Genome-wide identification of allelic expression in hypertensive rats

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SUPPLEMENTAL MATERIAL

Flow Chart and Hypothesis testing

Step 1.

Procedure: Determine gene expression in 1 WKY line and 3 SHR lines at 4 weeks of age.

Test hypothesis that genes can be identified that are differentially expressed in all SHR lines compared with WKY and that the directional difference of expression compared with WKY is the same in all SHR lines tested

Step 2.

Procedure: Repeat gene expression analysis in same animals, but at age 8wks, age 12wks and age 18wks.

Test same hypothesis as Step 1, but for each new age added/

Step 3.

Procedure: Examine expression data obtained in Steps 1 and 2.

Test hypothesis that a set of genes can be identified in which differential expression identified in Steps 1 and 2 is present at all ages tested for that gene.

Step 4.

Procedure: Amplify genomic DNA from genes identified in Step 3 that show differntial expression in all SHR lines compared to WKY and screen PCR amplified DNA from WKY and SHR by dHPLC to determine whether variation between WKY and SHR can be identified. When variation is identified, resequence the amplified DNA to identify the explicit sequence change obtained through screening. Genotype 2 distinct WKY lines and 4 distinct SHR lines to identify the alleles present in the differentially expressed genes.

Test the hypothesis that some of the genes that demonstrate persistently differential expression between WKY and SHR lines exist as allelic variants and to identify which genes possess allelic variants for which SHR lines have all fixed one variant while WKY lines have all fixed the alternative variant.

Step 5.

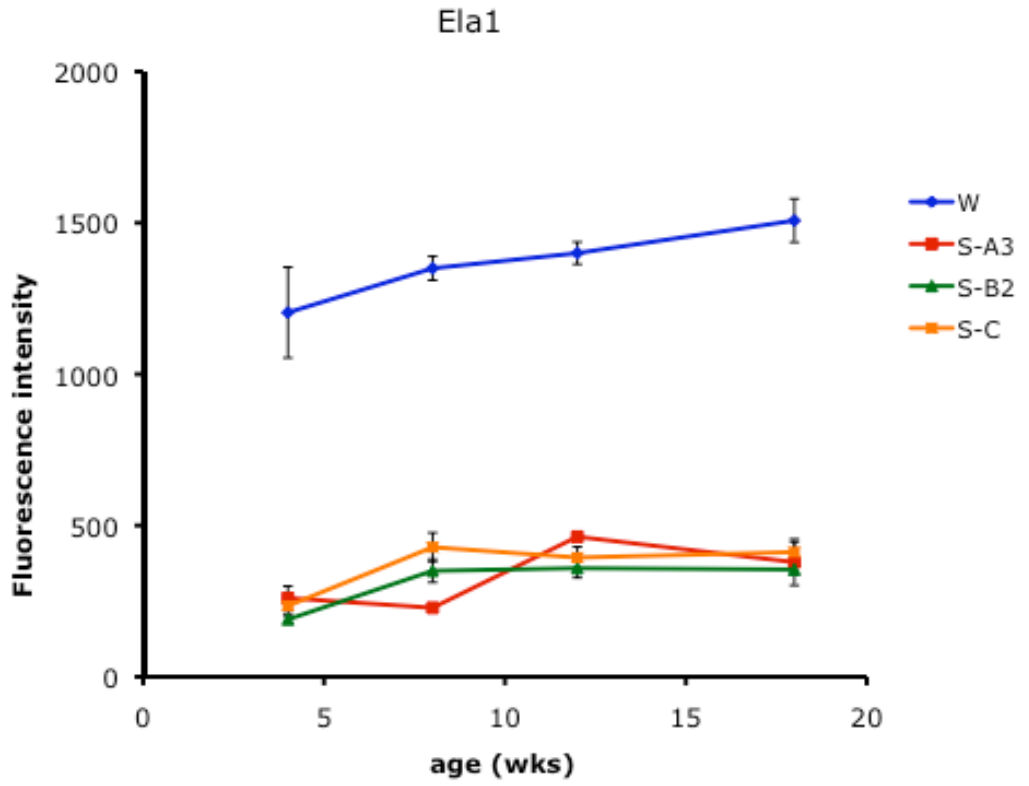
Procedure: Examine expression of differentially expressed genes in which all WKY and all SHR lines have fixed contrasting alleles in the F2 progeny of a WKY x SHR cross in which these alleles freely segregate.

