The Genetics of Cardiovascular Disease

Christian Delles, Martin W McBride, Sandosh Padmanabhan, Anna F Dominiczak

BHF Glasgow Cardiovascular Research Centre, Faculty of Medicine, University of Glasgow

Address for correspondence:

Prof. Anna F Dominiczak
BHF Glasgow Cardiovascular Research Centre
University of Glasgow
126 University Place
Glasgow, G12 8TA
Scotland, UK
Phone: +44 (141) 330 5420
Fax: +44 (141) 330 6997
E-mail: ad7e@clinmed.gla.ac.uk

Abstract

Recent advances in genotyping technology and insights into disease mechanisms have increased interest in the genetics of cardiovascular disease. Several candidate genes involved in cardiovascular diseases were identified from studies using animal models, and translation of these findings to human disease is an exciting challenge. There is a trend towards large-scale genome-wide association studies that are subject to strict quality criteria with regard to both genotyping and phenotyping. We will review some of the strategies developed to translate findings from experimental models to human disease and outline the need for optimizing global approaches to analyze such results. Findings from ongoing studies will be interpreted in the context of disease pathways instead of the more traditional focus on single genetic variants.

Introduction

The pathogenesis of cardiovascular diseases involves a complex interplay between environmental and genetic factors. The relative contribution of these factors to development of disease and manifestation of symptoms differs not only between disease entities but also between individual patients. Severe phenotypes can be observed as a result of mutations in single genes such as in monogenic forms of hypertension, but the majority of cardiovascular diseases involve mutations in a larger number of genes [1,2]. We will provide an overview of strategies to examine the genetic basis of human cardiovascular disease. This will include a translational approach from rodent to human genetics and recent findings from large-scale genotyping projects in man.

In recent years, we have witnessed substantial progress in unravelling the genetic determinants of cardiovascular disease. Better understanding of pathophysiological principles and recent genome-wide association studies (GWAS) have identified robust candidate genes involved in cardiovascular disease. Rapidly evolving tools such as chip-based genotyping technology [3,4] have enabled the genotyping of hundreds of thousands of single nucleotide polymorphisms (SNPs) within a short timeframe and at a fraction what a single SNP would have costed just a few years ago. This innovation has facilitated genetic studies in large cohorts in multicentre and multinational collaborations [5-11]. These advances in human population genetics have been complemented by a significant progress in our understanding of molecular genetics ranging from design of animal models to discovery of key elements of gene regulation.

The recent publication of results of GWAS into seven common diseases by the Wellcome Trust Case Control Consortium (WTCCC) [5] has elicited unprecedented interest in the genetic basis of human disease not only in the scientific community but also in the wider public arena. This interest has been driven by a number of reasons. First, WTCCC was the first large-scale genome scan using dense SNP markers across the genome. Previous genome scans were limited by the

inability to genotype a large number of markers and therefore used fewer but highly polymorphic markers in the form of microsatellites. In WTCCC the large number of SNPs (approximately 500,000) compensated for the lower genetic variability of SNPs. Secondly, this study identified novel genes and thereby new disease mechanisms in a range of diseases including bipolar disorder and coronary artery disease (CAD) [5,6]. Thirdly, and may be surprisingly, despite the comprehensive approach in WTCCC, there was failure to discover convincing candidate genes for other traits including essential (or primary) hypertension in this [5] and other studies [12]. In light of these developments, numerous reviews and commentaries on the genetic basis of cardiovascular disease have been published recently [1,13,14].

Significant progress in cardiovascular genetics can only be optimally achieved by using a translational approach, since any increase in understanding ultimately depends on knowledge of pathways and the gene-environment interplay, not heavily considered in single gene-focused research. This review therefore highlights recent developments in molecular, animal and human genetics, focusing on a variety of cardiovascular diseases with particular emphasis on hypertension and the pathophysiological roles of oxidative stress in hypertension. We propose that future progress in cardiovascular genetic will depend on our ability to understand pathways and the gene-environment interplay rather than on single gene-focussed research.

Rodent studies

Recent improvements in rat molecular toolbox have accelerated the identification of genes involved in complex diseases in the rat, with many of these identified genes showing successful translation and relevance to human diseases. These experiments in rats mostly relied on microarray gene expression profiling in biological tissues. However, even with the power of recent human genome-wide association studies to identify SNPs with genome-wide significance, it seems likely that to fully elucidate disease mechanisms, model organisms are required. Two

recent studies using mouse models modified the traditional forward genetic strategy, identifying the genetic basis from a phenotype, and identified gene networks that contributed to increased susceptibility to metabolic disease processes in humans such as obesity [15,16]. To date, essential hypertension has proven particularly resistant to this form of analysis. One further strategy to overcome limitations of functional genetic studies in humans is to take advantage of recombinant inbred strains that have been selectively bred over many generations and are inherently simpler models of disease, where gene expression is modulated while under complex control of the recipient genetic background [17,18]. This is especially relevant since recent developments in genome sequencing [19], and software development [20] have accelerated conserved genome analysis and translational studies between rat, mouse and human [21-23].

