no knock out exists presumably due to its requirement during development.

4.6.1. Caveats and limitations are: First, although human GWAS genes with missense mutations do not genetically prove by themselves to be the QTLs in questions, they provide entry points towards probable steps in 2 pathways contributed by each QTL. Molecular designs with viable gene-targeting of their codons [5] can test their functions on blood pressure in rodents.

4.6.2. Second, structural mutations are not necessarily the only molecular bases that can affect a pathway in question. *C10QTL5*/*VMP1* has no missense mutation in DSS rats. Despite of it, it can still be a BP QTL, because certain steps in epistatic module 1/pathway 1 may depend on a multi-component complex. One component can affect the function of the entire complex by altering its stoichiometry of the composition. As a result, the pathway as a whole is affected.

4.6.3. Finally, unlike the small segment harboring *C17QTL1*/*Chrm3*, the regions containing the remaining 5 QTLs are quite large (Figure 1). The number of QTLs in each of the 5 regions is under reported. At least 3 QTLs exist in the *C17QTL2*-residing interval. 2 QTLs at minimum lie in the segment bearing *C3QTL3* [34,35]. A minimum of 3 QTLs may be present in the *C3QTL1*-lodging region, because human GWAS genes exist on 3 separate CHROMOSOMES (Supplementary Table 1).

5. Conclusions

Studies of human epidemiology and animal models in polygenic hypertension have been accidentally divided into 2 practices and doctrines that conveniently and separately govern each. However, this artificial partition is not based on the physiology and mechanisms, and cannot hide an inconvenient truth that both mammalian orders share fundamental mechanisms in BP controls at least 90 million years in the making. The reproducible experimental evidence has reinforced the verdict that shared BP QTLs are causes of these mechanisms. QTL modularity mechanistically joins humans and rodents, suggests that multiple QTLs may function in a common pathway, and each is involved in a different step in the pathogenic pathway towards polygenic hypertension. This emerging paradigm encompasses not only humans, but also most other land mammals, and is a departure from the human-centric precept [3] which is reminiscent of geocentrism distorting heliocentricy of our solar system in cosmology.

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Conflict of interest

None.

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Supplementary Table 1: Rat QTLs and genes residing in the intervals harboring them that correspond to human GWAS genes trapped by congenic knock in genetics.

Rat QTL	Rat Gene	Rat Chr span	Human GWAS gene (SNPs, positions)	Human CHR
C10QTL1	*Tex14*	75252796..75376356	*TEX14*	17
	Rad51c	75376876..75403188	*RAD51C*	17
	Ppm1e	75421043..75552393	***PPM1E*** (intronic SNP rs304295) (intron1 SNP rs304298) (intron1 SNP rs1294296)	17
C10QTL5	***Vmp1***	76098589..76197394	***VMP1*** (intronic SNP rs264566)	17
C3QTL1	*Rpl24p7*	23175810..23176358	*RPL24P7*	3
	Nek6	28037221..28114175	*NEK6*	9
	Zeb2	35058725..35186767	*ZEB2*	2
	Acvr2a	38113728..38197531	*ACVR2A*	2
	Orc4	38201914..38240600	*ORC4*	2
	Mbd5	38600038..38663451	*MBD5*	2
	Cacnb4	43050530..43312292	*CACNB4*	2
	Stam2	43329060..43378948	*STAM2*	2
	Prpf40a	43778141..43839268	*PRPF40A*	2
	Arl6ip6	43839462..43867404	*ARL6IP6*	2
	Acvr1c	49392012..49462621	*ACVR1C*	2
C3QTL3	*Plcb1*	134211557..134908321	*PLCB1*	20
	Slx4ip	136489186..136545079	*SLX4IP*	20
	Jag1	136558688..136594108	*JAG1*	20
	Btbd3	137786843..137834225	*BTBD3*	20
	Snx5	144812043..144831955	*SNX5*	20
	Slc24a3	145760074..146259491	*SLC24A3*	20
	Id1	154619728..154621190	*HM13-AS1-ID1*	20
	Dnmt3b	155510828..155591435	***DNMT3B*** (intronic SNP rs6141767)	20
	Cdk5rap1	156207918..156241596	*CDK5RAP1*	20
	Cbfa2t2	156308253..156414187	*CBFA2T2*	20
	Pxmp4	156464180..156481427	*PXMP4*	20
	Itch	157055848..157117955	*ITCH*	20
	Dynlrb1	157129655..157150710	*DYNLRB1*	20
	Procr	157650408..157654716	*PROCR* (mis-sense SNP rs867186)	20
	Chd6-Ptprt	163164938..163324764	*CHD6-PTPRT*	20
	Jph2	165905606..165968938	*JPH2*	20
	Prex1	169494331..169575981	*PREX1*	20
	Kcnb1	170010756..170094507	*KCNB1*	20
	Nfatc2	171380993..171514802	*NFATC2*	20
C17QTL1	***Chrm3***		***CHRM3*** (intergenic rs2820037)	1