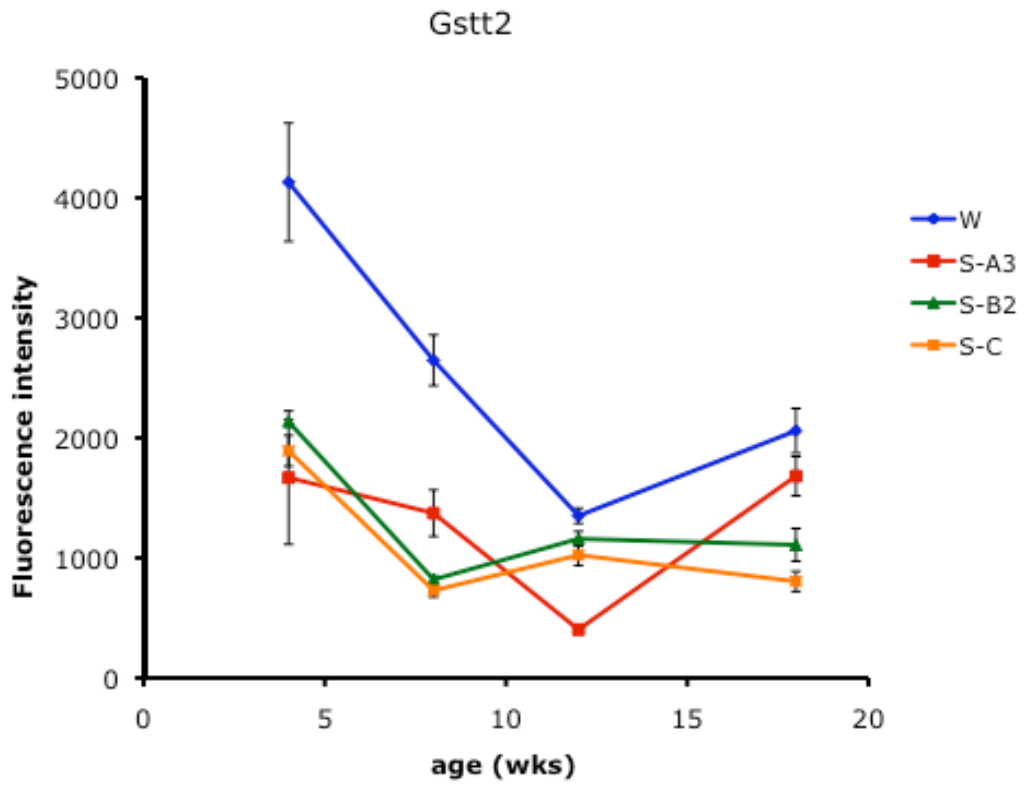
Test the hypothesis that among the differentially expressed genes in which all SHR and all WKY lines have fixed alternate alleles, that the expression of the gene in the F2 progeny will be correlated with the number of SHR alleles inherited.