Rat as a physiological model of human cardiovascular disease

Inbred strains of rodents have been used to study mammalian physiology and understand the contribution of specific genes to the pathogenesis of many diseases. The stroke-prone spontaneously hypertension rat (SHRSP), for example, is a well-characterized experimental model for human essential (or primary) hypertension, *i.e.* for the far more common polygenic form of hypertension. Similar to human disease, the genetic determination of hypertension in the SHRSP model is due to multiple gene-gene and gene-environment interactions [17]. The SHRSP develops a number of cardiovascular complications, including cardiac hypertrophy, stroke and endothelial dysfunction due to increased oxidative stress [24-29]. Although exceptionally high blood pressure is clearly a factor for end-organ damage in the SHRSP, genetic factors are also likely to contribute. Genome-wide linkage studies have been successful in localizing large chromosomal regions containing quantitative trait loci (QTL) for blood pressure regulation in the SHRSP [17]. In particular, two blood pressure QTL have been mapped to rat chromosome 2 [30]. This region of chromosome 2 is a classic example of a common or overlapping QTL, since

it has been implicated in several rat crosses. These two QTL, therefore, have become a focus for further congenic strategies in our [31] and other [32,33] laboratories. Speed congenic breeding protocols have generated strains, introducing various segments of chromosome 2 from the Wistar Kyoto (WKY) into the SHRSP rat and *vice versa* [31,34]. Transfer of the region of rat chromosome 2 containing QTL from WKY into an SHRSP genetic background lowered both baseline and salt-loaded systolic blood pressure by approximately 20 and 40 mmHg, respectively, in male congenic rats compared to the SHRSP parental strain (Figure 1). In the reciprocal WKY.SPGla2a congenic strain the blood pressure was increased by approximately 15mmHg. In combination with congenic strain construction in the SP.WKYGla2a and SP.WKYGla2c* strains, genome-wide microarray expression profiling identified glutathione S-transferase mu type 1 (*Gstm1)* as a positional candidate gene for spontaneous hypertension [23,34,35] and endothelial differentiation gene 1 (*Edg1)* and vascular cell adhesion molecule 1 (*Vcam1)* for salt sensitivity [36] (Figure 1, Text Box).

Candidate gene approach in human studies

In the last few years, exciting developments in rat genetics have integrated a variety of genetic mapping strategies, gene expression and computational analysis to accelerate gene discovery in rat cardiovascular disease including those involved in affecting left ventricular mass [37] and heart failure [38] with important implications for human disease. Robust candidate genes for human cardiovascular disease have been derived from experimental models such as the SHRSP and from pathophysiological considerations. For example, sodium homeostasis, oxidative stress and vascular remodelling are clearly involved in the pathogenesis of hypertension, and genes involved in these pathways are likely to be genetic candidates for hypertension. It has been shown without any doubt that *GSTM* genes are functional and positional candidate genes for human hypertension and possibly for other cardiovascular diseases (Text Box). Previous to work

in SHRSP and chromosome 2 congenic strains [34,35], *GSTM* genes were subject to association studies in humans [39-41] due to the fundamental role of the oxidative stress pathway in cardiovascular pathophysiology [42,43]. While there is agreement on the involvement of genetic variants of *GSTM*s in the development of cancer [44,45], studies on the role of *GSTMs* in cardiovascular diseases are less consistent [40,41]. This inconsistency suggests the necessity for setting quality criteria for genetic association studies. In fact, such criteria have been recently published by the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) Working Group on Replication in Association Studies; these criteria focus on the importance of replicating significant results in independent cohorts and demonstrating differential gene expression and functional analysis of genetic variants [46]. In accordance with these criteria, a definitive association study of *GSTM*s in hypertension has been conducted, associating a SNP in *GSTM5* with hypertension in a family-based cohort and differential expression of *GSTM5* in renal tissue of subjects with hypertension compared to normotensive controls. [47] However, these findings were not reproduced in an independent case-control cohort. Although this study linking *GSTM5* with hypertension is essentially negative, it demonstrates the need for replication despite the presence of functional or gene expression data.

Variants of other genes have been more consistently found to be associated with cardiovascular disease. For example, studies have attempted to identify candidate genes for essential hypertension by extrapolating knowledge of the pathogenesis of monogenic forms of hypertension. A recent study in the general population, *i.e.* in a cohort not enriched for hypertension, suggests an association of variants in such genes including *KCNJ1*, encoding the potassium channel ROMK1, with the level of blood pressure [48] (Text Box). Many rare alleles that alter renal salt handling in blood pressure variation have also been identified in the Framingham Heart Study [49]. However, these results must be interpreted with caution, as the

functional or molecular link between polymorphisms and changes in gene and protein expression have not yet been examined in these studies.

