Published in final edited form as:
Hypertension. 2010 May ; 55(5): 1231–1238. doi:10.1161/HYPERTENSIONAHA.109.141663.

Whole Genome Survey of Copy Number Variation in the Spontaneously Hypertensive Rat: Relationship to Quantitative Trait Loci, Gene Expression and Blood Pressure

Fadi J Charchar[1,2], **Michael Kaiser**[2], **Andrew J Bingham**[2], **Nina Fotinatos**[1], **Fahima Ahmady**[1], **Maciej Tomaszewski**[2], and **Nilesh J Samani**[2]

[1]School of Science and Engineering, University of Ballarat, Victoria, Australia

[2]Department of Cardiovascular Science, University of Leicester, Leicester, UK

Abstract

Copy number variation has recently emerged as an important genetic mechanism leading to phenotypic heterogeneity. The aim of our study was to determine whether copy number variants (CNVs) exist between the SHR and its control strain - the Wistar-Kyoto (WKY) rat, whether these map to quantitative trait loci (QTLs) in the rat and whether CNVs associate with gene expression or blood pressure (BP) differences between the two strains. We performed a comparative genomic hybridization (CGH) assay between SHR and WKY strains using a whole-genome array. In total, 16 CNVs were identified and validated (6 due a relative loss of copy number in the SHR and 10 due to a relative gain). CNVs were present on rat autosomes 1, 3, 4, 6, 7, 10, 14 and 17 and varied in size from 10 kb to 1.6 Mb. Most of these CNVs mapped to chromosomal regions within previously identified QTLs, including those for BP in the SHR. Transcriptomic experiments confirmed differences in the renal expression of several genes (including Ms4a6a, Ndrg3, Egln1, Cd36, Sema3a, Ugt2b and Idi21) located in some of the CNVs between SHR and WKY rats. In F_2 animals derived from an SHR x WKY cross, we also found a significant increase in BP associated with an increase in copy number in the Egln1 gene. Our findings suggest that CNVs may play a role in the susceptibility to hypertension and related traits in the SHR.

Keywords

DNA Copy Number Variations; Hypertension; Inbred SHR, Genetics; Gene Expression; Microarray Analysis; Blood Pressure

Introduction

High blood pressure (hypertension) is a major risk factor for coronary, cerebrovascular and renal disease. Most cases of hypertension have unknown aetiology and are thus classified as essential hypertension. Hypertension has a significant genetic contribution. Despite the

Corresponding Author: Fadi J Charchar, School of Science and Engineering, University of Ballarat, University Drive, Mt, Helen, Ballarat, Australia, 3350. Phone: +61 3 5327 6098; Fax: +61 3 5327 9240; f.charchar@ballarat.edu.au.

Disclosures
None

Europe PMC Funders Author Manuscripts

progress made towards understanding of rare monogenic forms of hypertension in humans, the genetic background of essential hypertension remains poorly understood.1 The spontaneously hypertensive rat (SHR) is one of the most widely used genetic models for hypertension. The SHR model is characterized by hypertension, insulin resistance, hypertriglyceridemia and hypercholesterolemia. Genetically the SHR was derived in 1963 from inbreeding Wistar rats with the highest blood pressures.2 Using linkage analysis, there have been multiple efforts to map genes influencing BP and related phenotypes in the SHR. These efforts have resulted in the successful identification of several chromosome regions containing quantitative trait loci (QTLs) regulating BP or related cardiovascular and metabolic phenotypes in the SHR.3 Yet, despite many experiments very few genes that underlie these QTLs have been unambiguously identified.4 Copy number variations (CNVs) defined as gains and losses of DNA typically over one kilobase (kb) and up to several megabases (Mb) are being increasingly recognized as a source of differences in genomic sequence,5-8 and have been proposed as a possible mechanism for phenotypic variation in humans,8, 9 the mouse and recently - the rat.10-12

The aim of this study was to first identify differences in copy number between the genome of the SHR and its most commonly used control strain - the Wistar Kyoto rat (WKY). We then determined whether CNVs map to known QTLs and whether genes located within these CNV regions are differentially expressed between the SHR and WKY. Finally, we investigated the relationship of one of the identified CNVs to BP in a genetic cross between the SHR and WKY.

Methods

Samples

Genomic DNA was prepared using the DNeasy kit (DNeasy; Qiagen, Mississauga, Canada) from livers and kidneys of 3 SHR and 3 WKY male rats aged 8 to 10 weeks obtained from the research colony maintained at The University of Leicester (all animals were initially obtained from the breeding stock of Charles River Laboratories, Margate,UK). Total RNA was extracted from rat kidney, spleen and livers using Qiagen RNeasy kits (Qiagen, Mississauga, Canada) according to the manufacturer's instructions. Total RNA yield was quantified by UV spectrophotometry and RNA integrity was verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA).