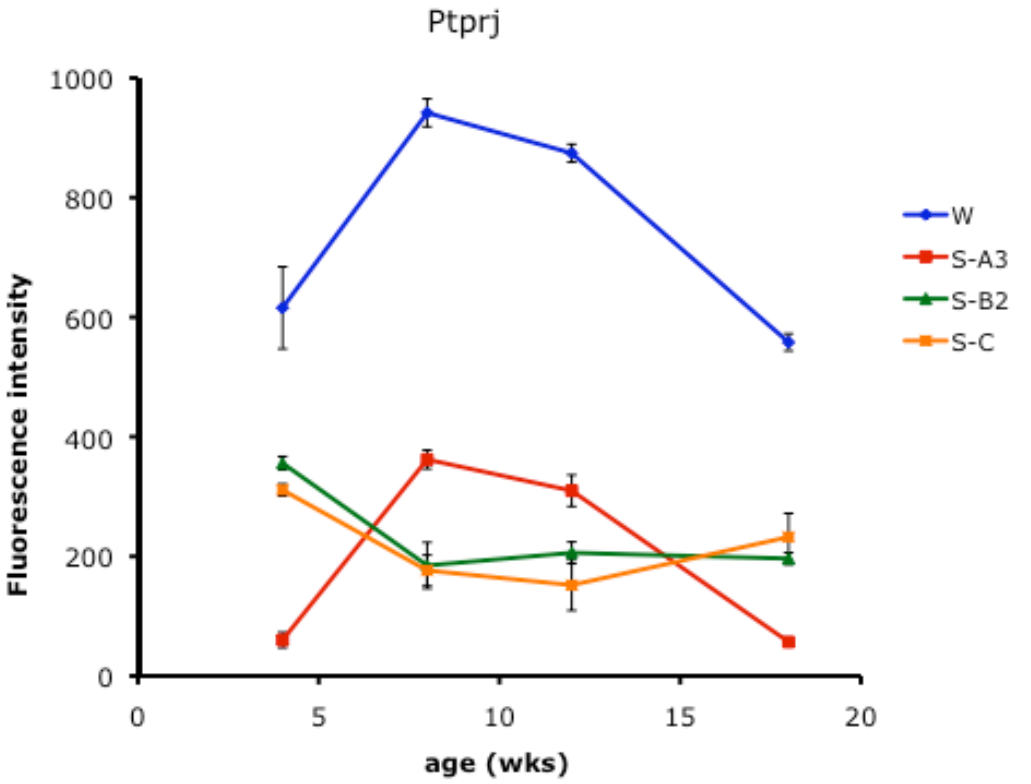
Step 6.

Further procedures: Bioinformatic analysis to determine whether those genes, in which SHR and WKY lines have fixed alternative alleles for which the expression level of the gene is determined by the allele inherited, lie in genomic blocks that have been inherited identical by descent in all SHR lines for which microsatellite and SNP genotypes are available.

Expression of SHR differentially expressed genes in individual SHR lines at 4 ages, compared with WKY.