C17QTL2	*Phactr1*	25209368..25713282	*PHACTR1*	6
	Bmp6	28870345..29029564	*BMP6*	6
	Rreb1	29453342..29632680	***RREB1*** [intronic SNPs rs4960295 and rs2151942, missense mutation rs1334576 (Gly195Arg)]	6
	Gmds	35298853..35824292	*GMDS*	6
	Foxc1	35833707..35837690	*FOXC1*	6
	Hfe	45515737..45523531	*HFE*	6
	Sugct	48313139..49097298	*SUGCT*	7
	Arhgap12	51975184..52089094	*ARHGAP12*	10
	Bambi	54939068..54943633	*BAMBI*	10
	Epc1	55332012..55423030	*EPC1*	10

Footnote for table: Fig. 1 of the text defines the chromosome regions containing QTLs and the gene residing in the intervals containing these QTLs. Human GWAS genes are all from (*Nat Genet* 2018;50:1412), except for *CHRM3* marked by rs2820037, which was taken from (*Nature* 2007;447:661). *C17QTL1*/*Chrm3* is adapted from (*Hypertension* 2018; 72:755). Only the genes underlined were analyzed further in the current work, because they are functional candidate genes for the human QTLs. ***CHRM3***, muscarinic cholinergic receptor 3; ***DNMT3B***, DNA methyltransferase 3 beta; ***PPM1E***, protein Phosphatase, Mg2+/Mn2+ Dependent 1E; ***RREB1***, ras responsive element binding protein 1; ***VMP1***, **Vacuole membrane protein 1**. CHR, Chromosome.