Oligonucleotide CGH array hybridization (oligo-aCGH)

Unamplified genomic DNA (1 μg) was labeled with Cy3 (SHR) or Cy5 (WKY). We performed comparative genomic hybridization using long oligonucleotide arrays containing 385,000 isothermal probes, 45–75-mer, spanning the rat reference genome with a median spacing of ~ 5 kb (NimbleGen Systems). The oligonucleotide design, array fabrication, DNA labeling, aCGH experiments, data normalization and calculations of copy number ratio (using log2 of the ratio of signal from the fluorophores Cy3 and Cy5) were performed at NimbleGen according to recommended and published procedures. CNVs were identified based on the relative intensity of log2 ratio profiles of each experiment, using the circular binary segmentation algorithm from Olshen *et al.13* Our criteria of three or more probes in a

segment, mean amplitude of log2 shift across segment ± 0.5 were used to define the final set of high confidence CNV calls. The BLAT hit count was defined as the number of matches in which the probe sequence identity × length of matching sequence/length of the probe was greater than or equal to 0.9. Gene annotation and overlap were determined using Ensembl GeneBuild 3.4.

Validation of array data using quantitative real-time PCR (qPCR)

To validate CNVs detected by oligo-aCGH, qPCR assays were developed to measure copy number in implicated chromosomal regions relative to a control region of invariant copy number in both strains (selected based on aCGH profile) in DNA extracted from the kidney.14 Relative copy numbers were determined by qPCR using Power SYBR Green chemistry and the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, USA). Pre-designed QuantiTect primers (Qiagen, USA) were used when available or primers were designed using ABI Primer Express Software (version 2.0) and the Ensembl Rat GenomeBuild 3.4 (Online Supplementary Table 1, please see http://hyper.ahajournals.org). Each assay was performed in triplicate using 20 μl reactions containing 10 μl of Power SYBR Green PCR Master Mix (2X) (Applied Biosystems, USA), 200 nM concentration of forward and reverse primer and 20 ng of genomic DNA extracting from kidneys to confirm the presence of the same CNVs in DNAs from different tissues. Melting curve analysis and sequence analysis were performed to check PCR product specificity. Amplification was performed according to the following conditions: one cycle at 95 °C for 10 min, 50 cycles at 95 °C for 15 s and 60 °C for 1 min. Experiments were performed on the test and control primers to verify comparable efficiency in amplification prior to analysis of copy number in the strains. Mean DNA starting quantities and standard deviations were estimated based on threshold cycle differences between the control and test loci.14 Primer sequences are available in Supplementary Material, (online Table S1, please see http://hyper.ahajournals.org).

Gene expression analysis

To evaluate whether mRNA levels of genes that lie within CNV regions are differentially expressed between SHR and WKY, we used qPCR as described previously.15 In order to avoid inter-strain variation of primer binding efficiency, we performed melting curve analysis and only used pairs of primers with similar binding efficiencies determined from a dilution curve. mRNA levels of test genes were compared to those for β-actin of the same sample and the results displayed are the relative level of renal expression in SHR compared to the WKY control strain. We initially measured mRNA levels in the kidney because of the known role of this organ in the regulation of BP.16 We also measured mRNA levels in the heart and spleen for a broader assessment of CNV-related changes in mRNA expression. We used QuantiTect predesigned and validated primer sets for each gene (Qiagen).

Association analysis of Egln1 gene copy number and BP in a SHR x WKY F_2 population

We measured the copy number of Egln1 (CNV1b) in 229 F_2 rats derived from a cross of SHR and WKY rats. The generation, characterization and BP measurement of this F_2 cross, which was derived from the same SHR and WKY colonies as animals analyzed for CNVs in this study, have been described in detail previously.17 Briefly, indirect systolic BP in the tail

artery was measured by tail plethysmography at 20 weeks of age in rats on a normal diet. To determine relative copy number in F_2 rats we performed qPCR in triplicate and determined the normalized relative copy number as stated above.

Statistical analyses

Statistical analysis of comparisons between SHR and WKY genes and copy number was undertaken using paired and unpaired t-tests where appropriate; otherwise, the Mann–Whitney test was used. To determine whether there is any difference in systolic BP between F_2 rats that carry different Egln1 or Ugt2b copy numbers we used one-way ANOVA and Kruskal-Wallis test with Dunn's Multiple Comparison Test.

Results

High-resolution comparative genomic hybridization analysis

The three comparative genomic hybridizations consistently revealed 16 CNVs on eight SHR Chromosomes (1, 3, 4, 6, 7, 10, 14 and 17) (Figure 1, Table 1). Six of these regions were due to a relative loss of DNA event in the SHR and 10 regions exhibited an increase in copy number in the SHR. The segments varied in size (range: 10 kb to 1.6 Mb; mean - 250 kb). Changes on the Y chromosomes were not analyzed because of lower probe density and greater mapping uncertainty for these regions in the current assembly.

Validation of CNVs

To validate CNVs at each locus we developed a real-time PCR assay to quantitatively determine whether these regions show copy number variation. Figure 2 shows the real time PCR results which confirmed the CNVs in all regions. In those regions where there was a gain of DNA event in the SHR compared to the WKY, the increase in copy number was between 2 to 4 copies. We also determined which of our CNVs overlapped with CNVs detected by Guryev *etal.12* and found that eight of our CNVs (1b, 3b, 4a, 6a, 7b, 7c, 10a, 17a) were detected in both studies.