Robust evidence associates *WNK1* and *WNK4* genes, encoding serine-threonine kinases and involved in renal electrolyte homeostasis, with the level of blood pressure in a general population sample and in patients with severe hypertension [50,51] (Text Box). With regard to the oxidative stress pathway, variants of *CYBA* encoding the p22phox subunit of NADPH oxidase have been found to be associated with hypertension [52], vascular superoxide production [53,54], and intermediate cardiovascular phenotypes such as increased carotid intima-media thickness and vascular stiffness [54,55] (Text Box). Consistent association of a genetic variant and the immediate function of its gene product affecting a cardiovascular trait and intermediate phenotypes provides strong evidence for a functional role of that gene in the pathophysiology of cardiovascular disease. Other promising functional candidate genes for cardiovascular disease include *ACE* and *ADD1*, which encode for proteins that are critically involved in pathophysiology of hypertension such as the renin-angiotensin-aldosterone system and Na^+/K^+ ATPase activity [reviewed in 56,57] (Text Box), but they did not produce significant signals in recent genome-wide scans [5,6,12].

Genome-wide association studies in human cardiovascular disease

In contrast to candidate gene studies, GWAS initially are not hypothesis-driven, *i.e.* they are not necessarily suited to detect an association of well-established candidate genes with cardiovascular disease, since the effect of even strong candidate genes might be relatively low or not detectable if only a limited set of phenotypes are examined. The major challenge in GWAS is therefore to provide adequate power to detect disease-specific genetic variants [58]. Factors influencing power and sample size determinants include expected effect size, the degree of gene-gene and gene-environment interactions, and the number of genetic variants that are necessary to

cause disease. In most cardiovascular traits, we assume involvement of several, maybe hundreds of genes with individual odds ratios of 1.2-1.3 or less [1]. Current GWAS are powered according to this assumption, and the promising results of GWAS in CAD and diabetes [5,6,11] confirm the validity of these considerations.

Interpreting GWAS results that do not result in significant associations of genetic variants with disease is challenging. For example, the lack of significant associations of SNPs with hypertension in the WTCCC and the British Genetics of Hypertension (BRIGHT) study [59, 12] [5] triggered discussions about genetics of cardiovascular diseases. However, a negative GWAS does not exclude involvement of genetic factors in the pathogenesis of, for example, hypertension. In fact, family and twin studies have established that genetic factors are important determinants of hypertension [1]. A negative result is more likely due to wrong assumptions with regard to effect size and number of potentially involved genes. In this sense, GWAS are not completely hypothesis-free but instead dependent on a hypothesis of effect sizes and number of genes and pathways potentially involved.

The role of phenotyping in genetic studies

Recent advances in genotyping technology yielded increasingly robust genotyping procedures . Therefore, the chance to find significant associations between genetic variants and a specific phenotype increasingly depends on the quality of phenotyping. A clearly defined and measurable phenotype increases the chances for detecting genotype-phenotype associations. For example, the extremely strong association (up to P=4.95 x 10^{-10}) found between complement factor H gene (*CFH)* variants such as rs380390 or Tyr-402His and age-related macular degeneration [60,61] not only depended on the extraordinarily strong gene effect but also on the clear detection of presence of the phenotype. In other words, investigators were sure that all cases had macular degeneration whereas all control subjects clearly did not have macular degeneration.

In cardiovascular diseases, phenotyping is more challenging. For example, it is difficult to uniformly define the phenotype "stroke" in patients with a cerebrovascular event, as this phenotype may range from modest to severe extremes, *e.g.* from ischaemic to haemorrhagic stroke, from transient ischaemic attacks to established cerebral infarction, and from small infarcted areas to areas involving large parts of the brain. Additional phenotypes can be observed depending on the mode of imaging; for example, in comparison to computed tomography, magnetic resonance imaging more sensitively detects the penumbra that is also part of the phenotype "stroke". Unsurprisingly, GWAS addressing stroke have so far only produced preliminary results and are one of the major challenges in cardiovascular genetics [62,63]. It is also difficult to define a uniform phenotype in CAD patients for genetic studies. It is even more difficult to definitely exclude presence of CAD in controls, since such an exclusion would rely on invasive techniques that are clearly unethical in presumably healthy subjects. This potential "caseness" of controls has to be considered in power and sample size calculations of genetic studies [5].