Supplementary Table 2. Amino acid alignment for muscarinic cholinergic receptor 3 (M3R) among Tasmania devils (marsupial mammals), humans and rats (both placental mammals).

```
Tasmania_M3R      MTLHSNNTTSSSFVNISTSWTQGSSGPGLPP----HYGSYNVSQASEAFSN-NATNNDPL       55
Hs_M3R            MTLHNNSTTSPLFPNISSSWIHSPSDAGLPPGTVTHFGSYNVSRAAGNFSSPDGTTDDPL       60
DSS_Rat_M3R       MTLHSNSTTSPLFPNISSSWVHSPSEAGLPLGTVTQLGSYNISQETGNFSSND-TSSDPL       59
                  ****.*.***  * ***:** :. *  ***     : ****:*: :  **. : *..***

Tasmania_M3R      GGHTVWQVVLIALLTGILALVTIIGNILVIVAFKVNKQLKTVNNYFLLSLACADLIIGVI       115
Hs_M3R            GGHTVWQVVFIAFLTGILALVTIIGNILVIVSFKVNKQLKTVNNYFLLSLACADLIIGVI       120
DSS_Rat_M3R       GGHTIWQVVFIAFLTGFLALVTIIGNILVIVAFKVNKQLKTVNNYFLLSLACADLIIGVI       119
                  ****:****:**:***:**************:****************************

Tasmania_M3R      SMNLFTTYIIMNRWALGNLACDLWLSIDYVASNASVMNLLVISFDRYFSITRPLTYRAKR       175
Hs_M3R            SMNLFTTYIIMNRWALGNLACDLWLAIDYVASNASVMNLLVISFDRYFSITRPLTYRAKR       180
DSS_Rat_M3R       SMNLFTTYIIMNRWALGNLACDLWLSIDYVASNASVMNLLVISFDRYFSITRPLTYRAKR       179
                  *************************:***********************************

Tasmania_M3R      TTKRAGMMIGLAWIISFILWAPAILFWQYFVGERTVPPGECFIQFLTEPTITFGTAIAAF       235
Hs_M3R            TTKRAGVMIGLAWVISFVLWAPAILFWQYFVGKRTVPPGECFIQFLSEPTITFGTAIAAF       240
DSS_Rat_M3R       TTKRAGVMIGLAWVISFVLWAPAILFWQYFVGKRTVPPGECFIQFLSEPTITFGTAIAAF       239
                  ******:******:***:**************:*************:*************

Tasmania_M3R      YMPVTIMTILYWRIYKETEKRTKELAGLQASGSEAEAAHFVNPTGSSRSCSSYELQQQTL       295
Hs_M3R            YMPVTIMTILYWRIYKETEKRTKELAGLQASGTEAETENFVHPTGSSRSCSSYELQQQSM       300
DSS_Rat_M3R       YMPVTIMTILYWRIYKETEKRTKELAGLQASGTEAEAENFVHPTGSSRSCSSYELQQQGV       299
                  *********************************:***: :**:**************** :

Tasmania_M3R      KRSSRRKYGRCHFWFTTKSWKPSAEQMDQEHSSSDSWNNNDAAASLENSASSDEEDIGSE       355
Hs_M3R            KRSNRRKYGRCHFWFTTKSWKPSSEQMDQDHSSSDSWNNNDAAASLENSASSDEEDIGSE       360
DSS_Rat_M3R       KRSSRRKYGRCHFWFTTKSWKPSAEQMDQDHSSSDSWNNNDAAASLENSASSDEEDIGSE       359
                  ***.*******************:*****:******************************

Tasmania_M3R      TRAIYSIVLKLPGHSTILNSTKLPSSEDLQGSEEELQKADLEKKTNKLQAQKSMEDGGSF       415
Hs_M3R            TRAIYSIVLKLPGHSTILNSTKLPSSDNLQVPEEELGMVDLERKADKLQAQKSVDDGGSF       420
DSS_Rat_M3R       TRAIYSIVLKLPGHSSILNSTKLPSSDNLQVSNEDLGTVDVERNAHKLQAQKSMGDGDNC       419
                  ***************:**********::**  :*:*  .*:*:::.*******: **..

Tasmania_M3R      RKSFAKLPIQLESTVEAAKTPEAISSVAKTTAALPLSFKEATLAKRFALKTRSQITKRKR       475
Hs_M3R            PKSFSKLPIQLESAVDTAKTSDVNSSVGKSTATLPLSFKEATLAKRFALKTRSQITKRKR       480
DSS_Rat_M3R       QKDFTKLPIQLESAVDTGKTSDTNSSADKTTATLPLSFKEATLAKRFALKTRSQITKRKR       479
                   *.*:********:*:::.** :. **. *:**:****************************

Tasmania_M3R      MSLIKEKKAAQTLSAILLAFIITWTPYNIMVLVNTFCKSCIPKTYWNLGYWLCYINSTVN       535
Hs_M3R            MSLVKEKKAAQTLSAILLAFIITWTPYNIMVLVNTFCDSCIPKTFWNLGYWLCYINSTVN       540
DSS_Rat_M3R       MSLIKEKKAAQTLSAILLAFIITWTPYNIMVLVNTFCDSCIPKTYWNLGYWLCYINSTVN       539
                  ***:*********************************.******:***************

Tasmania_M3R      PMCYALCNKTFRTTFKMLLLCQCDKRKRRKQQYQQRQSVIFHKRVPQEAS         585
Hs_M3R            PVCYALCNKTFRTTFKMLLLCQCDKKKRRKQQYQQRQSVIFHKRAPEQAL         590
DSS_Rat_M3R       PVCYALCNKTFRTTFKMLLLCQCDKRKRRKQQYQQRQSVIFHKRVPEQAL         589
                  *:***********************:******************.*::*:
```