Genes located within CNV regions and their expression

11 of the 16 identified CNVs contain annotated or putative genes (Table 2). We compared expression of these genes in the kidney, heart and spleen between SHR and WKY rats. Some of the genes did not have detectable expression in these tissues. However, others showed significant differential expression between the two strains (Table 2). Notably, Ms4a6a located in CNV1a (first CNV on Chromosome 1) had markedly decreased expression in all three tissues in the SHR compared with the WKY consistent with the loss of DNA in the SHR (Figure 3). CNV1b which causes a gain of the Egln1 gene in the SHR was associated with significantly increased mRNA levels of this gene in the kidney and heart of the SHR rat compared to WKY (Figure 3). We did not detect any expression of Egln1 in the spleen. Within CNV3a (Chromosome 3) lies the Ndrg3 (N-*myc* downstream regulated gene 3) involved in cell differentiation. The expression of the Ndrg3 was significantly decreased in the SHR kidney, heart and spleen. CNV4a harbors the Cd36 predicted gene. We observed decreased expression of this gene in the SHR kidney and spleen as previously described by other groups (data not shown).18 CNV4b contains a putative gene similar to NEDD4-

binding protein 1 which was expressed at very low levels in both the SHR and WKY but showed a trend towards increased expression in the SHR (data not shown). Also in the same region is the Sema3a gene which shows increased expression in the SHR kidney and heart. CNV14a contains the Ugt2b gene thought to be a glucosyl transferase. mRNA levels of this gene were significantly higher in the kidney of the SHR compared to the WKY. CNV17a contains two genes Idi21 (isopentenyl diphosphate delta-isomerase) involved in cholesterol synthesis and Similar to Nucleolar GTP-binding protein 1 involved in chronic renal failure. Expression of Idi21 was significantly up-regulated in the SHR kidney (Figure 3).

CNV location within rat QTLs and orthologous regions in humans

Of the 16 confirmed CNVs, several (1a, 1b, 3b, 4a, 4b, 7b, 7c, 10a) are located within BP QTLs mapped in the SHR. (Table 3). Furthermore, 1b, 3b and 4b CNVs map to QTLs in crosses derived from SHR and WKY strains.19-22 Both 1a and 1b CNVs on Chromosome 1 map to the BP QTL in our previously constructed WKY.SHR-Sa (D1Wox19-D1Mit2) strain (Figure 4). CNV1b is located 4707195 bp from the boundary of our minimal SISA congenic strain (Figure 4).19,21 Other CNVs (3a, 7a, 10a, 14a) map to QTLs for related traits such as vascular growth, urinary albumin excretion and cholesterol level in the SHR. 3a, 10b and 17a CNVs also map to known BP QTLs in strains other than SHR.

When we searched The Copy Number Variation Discovery Project for orthologou*s* regions of these stretches of DNA in humans we found that CNVs on Chromosome 1, 6 and 14 have orthologous regions in humans with putative structural variations as described in the database of genomic variants [http://www.sanger.ac.uk/humgen/cnv/42mio/ and http://projects.tcag.ca/variation/] (Table 3).

Egln1 CNV and BP

We studied the association between copy number in CNV1b (Egln1 gene) and BP, in a large F_2 cross from SHR and WKY (Figure 5). There was a significant difference in systolic blood pressure (SBP) in the F_2 animals that carry different copy numbers of the Egln1 gene (ANOVA $P. = 0.0083$). The mean SBP of animals with one copy of the Egln1 gene was 155.5 ± 17.2 mmHg while animals which have more than one copy on only one chromosome was 157.0 ± 13.7 mmHg, animals with two duplications had a mean SBP of 163.5 ± 13.9 mmHg.

Discussion

In this comparative genome hybridization analysis of the SHR and the WKY strains we have identified new and validated existing CNVs in the rat. Of the 16 CNVs detected, eight were previously revealed in the study by Guryev et al. that compared the SHR and the Brown Norway strain.12 The seven additional CNVs detected by our experiments may have arisen in the SHR since the divergence from the WKY strain. We also demonstrate that in many instances copy number variation is associated with changes in expression of the genes located in the CNV regions. Our results highlight protein-coding genes within the CNVs that may contribute to the pathogenesis of disease in the SHR. Kidney, liver and spleen mRNA analysis of genes revealed changes in eight genes that overlap with the detected CNVs.

However, some of the genes did not show expression change which paralleled the copy number status of the genomic segment. Such observations are in line with findings by Guryev et al.12 and others that show that increased copy number can be positively 23, 24, negatively 25 or neutrally correlated with gene expression. Changes in expression may be caused by a loss of DNA that can delete a transcriptional repressor or duplication in DNA in a gene enhancer. Other possible regulatory elements that may be lost or duplicated are microRNAs (miRNA) or miRNA targets which regulate gene expression. Lack of SHR-WKY differences in tissue expression of genes showing a distinct pattern of CNV may be explained by one of the following: i) regulation of these genes is not affected by the rearrangement, ii) expression was measured in an inappropriate tissue, iii) feedback mechanisms exist to correct for any changes in copy number.25 Our data therefore supports previous findings that some but not all CNVs influence the expression profile of genes in a way that may affect pathogenesis of disease.