In contrast to the phenotypes of stroke and CAD, hypertension can be diagnosed qualitatively and quantitatively with high precision using non-invasive techniques at low cost and without potentially harmful imaging techniques involving radiation. Nevertheless, only few genetic studies on hypertension have benefited from accurate phenotyping which should ideally include standardized office blood pressure readings in the absence of antihypertensive medication and 24-hour ambulatory blood pressure monitoring in both cases and controls. The fact that WTCCC did not find significant genetic associations for hypertension [5] is at least in part attributable to the lack of precise blood pressure phenotyping in control subjects [1]. Nevertheless, the WTCCC [5] and BRIGHT study [12] benefit by selecting cases at the top end of the blood pressure distribution in a given population, since selecting extreme phenotypes increases the chance of finding a genetic determinant. In our opinion, selecting extreme phenotypes, however, should

also be done for control subjects. In the first instance control subjects should undergo the same phenotyping as cases. In the case of hypertension for example, one would expect at least the same protocols for blood pressure measurement being applied to cases and controls. Ideally, however, control subjects would not only be "not hypertensive", but in fact should have normal blood pressure, for example 120/70 mmHg (and not for example, high normal blood pressure such as 135/85 mmHg). One could also ask for further evidence of absence of cardiovascular disease in control subjects, such as absence of end organ damage including hypertensive retinopathy, microalbuminuria and left ventricular hypertrophy, and follow-up data without evidence for cardiovascular events. We think that this concept of well characterised and "absolutely normal" control subjects ("hypercontrols") will increase the chances of finding genetic associations by increasing the phenotypic difference between cases and controls in hypertension and other cardiovascular diseases.

Increasing phenotype stringency also facilitates the determination of pathophysiological links between genes and cardiovascular traits. For example, assessing inflammatory markers at the time of blood pressure measurement could help confirm the functional relevance of any establishd genetic associations. Indeed, GWAS has been successful in identifying biomarkers such as lipids [9-11] and urate levels [10] in patients with diabetes and cardiovascular disease.

In most cases, the original study may not have been powered for studies into additional phenotypes, and such data must then be derived from secondary analyses. The more phenotypes and subphenotypes/ intermediate phenotypes measured, the greater the risk for false positive findings when these phenotypes are subject to genetic association studies using the same genetic markers [64]. Therefore, while detailed characterisation of additional and intermediate phenotypes are suggested, it may benefit researchers to focus on the precise characterisation of the primary phenotype. In that sense, a study into the genetics of hypertension would benefit

more from a second blood pressure reading than from measurement of an inflammatory marker *e.g.*, C-reactive protein.

Pathways versus single gene effects

The stringent criteria for significance in GWAS are somehow artificial, as they primarily derive from the view that a single gene is entirely responsible for the resulting phenotype. It is more likely that genes and their gene products interact with each other and with environmental factors in highly redundant pathways to contribute to a phenotype. As previously discussed, a recent study excluded the significant association between *GSTM* genes and hypertension in humans [47], but other studies have shown significant effects of *GSTM* genotype in the presence of mutations of other *GST* genes [65]. With GST enzymes being involved in detoxification of free radicals, it is likely that mutations in more than one *GST* gene are required to cause a certain phenotype, as other enzymes could compensate for one dysfunctional gene product. It is also possible that mutations only in the presence of free radicals, *e.g.* derived from smoking or other toxins, will lead to a phenotype, as under normal conditions even dysfunctional enzymes could cope with the amount of free radicals generated. While some studies may be underpowered to examine such complex interactions, it is possible that even though two or three genetic variants within the same pathway show association with a phenotype at only borderline significance, these genes may be as relevant to pathogenesis as a single genetic variant in the same pathway with a highly significant *P*-value (Table 1).

Current studies on the genetics of cardiovascular disease are performed by large consortia of researchers on thousands of patient samples. It is now common practice that patient and control cohorts are "reshuffled" and subject to more than one study. For example, the cases in the WTCCC [5] derive from existing cohorts and have been analysed using different genotyping technology and/or different control subjects in the past [12,50]. While the detailed statistical

implications of this practice are beyond the scope of this review, the amount of available genotypic and phenotypic data in publicly accessible databases constitutes an enormously rich resource of data. Even within a single study such as the WTCCC [5], researchers are able to access data and investigate the association of genetic variants across a variety of cardiovascular and other diseases. Such comparisons offer additional information about significance of results. Table 1 shows that certain genes are involved in a number of diseases that are expected to share the same pathogenetic pathways, *e.g.* CAD and rheumatoid arthritis in both of which inflammation plays a role in the disease process. Even if associations are only of nominal significance, occurrence of associations between genotype and phenotype in more than one disease points towards a "true" finding. However, biostatistical methods still need to be developed to make use of such information, as our current abilities to analyse large datasets of mainly descriptive nature are limited. It is clear that such analysis would only be suitable to generate hypotheses, but the extent of any validation and replication studies needs to be evaluated.

Current and future trends in cardiovascular genetics

The dramatic improvement in genotyping technology constitutes a unique opportunity to conduct large-scale collaborative studies on the genetics of cardiovascular disease. Standardisation of phenotyping and quality control is a prerequisite for interpretation but also offers the possibility of pooling of datasets from different investigators in order to create larger cohorts. Also, only if genetic studies follow similar quality criteria, there is potential for meta-analysis of results of different studies. The vast majority of genetic studies are currently performed in case-control settings, primarily aimed at transmission of disease alleles that can be ideally assessed in family-based studies [66]. Large-scale genotyping and the presence of complex pathophysiological networks, however, demands new biostatistical methods to analyse the datasets. At present, it

seems that advances in genotyping technology run at a faster pace than development of analysis methods, with an urgent need for development of new statistical and mathematical tools.