Footnote for table: * denotes an overall amino acid identity (92.3%) between humans and rats, (90.8%) between humans and Tasmania Devils and (90.3%) between rats and Tasmania Devils. Underlined codons indicate the domain involved in M3R signaling. The single missense mutation in DSS rat is shaded at ***M*** and alters the M3R signaling, and is associated with BP changes. Probable human M3R missense mutations are marked and curated from https://www.ncbi.nlm.nih.gov/snp/?term=chrm3+missense. Only those human mutations with minor alleles that were observed at least 2 times in the tested populations are given.

Supplementary Information:

Supplementary Table 3: Mutation survey of genes in rat QTL-residing intervals that capture human GWAS genes by congenic knock in genetics

Rat QTL name	Rat/human GWAS Gene	Mutation detected Lew/ DSS	Change in amino acid (AA) Lew/DSS	RGD (AA) Lew/DSS	Human GWAS SNP
C3QTL1	*Acvr2a*	T720G	No	No	
	Acvr1c	5'UTR	No	No	
C3QTL3	*Plcb1*	5'UTR	No	No	
	DNMT3B	**A238G**	**Met80Val**	**Mel80Val**	rs6141767 (intronic)
	Cbfa2t2	3'UTR	No	No	
	Pxmp4	C290T	No	No	
	Procr	CGCdel20 (un confirmed)	SerDel20 (un-confirmed)	No	
	Jph2	C1851T	No	No	
C10QTL1	*Tex14*	C141G G2592A T3423C C4215T	NO NO NO No		
	Rad51c	No			
	PPM1E	**C335G** Confirmed by sequencing	**Pro112Arg** Confirmed by sequencing	No	Multiple intronic SNPs
C10QTL5	*Vmp1*	G1101A	No	No	Intronic rs264566
C17QTL2	***RREB1***	G1155A **G1168A** C1290A T1991C C2052T C2277T **A2725G** A3594G	No **Ala390Thr** No Phe664Ser (unconfirmed) No No **Ile909Val** No	No **Ala390Thr** No No No No **Ile909Val** No	rs4960295 (intronic) rs2151942 (intronic) rs1334576 (missense Gly195Arg)
	Foxc1	5'UTR	No	No	
	Arhgap12	T711G	No	R91K (un-confirmed)	rs867186 (missense)

Footnote to Table: Gene locations are indicated on the map in Fig. 1. The position of a mutation enumerates from the ATG start codon of that gene. The amino acid position begins from the first methionine. Confirmed mutations are indicated by bold letters. ***DNMT3B***, DNA methyltransferase 3 beta; ***PPM1E***, *protein phosphatase, Mg2+/Mn2+ dependent 1E*; ***RREB1***, ras responsive element binding protein 1; No Copy Number Variation (CNV) had been found for those genes from total genome sequencing of DSS and Lewis rats based on our current work and those of the rat genome database (RGD).