Potential relevance to BP QTLs in SHR

Several CNVs (1a, 1b, 3b, 4a, 4b, 7b, 7c, 10a) we identified are located within BP QTL regions identified in crosses of the SHR with other rat strains. For example, the CNV on Chromosome 4 includes a predicted Cd36 gene; Cd36 is a duplicated gene that has previously been identified as a cause of defect in insulin action, fatty acid metabolism and hypertension in the SHR.18, 26 The QTLs containing CNVs on Chromosomes 1b, 3b and 4b have been directly detected in crosses between SHR and WKY. To examine the involvement of one of these CNVs in BP in the SHR, we took forward CNV1b, which contains the Egln1 (EGL nine homolog 1) gene. Egln1, also known as HPH-2 (HIF-prolyl hydroxylase gene), showed increased expression in the kidney of the SHR (Table 2), and in previous studies, using renal transplantation, we have shown that the BP QTL located in this region on chromosome 1b at least partly mediates its effect via the kidney 16 The protein product of Egln1 is a hydroxylase enzyme which promotes degradation of the HIFs (Hypoxia-induced factors) subunits,27 a study by Li et al. demonstrated that blunting this protein was associated with an increase of BP.28 The observed increase in HPH gene expression in the SHR could therefore lead to an augmented degradation of HIF-1α - the key molecule that regulates adaptation to hypoxia, protects renal medulla from ischemia and thus controls renal sodium excretion. Consistent with this, we observed a significant association of Egln1 copy number with BP in the expected direction in an F_2 cross derived from a cross of SHR and WKY rats, providing strong support for a possible causal involvement of this CNV with BP in the SHR.

CNVs in the rat can be successfully exploited to model CNVs apparent in the human genome as shown for the CNV in the Fcgr3 gene.29 The human orthologs of the Ms4a6a and HPH-2 contained within the rat CNVs identified in this study lie within regions with reported germline copy number polymorphisms in humans. Human orthologs of the olfactory receptor genes are also known to be duplicated in humans and other mammalian species.30 Conservation of CNVs across species suggests that selective pressure may drive acquisition or retention of specific gene dosage alterations. In fact, the study by Guryev et al. which surveyed over 10 rat models showed that human and rat CNV regions share more in common than the mouse, reinforcing the importance of the rat as a model organism for

studying phenotypic effects of structural variations relevant to complex disease in humans.[12]

A limitation of our study is that the number of CNVs detected are an underestimate of the real number of structural variants in SHR, as many genomic regions were not effectively surveyed owing to technical limitations (for example, probe density or non-random distribution) of the technology platform that was used. Newer arrays with higher densities and next-generation sequencing platforms utilizing paired-end tag sequencing may resolve this limitation.[31] Another limitation of the study is that we could not accurately size the CNV regions detected since probes are not equally distributed along the rat genome due to various factors such as repeat elements. Probes may therefore fall far from the border of each CNV region. The borders may be determined by newer arrays with higher probe density or custom arrays. It is also possible that somatic mutations that arise in specific tissues may contribute to cardiovascular disease; however this was not a focus for our study which was designed to investigate germ-line transmitted CNVs. Finally, while we show that several CNVs map to QTL regions in the SHR and some have a significant effect on expression of coincident genes, further studies are required to support their phenotypic relevance. Even where a direct association with BP (or another trait) is shown, as we have for the CNV containing Egln1 further investigation including ultimately genetic manipulation of copy number, is required to provide definitive proof of their causal involvement.

Perspectives

Human genomic variation exists in many forms; copy number variations are a form of variation in which large amounts of DNA are either deleted or duplicated. Such CNVs have not yet been systematically studied with regard to their relevance to hypertension. In the present study we examined the DNA of the spontaneously hypertensive rat model for CNVs. We demonstrated that there are CNVs that exist in this model compared with its control strain the Wistar-Kyoto rat, which coincide with BP QTLs and may contribute to the pathogenesis of its hypertension. This provides a hypothesis for human genetic research on the involvement of CNVs in hypertension which will require large-scale studies that comprise well-phenotyped cohorts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This study makes use of data generated by the Genome Structural Variation Consortium whom we thank for pre-publication access to their CNV discovery [and/or] genotyping data, made available through the websites http://www.sanger.ac.uk/humgen/cnv/42mio/ and http://projects.tcag.ca/variation/ as a resource to the community.

Sources of Funding

This work was supported by a Wellcome Trust Functional Genomics Programme Grant in Cardiovascular Disease and The University of Ballarat Research Grant. FC was supported by the British Heart Foundation. NJS holds a British Heart Foundation Chair.