Recent trends in genetics and epigenetics will play a major role for genetic studies of cardiovascular disease within the next few years. For example, sub-microscopic re-arrangements of small fragments of DNA (copy number variation) have not yet been examined in genotyping studies but are believed to play an important role in overall genetic variation and possibly in pathophysiology of cardiovascular and other diseases [67]. Also, epigenetic mechanisms, including regulation by microRNAs, cause differential gene activity in the absence of DNA mutation [68]. These will be exciting targets for future studies. Finally, high-throughput technology is now also available for analyzing protein expression, and proteomics approaches will open new avenues of cardiovascular research [69,70]. All these recent trends will contribute to the impact of genetic research on treatment and prevention of cardiovascular disease. First steps into cardiovascular pharmacogenomics have been made [71-73], and evolving technologies give confidence that many more such steps will follow in the near future.

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Text Box. Pathomechanisms of hypertension.

Electrolyte homeostasis

Impaired renal sodium and potassium balance is one of the most important factors in the development of hypertension. The renin-angiotensin-aldosterone system plays a crucial role in this context. Monogenic forms of hypertension are characterized by mutations of genes that are involved in renal electrolyte transport and recent data suggest that variants in these genes could also contribute to the pathogenesis of essential (primary) hypertension.

- Adducin is a cytoskeleton protein that is associated with binding of the Na^+/K^+ ATPase to the cytoskeleton. Variants of the *ADD1* gene have been shown to stimulate Na^+/K^+ ATPase, therby leading to sodium retention and hypertension [56,57].
- WNK1 and WNK4 are serin-threonine proteases expressed in the distal nephron. Mutations in *WNK1* and *WNK4* genes have been shown to be associated with high blood pressure [50,51].
- ROMK1 is an inwardly rectifying potassium channel. Mutations in the *KCNJ1* gene cause a form of Bartter syndrome, a monogenic form of hypertension. Recently, variants of *KCNJ1* have been found to be associated with essential (primary) hypertension [48].

Vascular function and structure

Changes in vascular function and structure are found early in the development of hypertension and other cardiovascular diseases and are both cause and consequence of disease, particularly with regard to end organ damage including coronary artery disease and nephropathy.

- Endothelial differentiation gene 1 encodes a G-protein-coupled receptor and is highly expressed in endothelial cells. It is involved in endothelial cell differentiation and causes cell-cell adhesion. In rat, *Edg1* is a functional and positional candidate gene for hypertension [36].
- Vascular cell adhesion molecule 1 is a cell surface sialoglycoprotein that is expressed by activated endothelium. It mediates leukocyte-endothelial cell adhesion and is implemented in the pathogenesis of atherosclerosis. In rat, *Vcam1* is a functional and positional candidate gene for hypertension [36].

Oxidative stress

Oxidative stress is an imbalance between production of reactive oxygen species such as superoxide anion and defences against these free radicals. Excess of free radicals causes damage to proteins, lipids and DNA and leads to changes in vascular function and structure. One of the earliest measurable consequences of oxidative stress in humans is impaired endothelium-dependent vasodilation, a common finding in cardiovascular diseases.

- NAD(P)H oxidase is a membrane-bound enzyme complex and a major source of vascular superoxide generation. Polymorphisms in the *CYBA* gene encoding the p22phox subunit of NAD(P)H oxidase have been associated with increased superoxide production and essential hypertension [52-55].
- Glutathione S-transferases are involved in the detoxification of a number of endogenous and exogenous compounds. Amongst the classes of GST enzymes the mu class is particularly involved in detoxification of free radicals. GSTM enzymes protect against oxidative stress, and or reduced expression of *Gstm1* has been found to be associated with hypertension in rats [23,34,35].

This text box shows major mechanisms but is by no means a comprehensive overview of the pathogenesis of hypertension. The genes in the text box have been selected as examples and are also mentioned in the text and many other genes are involved in these pathways. For further reading see [1,13,14].