Ptprj
1000
800
600
400
200
0
Fluorescence intensity
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5
10
15
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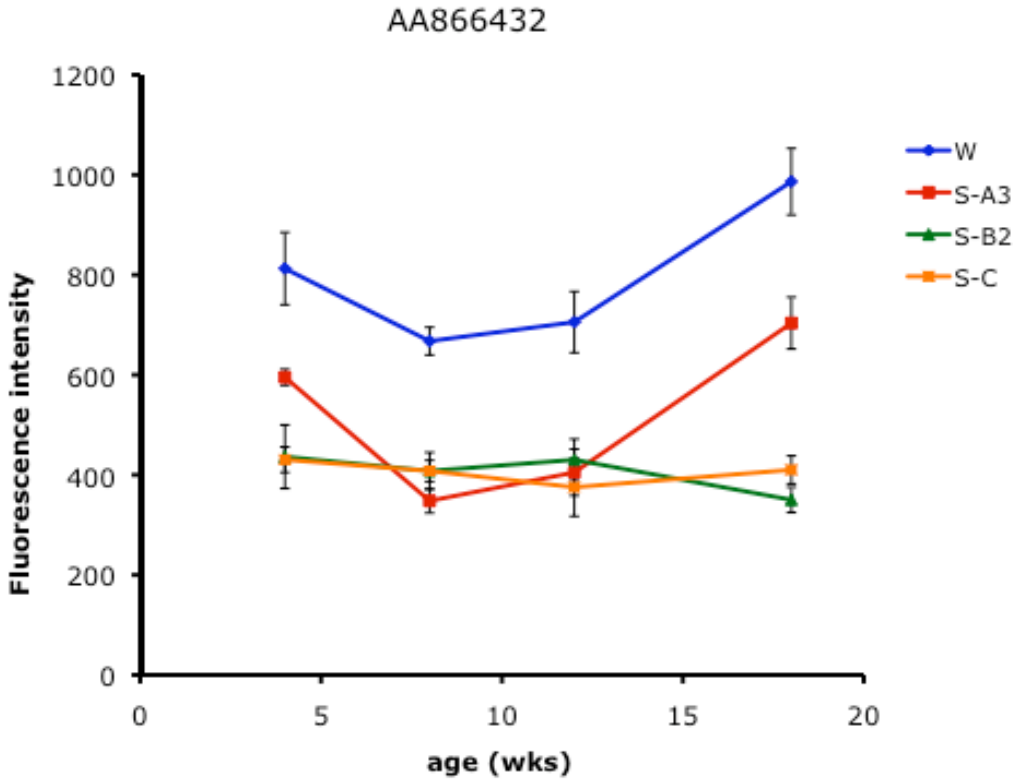
age (wks)
W
S-A3
S-B2
S-C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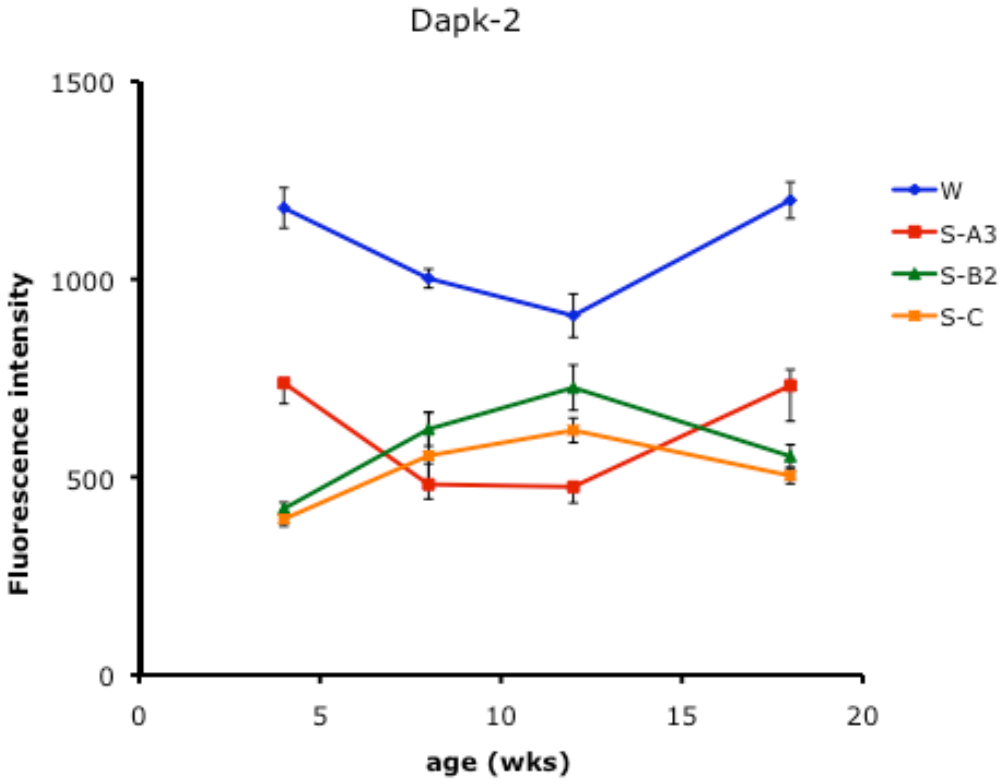
Dapk-2
1500
1000
500
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Fluorescence intensity
0
5
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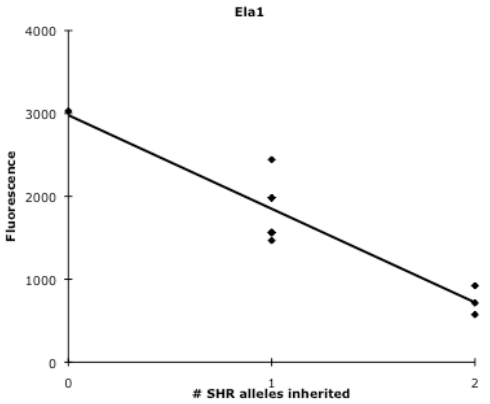
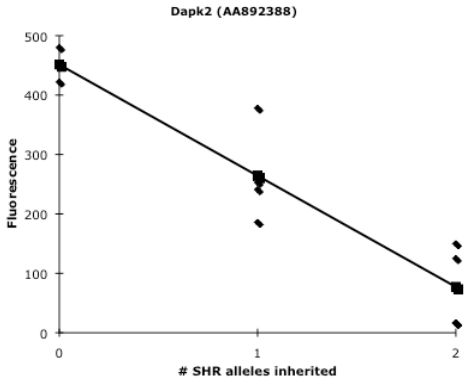
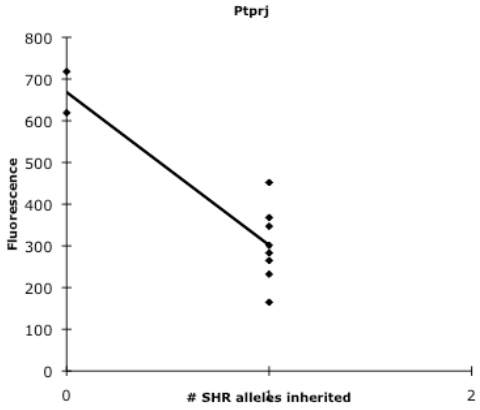
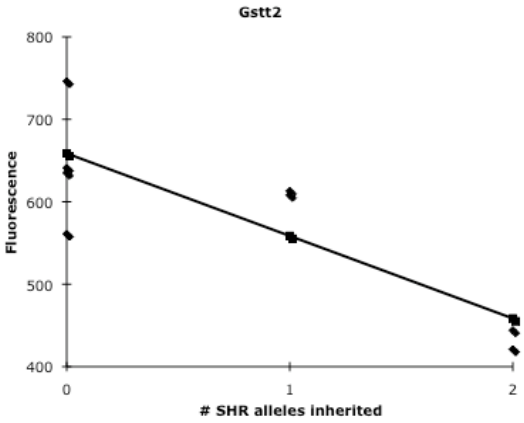
15
20
age (wks)
W
S-A3
S-B2
S-C