Europe PMC Funders Author Manuscripts

References

1. Charchar FJ, Zimmerli LU, Tomaszewski M. The pressure of finding human hypertension genes: new tools, old dilemmas. J Hum Hypertens. 2008; 22:821–828. [PubMed: 18633428]
2. Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. Jpn Circ J. 1963; 27:282–293. [PubMed: 13939773]
3. Pravenec M, Kren V. Genetic analysis of complex cardiovascular traits in the spontaneously hypertensive rat. Exp Physiol. 2005; 90:273–276. [PubMed: 15728137]
4. Cowley AW Jr. The genetic dissection of essential hypertension. Nat Rev Genet. 2006; 7:829–840. [PubMed: 17033627]
5. Sebat J, Lakshmi B, Troge J, Alexander J, Young J, Lundin P, Maner S, Massa H, Walker M, Chi M, Navin N, Lucito N, Healy J, Hicks J, Ye K, Reiner A, Gilliam CC, Trask B, Patterson N, Zetterberg A, Wigler M. Large-scale copy number polymorphism in the human genome. Sci. 2004; 305:525–528.
6. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, GonzÃ¡lez JR, GratacÃ²s M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME. Global variation in copy number in the human genome. Nature. 2006; 444:444–454. [PubMed: 17122850]
7. Bailey JA, Gu Z, Clark RA, Reinert K, Samonte RV, Schwartz S, Adams MD, Myers EW, Li PW, Eichler EE. Recent segmental duplications in the human genome. Sci. 2002; 297:1003–1007.
8. Cooper GM, Nickerson DA, Eichler EE. Mutational and selective effects on copy-number variants in the human genome. Nat Genet. 2007; 39:S22–S29. [PubMed: 17597777]
9. Pollex RL, Hegele RA. Copy number variation in the human genome and its implications for cardiovascular disease. Circulation. 2007; 115:3130–3138. [PubMed: 17576883]
10. Cutler G, Marshall LA, Chin N, Baribault H, Kassner PD. Significant gene content variation characterizes the genomes of inbred mouse strains. Genome Res. 2007; 17:1743–1754. [PubMed: 17989247]
11. Egan CM, Sridhar S, Wigler M, Hall IM. Recurrent DNA copy number variation in the laboratory mouse. Nat Genet. 2007; 39:1384–1389. [PubMed: 17965714]
12. Guryev V, Saar K, Adamovic T, Verheul M, van Heesch SA, Cook S, Pravenec M, Aitman T, Jacob H, Shull JD, Hubner N, Cuppen E. Distribution and functional impact of DNA copy number variation in the rat. Nat Genet. 2008; 40:538–545. [PubMed: 18443591]
13. Olshen AB, Venkatraman ES, Lucito R, Wigler M. Circular binary segmentation for the analysis of array-based DNA copy number data. Biostatistics. 2004; 5:557–572. [PubMed: 15475419]
14. Lee AS, Gutierrez-Arcelus M, Perry GH, Vallender EJ, Johnson WE, Miller GM, Korbel JO, Lee C. Analysis of copy number variation in the rhesus macaque genome identifies candidate loci for evolutionary and human disease studies. Hum Mol Genet. 2008; 17:1127–1136. [PubMed: 18180252]
15. Tomaszewski M, Charchar FJ, Lynch MD, Padmanabhan S, Wang WY, Miller WH, Grzeszczak W, Maric C, Zukowska-Szczechowska E, Dominiczak AF. Fibroblast Growth Factor 1 Gene and Hypertension. From the Quantitative Trait Locus to Positional Analysis. Circulation. 2007; 116:1915–1924. 1915. [PubMed: 17909102]
16. Clemitson JR, Pratt JR, Frantz S, Sacks S, Samani NJ. Kidney specificity of rat chromosome 1 blood pressure quantitative trait locus region. Hypertension. 2002; 40:292–297. [PubMed: 12215469]
17. Samani NJ, Lodwick D, Vincent M, Dubay C, Kaiser MA, Kelly MP, Lo M, Harris J, Sassard J, Lathrop M, Swales JD. A gene differentially expressed in the kidney of the spontaneously hypertensive rat cosegregates with increased blood pressure. J Clin Invest. 1993; 92:1099–1103. [PubMed: 8349793]