Table 1

Gene	Chromosome	BD	CAD	CD	HTN	RA	T1D	T2D
CRP	1	0.33489	0.179421	0.395047	0.288228	0.0653446	0.0758595	0.160982
GSTM3	1	0.798069	0.13995	0.342538	0.419983	0.823041	0.949932	0.546322
IL6R	1	0.0448555	0.00182706	0.001376	0.0600474	0.00142976	0.00851496	0.0265767
IL1B	2	0.288579	0.564475	0.341337	0.872045	0.254369	0.333314	0.163601
SOD3	4	0.223077	0.192229	0.0601649	0.106382	0.363473	0.0163271	0.0185113
IL6ST	5	0.949803	0.813763	0.889323	0.771115	0.657419	0.473425	0.921231
SOD2	6	0.0915653	0.000536518	0.0683838	0.291131	0.00459882	0.162744	0.175661
NOS3	7	0.739966	0.947511	0.328744	0.664842	0.551039	0.83784	0.909137
PON1	7	0.274734	0.139083	0.0830213	0.0205941	0.0485964	0.0258393	0.0774826
PON2	7	0.079956	0.205913	0.0277005	0.0363002	0.0118318	0.224679	0.0569649
PON3	7	0.421571	0.319962	0.210634	0.177386	0.412726	0.241487	0.0790662
GSR	8	0.398653	0.0793362	0.62712	0.185672	0.325371	0.0590146	0.400362
CAT	11	0.316104	0.0251473	0.364005	0.215003	0.384584	0.15847	0.365068
NOS1	12	0.012322	0.0983362	0.10515	0.0239134	0.331539	0.396458	0.36203
CYBA	16	0.659232	0.635098	0.554587	0.440936	0.785349	0.782325	0.388056
MPO	17	0.05805	0.236249	0.410893	0.0517084	0.131433	0.154636	0.331649
NOS2A	17	0.0840542	0.0568331	0.0139903	0.0731338	0.210996	0.0236865	0.0512084
KLK1	19	0.110755	0.293647	0.654226	0.0160403	0.452522	0.0685839	0.035118
GSS	20	0.0122597	0.186009	0.195624	0.178708	0.0194835	0.259212	0.43203
HMOX1	22	0.381948	0.876874	0.207927	0.133741	0.398465	0.967978	0.302908

Comparison of most-significant oxidative stress/inflammatory pathway gene-centric hits from the Wellcome Trust Case Control Consortium (WTCCC) study across the range of disease phenotypes studied.

P-values were downloaded from the WTCCC website (www.wtccc.org.uk) for the seven common diseases investigated (BD, bipolar disorder; CAD, coronary artery disease; CD, Crohn's disease; HTN, hypertension; RA, rheumatoid arthritis; T1D, type 1 diabetes; T2D, type 2 diabetes) [5]. Light grey shading indicates $P<0.05$, dark grey shading indicates $P<0.005$.

This approach highlights that even in the absence of genome-wide significance levels, there are patterns of nominal significance across different diseases that can advise and direct further pathway-directed search for causative genes. For example, nominal significance of interleukin-6 receptor gene (*IL6R*) in most diseases highlights the crucial importance of this gene in the pathophysiology of a number of disorders. Mitochondrial superoxide dismutase gene (*SOD2*) is associated with CAD and RA. In both diseases, reactive oxygen species play a particularly strong role. Absence of nominal significance for *SOD2* in BD, in which oxidative stress is thought to play a less prominent role, is supportive of these data. Absence of any significant results for p22phox subunit of NADPH oxidase (*CYBA*) is more difficult to interpret, but the fact that there was no nominal significance in any of the disorders indicates that this may be a less important member of the disease causing pathway.

Figure Legends

Figure 1. Chromosome 2 genetic map and congenic substrains generated on the SHRSP genetic background.

The bars represent chromosome 2 genotypes for each of the congenic substrains. Dark grey bars indicate regions of Wistar-Kyoto (WKY) homozygosity. Lighter grey bars represent regions of stroke-prone spontaneously hypertensive rat (SHRSP) homozygosity. The various introduced WKY regions completely overlap the original SP.WKYGla2a congenic interval. For each substrain, averaged difference in baseline and salt-loaded systolic blood pressure compared to SHRSP, measured by radiotelemetry, is illustrated below their respective genotype. SP.WKYGla2a n=8, SP.WKYGla2c* n=12, SP.WKYGla2b n=6, SP.WKYGla2e n=8, SP.WKYGla2f n=8, SP.WKYGla2g n=5, SP.WKYGla2i n=5, SP.WKYGla2k n=7.

* $P<0.05$ compared to baseline and salt-loaded systolic blood pressure respectively, in male SHRSP using a linear mixed effects model. cM denotes centi Morgan, and Mb denotes megabases.

Further analysis showed that glutathione S-transferase mu type 1 (*Gstm1*), endothelial differentiation gene 1 (*Edg1)* and vascular cell adhesion molecule 1 (*Vcam1)* are candidate genes for hypertension and salt sensitivity, respectively. Position of these genes is indicated in the figure. Salt sensitivity was studied to specifically dissect genes that are involved in sodium homeostasis, which is one of the major pathogenetic mechanisms of hypertension.