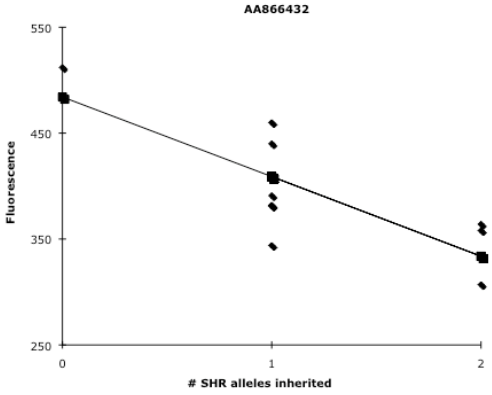
Relationship between expression levels (normalized fluorescence intensity) measured using Affymetrix E230A arrays and the allele inherited for each gene showing allelic expression specific to SHR lines. Determined using 12 F2 animals that were the progeny of a cross between SHR-A3 and WKY.











Rat BP QTL's overlapping genes with SHR expression alleles.

QTL's are numbered using the identifiers provided by the Rat Genome Database (http://rgd.mcw.edu).

QTL's marked with asterisk were identified in crosses using SHR progenitors.

Ptprj

Physical position: Chr3:74,693,135
Rat BP QTL 207, 208, Cluster 4*, 264, 251, 152

Ela1

Physical position: Chr7:139,515,742
Rat BP QTL 265, 266, 183

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Physical position: Chr8:11,151,250
Rat BP QTL 35*, 217

Dapk-2

Physical position: Chr8:70,581,319
Rat BP QTL 35*, 262, 253, 62*, 39,

Gstt2

Physical position: Chr20:13,221,934
Rat BP QTL None

Estimated Identity By Descent across SHR and WKY lines

Data on genome-wide SSLP allele size from the Allele Characterization Project (ACP) of the Rat Genome Project (http://rgd.mcw.edu/ACPHAPLOTYPER/) indicates that SSLP genotypes for WKY/OlaHsd, SHR/OlasHsd and SHRSP/Riv are available for all 3 strains at 3236 loci. All three strains share the same allele at 50.4% of these loci, reflecting the high level of relatedness among the progenitor animals that gave rise to these strains. Since all SHR lines are the progeny of a single pair of animals and were inbred through 8 generations before separation of isolated lines, the expected degree of allele sharing among any two SHR lines should be greater than between WKY and any SHR line. Allelic identity was observed at 79.0% of loci comparing SHR/OlaHsd with SHRSP/Riv, 51.5% of loci comparing WKY/OlaHsd with SHR/OlaHsd and 50.4% of loci comparing WKY/OlaHsd with SHRSP/Riv. Loci which are shared by both SHR lines, but differ from the WKY line are potential hypertension loci and account for 34.6% of loci for which the ACP has genotype information for all three strains.