18. Glazier AM, Scott J, Aitman TJ. Molecular basis of the Cd36 chromosomal deletion underlying SHR defects in insulin action and fatty acid metabolism. Mamm Genome. 2002; 13:108–113. [PubMed: 11889559]
19. Clemitson J, Dixon RJ, Haines S, Bingham AJ, Patel BR, Hall L, Lo M, Sassard J, Charchar FJ, Samani NJ. Genetic dissection of a blood pressure quantitative trait locus on rat chromosome 1 and gene expression analysis identifies SPON1 as a novel candidate hypertension gene. Circulation Research. 2007; 100:992–999. [PubMed: 17332427]
20. Mizutani K, Sugimoto K, Okuda T, Katsuya T, Miyata T, Tanabe T, Higaki J, Ogihara T, Yamori Y, Tsujita Y, Tago N, Iwai N. Kynureninase is a novel candidate gene for hypertension in spontaneously hypertensive rats. Hypertens Res. 2002; 25:135–140. [PubMed: 11924719]
21. Frantz SA, Kaiser M, Gardiner SM, Gauguier D, Vincent M, Thompson JR, Bennett T, Samani NJ. Successful isolation of a rat chromosome 1 blood pressure quantitative trait locus in reciprocal congenic strains. Hypertension. 1998; 32:639–646. [PubMed: 9774356]
22. Stoll M, Kwitek-Black AE, Cowley AW Jr, Harris EL, Harrap SB, Krieger JE, Printz MP, Provoost AP, Sassard J, Jacob HJ. New target regions for human hypertension via comparative genomics. Genome Res. 2000; 10:473–482. [PubMed: 10779487]
23. McCarroll SA, Altshuler DM. Copy-number variation and association studies of human disease. Nat Genet. 2007; 39:S37–S42. [PubMed: 17597780]
24. Somerville MJ, Mervis CB, Young EJ, Seo EJ, Campo M, Bamforth S, Peregrine E, Loo W, Lilley M, Pérez-Jurado LA, Morris CA, Scherer SW, Osborne LR. Severe expressive-language delay related to duplication of the Williams-Beuren locus. N Engl J Med. 2005; 353:1694–1701. [PubMed: 16236740]
25. Lee JA, Madrid RE, Sperle K, Ritterson CM, Hobson GM, Garbern J, Lupski JR, Inoue K. Spastic paraplegia type 2 associated with axonal neuropathy and apparent PLP1 position effect. Ann Neurol. 2006; 59:398–403. [PubMed: 16374829]
26. Pravenec M, Churchill PC, Churchill MC, Viklicky O, Kazdova L, Aitman TJ, Petretto E, Hubner N, Wallace CA, Zimdahl H, Zidek V, Landa V, Dunbar J, Bidani A, Griffin K, Qi N, Maxova M, Kren V, Mlejnek P, Wang J, Kurtz TW. Identification of renal Cd36 as a determinant of blood pressure and risk for hypertension. Nat Genet. 2008; 40:952–954. [PubMed: 18587397]
27. Marx J. Cell biology. How cells endure low oxygen. Science. 2004; 303:1454–1456. [PubMed: 15001751]
28. Li N, Chen L, Yi F, Xia M, Li PL. Salt-sensitive hypertension induced by decoy of transcription factor hypoxia-inducible factor-1alpha in the renal medulla. Circ Res. 2008; 102:1101–1108. [PubMed: 18356541]
29. Aitman TJ, Dong R, Vyse TJ, Norsworthy PJ, Johnson MD, Smith J, Mangion J, Roberton-Lowe C, Marshall AJ, Petretto E, Hodges MD, Bhangal G, Patel SG, Sheehan-Rooney K, Duda M, Cook PR, Evans DJ, Domin J, Flint J, Boyle JJ, Pusey CD, Cook HT. Copy number polymorphism in Fcgr3 predisposes to glomerulonephritis in rats and humans. Nature. 2006; 439:851–855. [PubMed: 16482158]
30. Young JM, Endicott RM, Parghi SS, Walker M, Kidd JM, Trask BJ. Extensive copy-number variation of the human olfactory receptor gene family. Am J Hum Genet. 2008; 83:228–242. [PubMed: 18674749]
31. Fullwood MJ, Wei CL, Liu ET, Ruan Y. Next-generation DNA sequencing of paired-end tags (PET) for transcriptome and genome analyses. Genome Res. 2009; 19:521–532. [PubMed: 19339662]

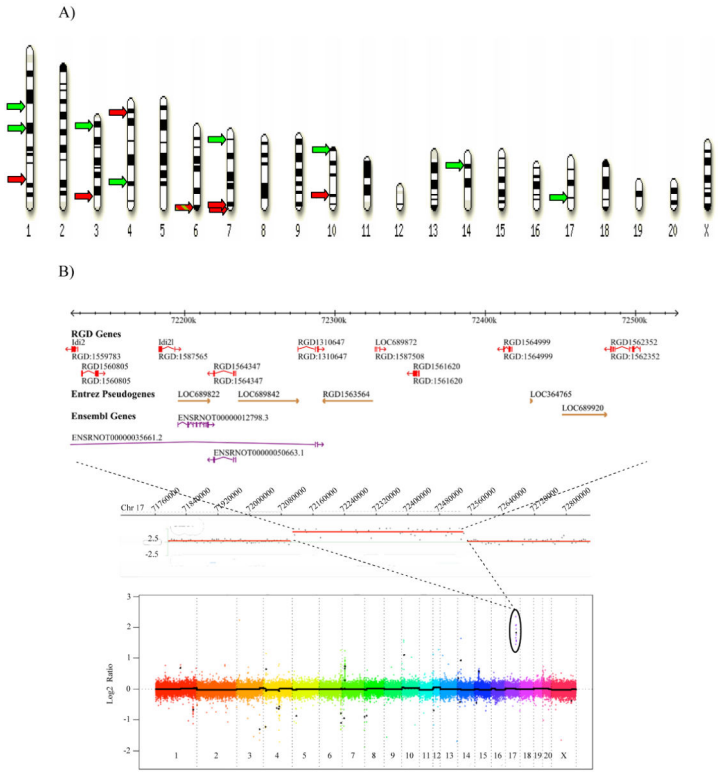

Figure 1.

A) Genomic locations of SHR CNVs. Location of 16 CNVs identified by aCGH experiments relative to the WKY strain. Green arrows and red arrows reflect gains and losses, respectively. B) Detection of CNVs in the SHR using oligonucleotide expression arrays. Log2 ratios are plotted with no moving average as a function of chromosomal position (Mb) for the DNA. Increased Log2 ratio is predictive of DNA duplication while decrease in Log2 ratio is indicative of DNA loss. An increase of Log2 ratio is evident for Chromosome 17 which are zoomed in the above boxes showing the Chromosome 17 region in more detail including gene content.