Supplementary Table 4: Amino acid alignment and missense mutations in codons DNA for methyltransferase 3 beta (DNMT3B) in humans and rats

```
Human_DNMT3B        MKGDTRHLNGEEDAGGREDSILVNGACSDQSSD-----SPPILEA-----IRTPEIRGRR      50
DSS_Rat_Dnmt3b      MKGDSRHLNEEEGASGYEDCIIVNGNCSDQSSDTKDAPSPPVLEAICTEPVCTPETRGRR      60
                    ****:**** **.*.* **.*:*** *******     ***:***     : *** ****

Human_DNMT3B        SSSRLSKREVSSLLSYTQDLTGDGDGE--DGDGSDTPVMPKLFRET---RTRSESPAVRT      105
DSS_Rat_Dnmt3b      SSSRLSKREVSSLLNYTQDVVGDGDGEADDGDGSDILMMPKLTRETKDARTPSESPAVRT      120
                    **************.****:.******  ******  :**** ***   ** ********

Human_DNMT3B        RNNNSVSSRERHRPSPRSTRGRQGRNHVDESPVEFPATRSLRRRATASAGTPWPSPPSSY      165
DSS_Rat_Dnmt3b      RNSNSISSLERQRTSPRITRGRQGRYHVQEYPVEFPATKSRRRRASSSASTPWSSPASIE      180
                    **.**:** **:* *** ******* **:* *******:* ****::**.*** ** *

Human_DNMT3B        LTIDLTDDTEDTHGTPQSSSTPYARLAQDSQQGGMESPQVEADSGDGDSSEYQDGKEFGI      225
DSS_Rat_Dnmt3b      LMEDV---------TPKSSSTPSVDLSQDGPQEGMDATQVDAESRDGDSTEYQDDKEFGI      231
                    *  *:         **:***** . *:**. * **:: **:*:* ****:****.*****

Human_DNMT3B        GDLVWGKIKGFSWWPAMVVSWKATSKRQAMSGMRWVQWFGDGKFSEVSADKLVALGLFSQ      285
DSS_Rat_Dnmt3b      GDLVWGKIKGFSWWPAMVVSWKATSKRQAMPGMRWVQWFGDGKFSEIAADKLVALGLFSQ      291
                    ****************************** ***************::*************

Human_DNMT3B        HFNLATFNKLVSYRKAMYHALEKARVRAGKTFPSSPGDSLEDQLKPMLEWAHGGFKPTGI      345
DSS_Rat_Dnmt3b      HFNLATFNKLVSYRKAMYHTLEKAMVRAGKTFPSRPGDSLEDQLKPMLEWAHGGFKPTGI      351
                    ********************:**** ********* ************************

Human_DNMT3B        EGLKPNNTQPVVNKSKVRRAGSRKLESRKYENKTRRRTADDSATSDYCPAPKRLKTNCYN      405
DSS_Rat_Dnmt3b      EGLKPNNKQPEVHKSKVRRSGSRNLEARRRENKSRRRTTIDFAASEYSTPPKRLKTNSYG      411
                    *******.** *:******:***:**:*: ***:****: * *:*:*.  *******.*.