Based on strain origin information in the ACP database, the SHRSP/Riv line is presumed to be the same lineage as our SHR-A3 line, while the SHR/OlaHsd line is distinct from SHRSP/Riv and is likely from the same lineage as the SHR/NCrl line used in our studies. The SHR-B2 and SHR-C lines that we have investigated in the present study are known to be independent lines from the two SHR lines in the ACP database and thus are likely to have inherited similar alleles at any loci at the same frequency (0.79) as SHR/OlaHsd and SHRSP/Riv. Thus we can estimate that the likelihood that any locus contains alleles identical by descent in SHR/OlaHsd, SHRSP/Riv, SHR-B2 and SHR-C is $(0.79)^4$ or 0.39. Given that differences in the frequency of allele sharing between any SHR line and any WKY line arise from the fact that these strains (i.e., SHR and WKY) arose from different founders, we can similarly estimate that the degree of

allelic identity between SHR-B2 and SHR-C with WKY will be similar to that between SHR/OlaHsd and SHRSP/Riv with WKY, i.e. approximately 50%. Thus, it is possible to estimate the likelihood at the genome-wide level that any single locus will share identity in all 4 SHR lines (SHR-A3, SHR-B2, SHR-C and SHR/N) and differ at the same locus with WKY as $(0.79)^4 \times 0.5 = 0.19$.

Notes on differentially expressed SHR genes.

Ptprj

This gene encodes a receptor-type protein tyrosine phosphatase. The protein consists of a large extracellular domain incorporating multiple fibronectin-like repeats. A single transmembrane pass connects this extracellular domain to an intracellular protein phosphatase domain. Two mouse knockout models of Ptprj have been reported [1]. One knockout was generated by replacing exons 3-5 with a beta-galactose-neomycin phosphotransferase cassette and produced no observed phenotype [2]. Another knockout model replaced the intracellular catalytic domain with enhanced green fluorescent protein. This resulted in an embryonic lethal phenotype with failure of vascularization, disorganized vascular structures and cardiac defects [3]. This phenotype might be explained by actions arising from the untargeted extracellular portion of the Ptprj molecule. Administration of antibody to the extracellular portion of Ptprj results in clustering of the ectodomain and inhibits vascular endothelial cell growth and blocks angiogenesis [4]. The antibody blocks phosphorylation of ERK1/2 kinases in the same way that activated Ptprj does. Evidence of a role of Ptprj in vascular development also comes from studies of the effects of morpholinos targeting the two Zebrafish isoforms of Ptprj (Dep1a and Dep1b) that revealed reduced arterial marker specification and increased venous marker specification [5].

Ptprj first came to be understood through its effects on contact-mediated inhibition of cell growth and is alternatively named density-enhanced phosphatase-1 (Dep1). In vascular endothelial cells, the function of Ptprj may depend on its ability to intercept signaling between vascular cadherin (VEC) and the VEGF recepotr 2 that is activated by VEC [6]. The signaling induced by this pathway is reduced by Ptprj, which may bind to the activated receptor, reducing its tyrosine phosphorylation and attenuating the activation of MAPK pathways driven by the activated receptor. Since VEGF is known to be involved in formation of blood vessels in both embryogenesis and in proliferative disease, Ptprj may play a critical role in modulating the pathway of vasculogenesis. In the kidney, VEGF has been shown to have functions in maintaining both renal vascular and tubular structure under normal conditions and antagonism of VEGF signaling results in reduced vascular formation and nephrogenesis in the superficial cortex [7]. Thus, Ptprj, through modification of renal VEGF signaling in SHR may have effects on blood pressure in SHR by altering both the vascular and renal tubular epithelium as a result of lower levels of expression than are found in WKY. The effect of reduced of Ptprj in SHR may result in increase VEGF receptor phosphorylation and increased signaling in this pathway.