Europe PMC Funders Author Manuscripts

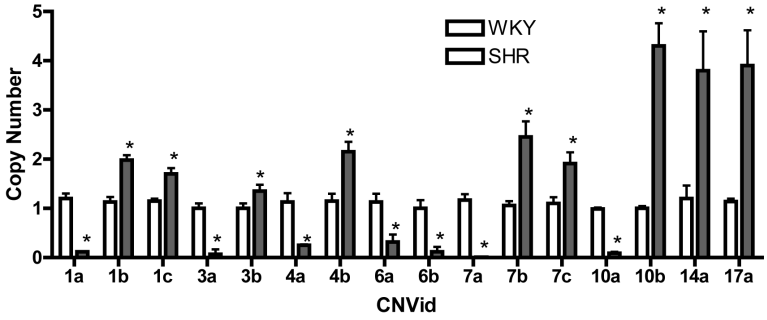

Figure 2.

Representative validation of CNV regions using real-time PCR. Results are the average of triplicate reactions in SHR and WKY. * $P<0.05$ SHR vs WKY.

Europe PMC Funders Author Manuscripts

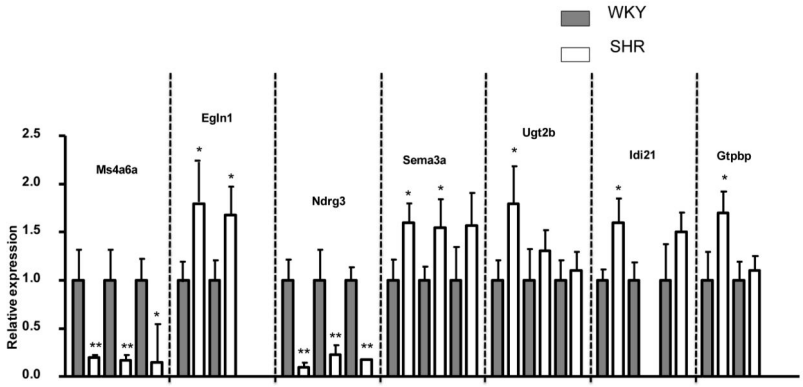

Figure 3.

Expression of genes in regions of gene duplications or deletions in kidney, heart and spleen. The graph depicts the mean ± SD for data from three replicates for both SHR and WKY. **P*<0.05; ***P*<0.01.

Europe PMC Funders Author Manuscripts

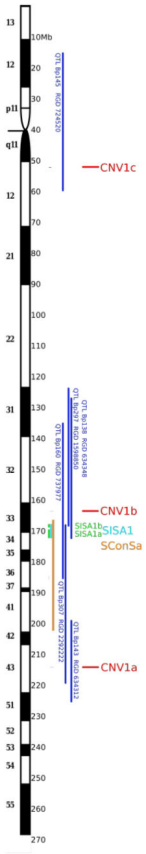

Figure 4.

A representative figure of CNVs located on Chromosome 1 in relation to BP QTLs and our congenic strains in the region.

Europe PMC Funders Author Manuscripts

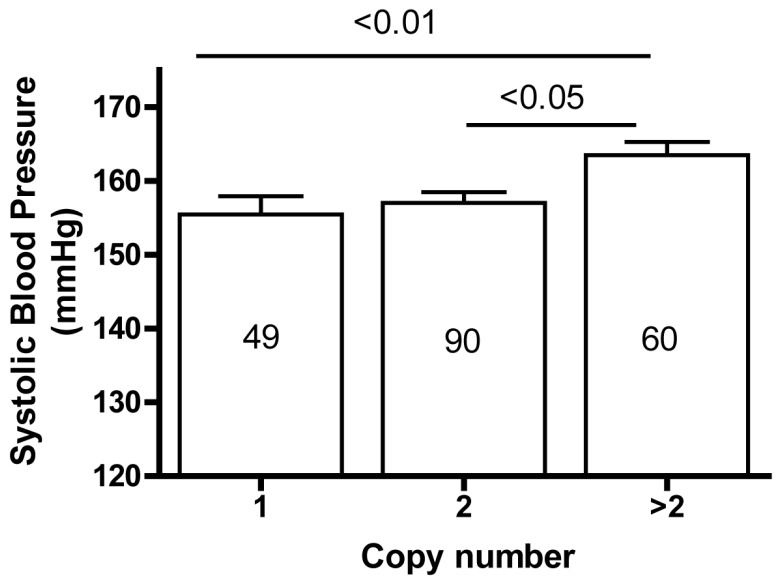

Figure 5.
Impact of CNVs in the Egln1 gene (open bars) on systolic BP in the F2 population at 20 weeks of age. The numbers on the bars represent the numbers of animals in the group.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 1

Summary of copy number variation regions detected in the SHR

CNV id (number indicates Chr)	Start (bp)	End (bp)	Size (bp)	Effect on SHR	Data Points	Mean -log2 ratio
1a	214,154,631	214,190,453	35,822	Loss	6	−1.03
1b	163,625,000	163,675,000	50,000	Gain	3	0.68
1c	52,225,000	52,375,000	150,000	Gain	3	0.65
3a	147,425,000	147,475,000	50,000	Loss	6	−1.44
3b	16,972,096	16,982,904	10,808	Gain	3	2.24
4a	135,515,50	136,595,02	107,952	Loss	13	−1.20
4b	17,125,000	17,795,000	70,000	Gain	4	0.68
6a	139,414,239	139,466,624	52,385	Loss	8	−0.80
6b	140,589,867	140,664,730	74,863	Gain	11	0.82
7a	142,925,000	142,972,551	47,551	Loss	22	−0.84
7b	16,596,577	17,484,548	887,971	Gain	50	0.82
7c	17,862,899	19,530,280	1,667,381	Gain	109	0.50
10a	83,473,206	83,515,064	41,858	Loss	9	−1.25
10b	48,455,195	48,469,795	14,600	Gain	11	0.85
14a	221,236,04	223,140,95	190,491	Gain	20	1.10
17a	72,123,819	72,528,542	404,723	Gain	37	1.80