Human_DNMT3B        NGKDRGDEDQSREQMASDVANNKSSLEDGCLSCGRKNPVSFHPLFEGGLCQTCRDRFLEL      465
DSS_Rat_Dnmt3b      -GKDRGEDEESRERMASDVTNNKGNLEDRCLSCGKKNPVSFHPLFEGGLCQSCRDRFLEL      470
                     *****:::::***:*****:***..*** *****:****************:********

Human_DNMT3B        FYMYDDDGYQSYCTVCCEGRELLLCSNTSCCRCFCVECLEVLVGTGTAAEAKLQEPWSCY      525
DSS_Rat_Dnmt3b      FYMYDEDGYQSYCTVCCEGRELLLCSNTSCCRCFCVECLEVLVGTGTAEDAKLQEPWSCY      530
                    *****:****************************************** :**********

Human_DNMT3B        MCLPQRCHGVLRRRKDWNVRLQAFFTSDTGL-EYEAPKLYPAIPAARRRPIRVLSLFDGI      584
DSS_Rat_Dnmt3b      MCLPQRCHGVLRRRKDWNMRLQDFFTTDPDLEEFEPPKLYPAIPAAKRRPIRVLSLFDGI      590
                    ******************:*** ***:* .* *:* **********:**************

Human_DNMT3B        ATGYLVLKELGIKVGKYVASEVCEESIAVGTVKHEGNIKYVNDVRNITKKNIEEWGPFDL      644
DSS_Rat_Dnmt3b      ATGYLVLKDLGIKVEKYVASEVCAESIAVGTIKHEGQIKYVNDVRKITKKNIEEWGPFDL      650
                    ********:***** ******** *******:****:********:**************

Human_DNMT3B        VIGGSPCNDLSNVNPARKGLYEGTGRLFFEFYHLLNYSRPKEGDDRPFFWMFENVVAMKV      704
DSS_Rat_Dnmt3b      VIGGSPCNDLSNVNPARKGLYEGTGRLFFEFYHLLNYTRPKEGDNRPFFWMFENVVAMKV      710
                    ************************************:******:***************

Human_DNMT3B        GDKRDISRFLECNPVMIDAIKVSAAHRARYFWGNLPGMNRPVIASKNDKLELQDCLEYNR      764
DSS_Rat_Dnmt3b      NDKKDISRFLACNPVMIDAIKVSAAHRARYFWGNLPGMNRPVIASKNDKLELQDCLEFSR      770
                    .**:****** ***********************************************:.*

Human_DNMT3B        IAKLKKVQTITTKSNSIKQGKNQLFPVVMNGKEDVLWCTELERIFGFPVHYTDVSNMGRG      824
DSS_Rat_Dnmt3b      TAKLKKVQTITTKSNSIRQGKNQLFPVVMNGKDDVLWCTELERIFGFPAHYTDVSNMGRG      830
                     ****************:**************:***************.***********

Human_DNMT3B        ARQKLLGRSWSVPVIRHLFAPLKDYFACE        853
DSS_Rat_Dnmt3b      ARQKLLGRSWSVPVIRHLFAPLKDYFACE        859
                    ****************************
```