However, the effects of Ptprj variation in SHR may be broader than the reduced expression we have observed to be associated with the SHR allele of Ptprj. In surveying the gene to detect sequence variation we discovered that SHR lines all possess a single SNP variation that leads to a non-synonymous replacement of alanine for threonine at position 39. Analysis of the predicted effects of this substitution using the InterProScan tool (www.ebi.ac.uk) indicates that this change alters one of the fibronectin type III domains that may have consequences for Ptprj activation. Finally, VEGF has been shown to play an important protective role in hypertension in which increased glomerular expression of VEGF occurs and drives signaling pathways that support glomerular integrity and prevent albuminuria. It is possible that the Ptprj allele expressed in SHR helps to protect these animals from the adverse renal effects of hypertension by maintaining the efficacy of VEGF signaling [8].

These observations regarding Ptprj function do not directly tie this gene to blood pressure regulation; however, they do strongly suggest that Ptprj function in the kidney may have important consequences for renal tubular and vascular development.

Elastase 1

Defects in the elastic component of the extracellular matrix of resistance arteries are known to be associated with hypertension. This suggests that the elastic component of resistance vessels is more than a simple reactive element allowing adaptation to fluctuations in arterial pressure, but rather molecular and genetic changes in elastin may contribute to vascular dysfunction and hypertension. Haploinsufficiency of the elastin gene in both mice and humans is associated with hypertension [9, 10]. In the SHR, altered elastin structure and organization occurs in resistance vessels and this disorganization precedes the elevation of blood pressure [11, 12]. Furthermore, manipulation of SHR vascular elastin so that it resembles in its organization that of WKY was associated with change in mechanical properties and lower blood pressure [13, 14].

Elastase 1 is a proteolytic enzyme with specificity for elastin and cleavage activity that extends to certain other proteins (e.g., hemoglobin, fibrin). Although pancreatic expression of elastase 1 is silenced in the human, the gene appears to be conserved and is expressed in at least one other tissue in humans [15]. While little knowledge of elastase function in kidney exists, analysis of expression array data indicates that it is abundantly expressed in the pancreas and kidney in rats and mice (http://genome.ucsc.edu). Thus, one potential mechanism for the involvement of this secreted proteolytic enzyme in the generation of hypertension is via the effect of secretion from the kidney on local renal vessels or perhaps even systemic effects of secreted renal elastase 1.

These observations suggest that elastase 1 may have the capacity to alter vascular function in a manner that would alter blood pressure regulation.

Dapk-2

The function of Dapk-2 is not well understood. There is evidence of Dapk-2's involvement in involvement of immune cell activation, and this might contribute to the degree of inflammation in kidney that is known to be involved in the pathogenesis of hypertension in SHR [16]. Death-associated protein kinases are involved in regulation of cell proliferation via apoptotic and autophagic cell death and has been shown to be down-regulated by beta-catenin and Tcf4 in order to induce anoikis of malignant epithelial cells [17, 18].

In the kidney, the closely related protein Dapk (Dapk-1) is upregulated in a number of renal injury models and apoptosis of injured cells may require the kinase activity of Dapk [19-22]. In renal injury, Dapk appears to increase apoptosis and reduce fibrosis [23]. It is unknown at present if renal Dapk-2 is involved in similar processes.

The limited knowledge of renal function of Dapk-2 at present precludes a well-developed hypothesis regarding its potential role as a source of hypertensive disease processes in SHR.

Gstt2

This gene is a member of the glutathione s-transferases that share general functions in detoxification of xenobiotics and reactive endogenous compounds. Little specific knowledge regarding the function of Gstt2 is known. It is expressed predominantly in liver, lung and kidney, though no reports of it distribution within the kidney have been published. Given that hypertension may have a pathogenic element arising from renal oxidative stress and that conjugation of glutathione to reactive oxidants can reduce redox stress, it is conceivable that Gstt2 might play a role in hypertensive mechanisms through this aspect of its function. However, the GST family is large it is can be questioned whether the moderately reduced expression of Gstt2 in SHR lines would be sufficient to produce a generalized shift in renal redox balance sufficient to effect blood pressure regulation.

Thus, any hypothesis regarding functional consequences of inheritance of a SHR allele of Gstt2 that contributes to the genesis of hypertension must await further understanding of its role with specific regions of the kidney and within specific elements of renal function.

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This expressed sequence tag has been mapped in the rat genome to a location in which there are no known rat genes. Further interpretation of the functional implications of this allelic expression signal will require some rudimentary understanding of which, if any, aspects of genomic function are reflected by this expression signal.

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