Data points refer to the probe number. Log2 ratio refers to the ratio of signal from the fluorophores Cy3 and Cy5. All CNVs were validated by qPCR.

Europe PMC Funders Author Manuscripts

Table 2

Gene located within the structural variation regions in the SHR

Region	Gene Name / ID	Function	Renal mRNA	*P* value
1a	Ms4a6a_predicted	IgE receptor beta chain	Down in SHR	0.003
1b	Egln1	hypoxia inducible factor prolyl hydroxylase	Up in SHR	0.021
1c	ENSRNOG00000039174	putative protein kinase	NA	NA
3a	Ndrg3	catalytic activity;	Down in SHR	0.004
	9830001H06Rik	cell differentiation contains a tropomyosin domain	NA	NA
3b	None	None	NA	NA
4a	Cd36 (predicted)	Cd36 (predicted)	Down in SHR	0.02
4b	ENSRNOG00000032279	similar to NEDD4-binding protein 1	Trend Up in SHR	0.61
	Sema3a	axon growth	Up in SHR	0.03
6a	None	None	NA	NA
6b	None	None	NA	NA
7a	None	None	NA	NA
7b	ENSRNOG00000039147	ubiquitin conjugating enzyme	NA	NA
	ENSRNOG00000030234	serine/threonine protein kinase	NA	NA
7c	ENSRNOG00000026990	olfactory receptor	NA	NA
	ENSRNOG00000039138	olfactory receptor	NA	NA
	ENSRNOG00000039136	vomeronasal receptor	NA	NA
	Vom2r57	vomeronasal receptor	NA	NA
10a	None, some predicted	none	NA	NA
10b	Ttc19	unknown/release of glutathione synthase	NA	NA
14a	Ugt2b	glucosyl/glucuronosyl transferases	Up in SHR	0.01
17a	ENSRNOG00000016217	nucleolar GTP-binding protein 1	Up in SHR	0.01
	Idi2l	isopentenyl diphosphate delta-isomerase	Up in SHR	0.02
	ENSRNOG00000033610	synthesis of lipophilic molecules	No change	0.72
	ENSRNOG00000031454	chronic renal failure gene protein	NA	NA
	ENSRNOG00000032838	isopentenyl-diphosphate delta isomerise	NA	NA
	ENSRNOG00000033149	isopentenyl-diphosphate delta isomerise	NA	

NA indicates no detectable expression in the tissues we tested

Table 3
Description of quantitative trait loci (QTL) in regions with detected structural variation

Region	QTL/eQTL	CNV in humans	CNV Guryev*
1a	***BP***	Yes	No
1b	***BP*** hepatocarcinoma susceptibility, hepatocarcinoma resistance, BW, RF, CM, urinary albumin excretion, triglyceride level, NIDDM, pancreatic morphology, kidney mass, hypercalciuria	Yes	Yes§
1c	BP	Yes	No
3a	NIDDM, IDDM, kidney mass, RF, BP, ***vascular growth***	No	No
3b	***BP***, CM	No	Yes§,†
4a	***BP***, GL, TL, BW	No	Yes§,† in SHR
4b	***BP***, GL	No	No
6a	PIA	Yes	Yes†,‡
6b	PIA	Yes	No
7a	***urinary albumin excretion***	No	No
7b	***BP***, HR	No	Yes†, ‡,§ in SHR
7c	***BP***, HR	No	Yes†, ‡
10a	***BP***, collagen induced arthritis, bone mineral density, proteinuria, PIA, CM, adjuvant induced arthritis, vascular elastic tissue fragility, ***stress response***, T-lymphoma susceptibility, BW, c inflammation , estrogen-induced pituitary tumorgenesis, oil induced arthritis, RF, bone structure & strength, ***eQTL cis fat***	No	Yes†
10b	BP, kidney mass, RF, joint inflammation	No	No
14a	mammary Ca susceptibility, serum renin concentration, BW, PIA, NIDDM, tongue tumor susceptibility, ***hypercholesterolemia***	Yes	No
17a	BP, RF, CM	No	Yes†,§

CHR - Chromosome; QTL - quantitative trait loci; BP - blood pressure; IDDM – type 1 diabetes mellitus; NIDDM - type 2 diabetes mellitus; RF - renal function; Ca - cancer; BW - body weight; HR - heart rate; GL - glucose; CM - cardiac mass. Items in italic and bold refer to QTLs in the SHR strain.

*refers to CNVs found in the SHR and other rat strains in the study by Guryev et al.

†aCGH Affymetrix RaEx

‡Nimblegen RN34_WG

§Whole gunshot sequence comparison 12