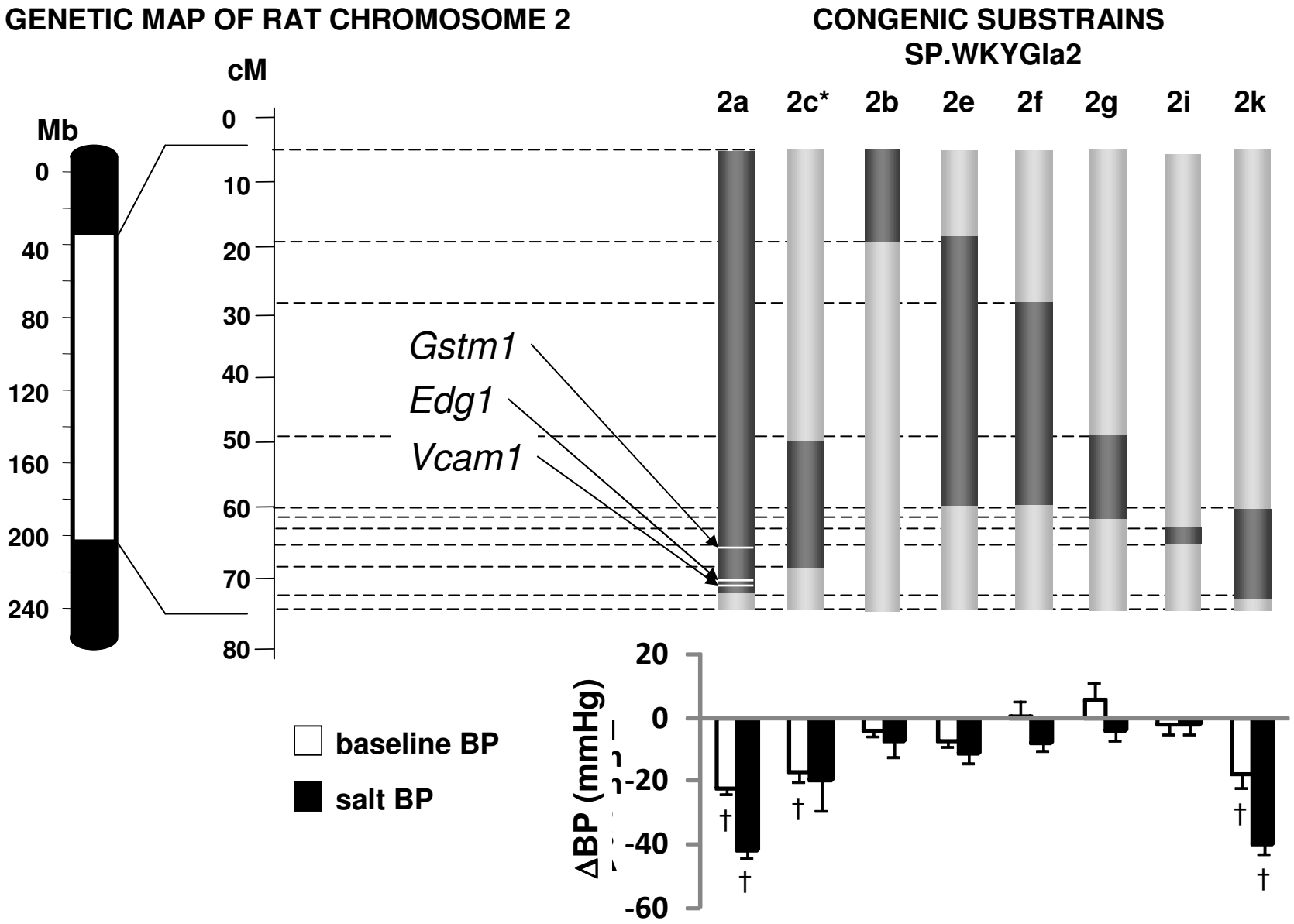
Figure 2. Translational approach in candidate gene studies.

Translation from genetic findings in rodents to human genetics is illustrated by studies on Glutathione S-transferase mu type 1 (*Gstm1*) in hypertension.

(A) A genome-wide scan in crosses of Wistar-Kyoto (WKY) and stroke-prone spontaneously hypertensive rat (SHRSP) revealed two quantitative trait loci (QTLs) for hypertension on rat chromosome 2 [30]. (B) Congenic strains have been designed replacing parts of chromosome 2 of SHRSP with parts of WKY chromosome 2 [31]. These strains were used confirm the effect of QTLs on the phenotype hypertension. (C) Microarray analysis showed differential expression of *Gstm1* between WKY, SHRSP and the congenic strain SP.WKYGla2c* with the congenic strain exhibiting similar *Gstm1* expression as WKY [34,35]. (D) Pathway analysis shows a crucial role of Gstm1 in detoxification of free radicals (X) by transferring them to glutathione (GSH). The figure is simplified, but still illustrates the complexity of the GSH pathway which can also be affected by variations of other genes. (E) Analysis of conserved synteny between rat chromosome 2 and human chromosome 1 showed five potential human orthologues of rat *Gstm1*. (F) These orthologues, *GSTM1* to *GSTM5*, were then subject to genetic association and linkage studies in humans [47].

The same approach can be applied to other cardiovascular traits and other candidate genes.