Footnote for table: * indicates amino acid identity (92%) between human and the rat. Probable human missense mutations (The Genomes Project et al. 2015) are shaded, which were curated from https://www.ncbi.nlm.nih.gov/snp/ and as of April 23, 2020. Only those missense mutations with minor alleles that were observed at least 2 times (marked

in red) in the tested populations are included with the validation status by 1000Genomes. Amino acids in blue indicate that the minor allele has been observed more than 10 times. The rat missense mutation is shaded in green. DSS, Dahl salt-sensitive rats.

Supplementary Table 5: Amino acid alignment and missense mutations in codons for protein phosphatase, Mg2+/Mn2+ dependent 1E (PPM1E) in humans and rats

```
Human_PPM1E       MAGCIPEEKTYRRFLELFLGEFRGPCGGGEPEPEPEPEPEPESEPEPEPELVEAEAAE      60
DSS_Rat_Ppm1e     MAGCIPEEKTYRRFLELFLGEFRGPCGGGEPEPEPESEPEP------EPEAELVAAEAAE      54
                  ************************************ ****      *** *** *****

Human_PPM1E       ASVEEPGEEAATVAATEEGDQEQDPEPEEEAAVEG---EEEEEGAATAAAAPGHSAVPEP      117
DSS_Rat_Ppm1e     ASGEDPGEDAATVEAAEEGVQDQDPEPEEEAVEEEAAAEGEEEEEEEEAAAPGHSAVRPP      114
                  ** *:***:**** *:*** *:*********. *    * ***     ********* **

Human_PPM1E       PPQLPPLPPLPRPLSERITREEVEGESLDLCLQQLYKYNCPSFLAAALARATSDEVLQSD      177
DSS_Rat_Ppm1e     QPQLPPLPPLPRPLSERITREEVEGESLDLCLQQLYKYNCPSFLAAALARATSDEVLQSD      174
                   ************************************************************

Human_PPM1E       LSAHYIPKETDGTEGTVEIETVKLARSVFSKLHEICCSWVKDFPLRRRPQLYYETSIHAI      237
DSS_Rat_Ppm1e     LSAHCIPKETDGTEGTVEIETVKLARSVFSKLHEICCNWVKDFPLRRRPQIYYETSIHAI      234
                  **** *********************************.************:*********

Human_PPM1E       KNMRRKMEDKHVCIPDFNMLFNLEDQEEQAYFAVFDGHGGVDAAIYASIHLHVNLVRQEM      297
DSS_Rat_Ppm1e     KNMRRKMEDKHVCIPDFNMLFNLEDQEEQAYFAVFDGHGGVDAAIYASVHLHVNLVRQEM      294
                  ************************************************:************

Human_PPM1E       FPHDPAEALCRAFRVTDERFVQKAARESLRCGTTGVVTFIRGNMLHVAWVGDSQVMLVRK      357
DSS_Rat_Ppm1e     FPHDPAEALCRAFRVTDERFVQKAARESLRCGTTGVVTFIRGNMLHVAWVGDSQVMLVRK      354
                  ************************************************************

Human_PPM1E       GQAVELMKPHKPDREDEKQRIEALGGCVVWFGAWRVNGSLSVSRAIGDAEHKPYICGDAD      417
DSS_Rat_Ppm1e     GQAVELMKPHKPDREDEKQRIEALGGCVVWFGAWRVNGSLSVSRAIGDAEHKPYICGDAD      414
                  ************************************************************

Human_PPM1E       SASTVLDGTEDYLILACDGFYDTVNPDEAVKVVSDHLKENNGDSSMVAHKLVASARDAGS      477
DSS_Rat_Ppm1e     SASTVLDGTEDYLILACDGFYDTVNPDEAVKVVSDHLKENNGDSSMVAHKLVASARDAGS      474
                  ************************************************************

Human_PPM1E       SDNITVIVVFLRDMNKAVNVSEESDWTENSFQGGQEDGGDDKENHGECKRPWPQHQCSAP      537
DSS_Rat_Ppm1e     SDNITVIVVFLRDMNKAVNVSEESDWTENSFQGGQEDGGDDKENHGECKRPWPQHQCSAP      534
                  ************************************************************

Human_PPM1E       ADLGYDGRVDSFTDRTSLSPGSQINVLEDPGYLDLTQIEASKPHSAQFLLPVEMFGPGAP      597
DSS_Rat_Ppm1e     ADLGYEGRVDSFTDRTSLSPGPQINVLEDPDYLDLTQIETSKPHSTQFLPPVEMIGPGAP      594
                  *****:*************** ********.********:*****:*** ****:*****

Human_PPM1E       KKANLINELMMEKKSVQSSLPEWSGAGEFPTAFNLGSTGEQIYRMQSLSPVCSGLENEQF      657
DSS_Rat_Ppm1e     KKA-YVNELIMEESSVTPSQPERSGAGELLVSFNLGSTGQQICRMENLSPVYSGLENEQF      653
                  ***  :***:**:.**  * ** *****: .:*******:** **:.**** ********

Human_PPM1E       KSPGNRVSRLSHLRHHYSKKWHRFRFNPKFYSFLSAQEPSHKIGTSLSSLTGSGKRNR-I      716
DSS_Rat_Ppm1e     KSPGKRASRLYHLRHHYSKRQRGFRFNPKFYSFFSAQESSRKIGISLSSLTRSGKRNKML      713
                  ****:*.*** ********: : **********:**** *:*** ****** *****: :

Human_PPM1E       RSSLPWRQNSWKGYSENMRKLRKTHDIPCPDLPWSYKIE         755
DSS_Rat_Ppm1e     RSSLPWRENSWEGYSGNMA-IRKRNNISCPDLPWDYKI-         750
                  *******:***:*** **  :** ::* ******.***
```