Figure 1


GENETIC MAP OF RAT CHROMOSOME 2
CONGENIC SUBSTRAINS
SP.WKYGla2
cM
Mb
0
10
20
30
40
50
60
70
80
0
40
80
120
160
200
240
2a
2c*
2b
2e
2f
2g
2i
2k
Gstm1
Edg1
Vcam1
baseline BP
salt BP
ΔBP (mmHg)
20
0
-20
-40
-60
†

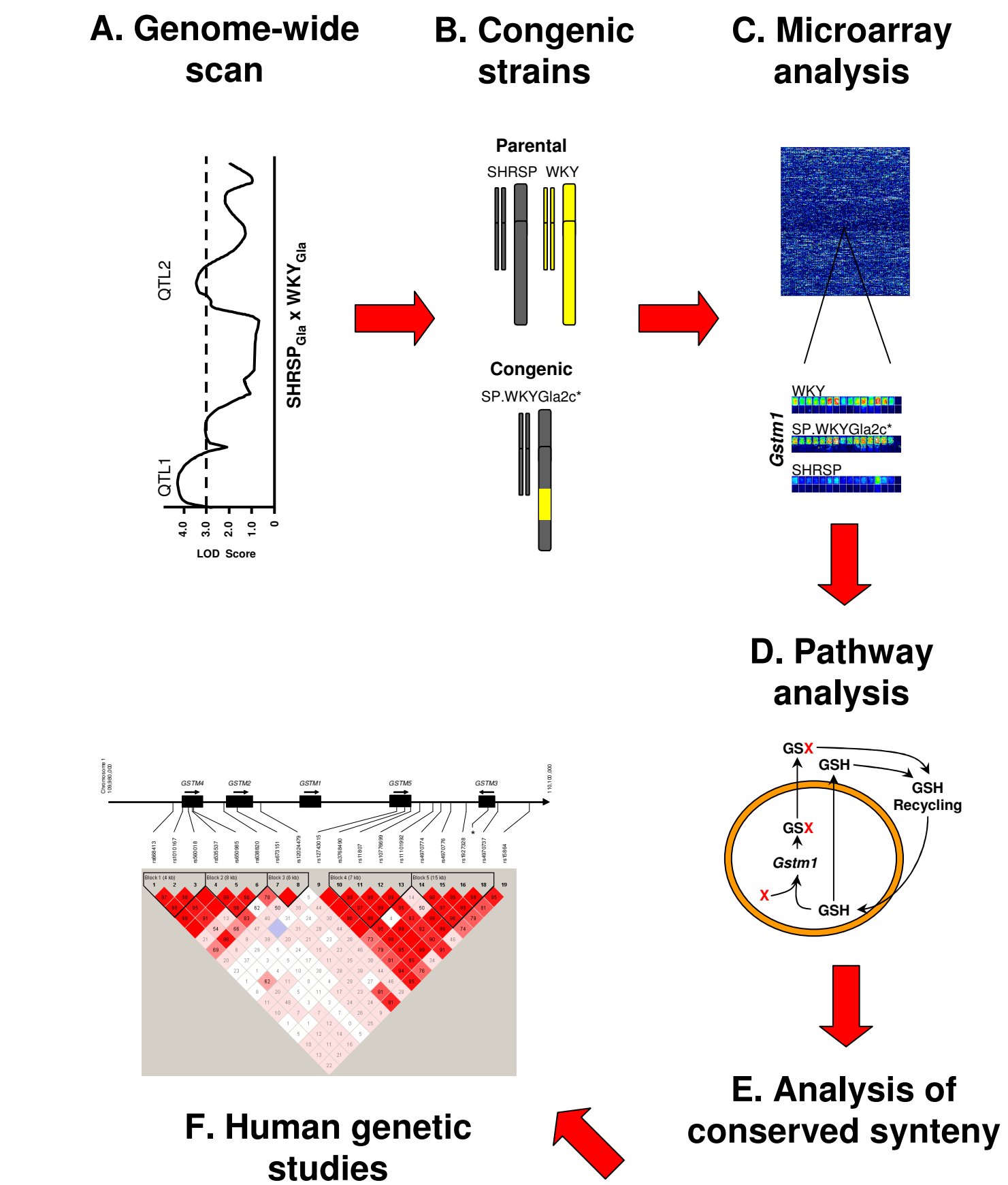

A. Genome-wide scan
B. Congenic strains
C. Microarray analysis
QTL2
QTL1
SHRSP$_{Gla}$ x WKY$_{Gla}$
4.0
3.0
2.0
1.0
0
LOD Score
Parental
SHRSP
WKY
Congenic
SP.WKYGla2c*
WKY
SP.WKYGla2c*
SHRSP
Gstm1
D. Pathway analysis
GSX
GSH
GSH Recycling
GSX
Gstm1
X
GSH
E. Analysis of conserved synteny
F. Human genetic studies
GSTM4
GSTM2
GSTM1
GSTM5
GSTM3
Rat Chr 2
Csf1
Gstm5
Gstm3
Gstm class
Gstm4
Gstm2
Gstm1
Gstm class
GNAT2
AMPD2
GSTM4
GSTM2
GSTM1
GSTM5
GSTM3
NM_139053
Human Chr 1

Figure 2