Footnote for table: * indicates amino acid identity (92%) between human and the rat. Probable human missense mutations (The Genomes Project et al., 2015) are shaded, which were curated from https://www.ncbi.nlm.nih.gov/snp/?term=ppm1e+missense and as of Nov. 12, 2018. Only those missense mutations with

minor alleles that were observed at least 2 times (marked in red) in the tested populations are included with the validation status by 1000Genomes. Amino acids in blue indicate that the minor allele has been observed more than 10 times. The rat missense mutation is shaded in green. DSS, Dahl salt-sensitive rats.

Supplementary Table 6: Amino acid alignment and missense mutations in codons DNA for vacuole membrane protein 1 (VMP1) in humans and rats

```
Human_VMP1        MAENGKNCDQRRVAMNKEHHNGNFTDPSSVNEKKRREREERQNIVLWRQPLITLQYFSLE   60
DSS_Rat_Vmp1      MAENGTNCDQRRGAMSKEQHNGSFTDPSSVNEKKRRDREERQNIVLWRQPLITLQYFSLE   60
                  *****.****** **.**:***.**********:**********************

Human_VMP1        ILVILKEWTSKLWHRQSIVVSFLLLLAVLIATYYVEGVHQQYVQRIEKQFLLYAYWIGLG   120
DSS_Rat_Vmp1      TLVVLKEWTSKLWHRQSMVVSFFLLLAALVATYYVEGAHQQYVQRIEKQFLLYAYWIGLG   120
                   **:**************:****:****.*:*******.**********************

Human_VMP1        ILSSVGLGTGLHTFLLYLGPHIASVTLAAYECNSVNFPEPPYPDQIICPDEEGTEGTIFL   180
DSS_Rat_Vmp1      ILSSVGLGTGLHTFLLYLGPHIASVTLAAYECNSVNFPEPPYPDQIICPDEEGTEGAISL   180
                  ************************************************************:* *

Human_VMP1        WSIISKVRIEACMWGIGTAIGELPPYFMARAARLSGAEPDDEEYQEFEEMLEHAESAQDF   240
DSS_Rat_Vmp1      WSIISKVRIEACMWGIGTAIGELPPYFMARAARLSGAEPDDEEYQEFEEMLEHAETAQDF   240
                  ************************************************************:****

Human_VMP1        ASRAKLAVQKLVQKVGFFGILACASIPNPLFDLAGITCGHFLVPFWTFFGATLIGKAIIK   300
DSS_Rat_Vmp1      ASRAKLAVQKLVQKVGFFGILACASIPNPLFDLAGITCGHFLVPFWTFFGATLIGKAIIK   300
                  ************************************************************

Human_VMP1        MHIQKIFVIITFSKHIVEQMVAFIGAVPGIGPSLQKPFQEYLEAQRQKLHHKSEMGTPQG   360
DSS_Rat_Vmp1      MHIQKIFVIVTFSKHIVEQMVAFIGAVPGIGPSLQKPFQEYLEAQRQKLHHRSEAGTAQG   360
                  *********:****************************************:** ** **

Human_VMP1        ENWLSWMFEKLVVVMVCYFILSIINSMAQSYAKRIQQRLNSEEKTK   406
DSS_Rat_Vmp1      ENWLSWTFEKLVVAMVCYFILSIINSMAQSYAKRIQQRLNSEEKTK   406
                  ****** ******.*********************************
```

Footnote for table: * indicates amino acid identity (96%) between human and the rat. Probable human missense mutations (The Genomes Project et al. 2015) are shaded, which were curated from https://www.ncbi.nlm.nih.gov/snp/ and as of April 23, 2020. Only those missense mutations with minor alleles that were observed at least 2 times (marked in red) in the tested populations are included with the validation status by 1000Genomes. Amino acids in blue indicate that the minor allele has been observed more than 10 times. DSS, Dahl salt-sensitive rats.