Dissecting the genetics of complex traits: lessons from hypertension*

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Summary of key findings

Newton-Cheh et al. (doi: 10.1038/ng.328) tested the hypothesis that the common variants in the candidate genes coding for the atrial natriuretic peptides (ANP) and brain natriuretic peptides (BNP) (NPPA and NPPB) are associated with blood pressure [1]. Firstly, in 1705 subjects from a general population cohort, 13 variants (single-nucleotide polymorphisms, SNPs) in this genetic locus were selected for association with higher ANP and BNP levels. Subsequently, a meta-analysis involving 14,743 Caucasian subjects confirmed the association for three SNPs. Next, in 29,717 subjects, these SNPs were found to be associated with lower blood pressure and lower risk for hypertension. The strongest effects were found for the SNPs coded rs5068 (minor allele frequency 6%) and rs198358 (19%) with reductions per allele of 0.9–1.5 mmHg systolic blood pressure (SBP) and 0.3–0.8 mmHg diastolic blood pressure (DBP), and odds ratios for hypertension of 0.85 and 0.90, respectively.

The Global Blood Pressure Genetics (Global BPgen) consortium performed a genome-wide association study (GWAS) of 2.5 million tested and imputed SNPs for association with blood pressure in 34,433 subjects from population-based cohorts (Newton-Cheh et al., doi: 10.1038/ng.361) [2]. Firstly, GWAS identified two SNPs reaching genome-wide significance (i.e. \( P < 5 \times 10^{-8} \)) and 24 SNPs just below genome-wide significance. Next, 12 SNPs were selected for confirmation by direct genotyping in an additional 71,225 European subjects, and 10 loci were tested in silico in the populations of the Cohorts for Heart and Aging Research in Groningen (CHARGE) consortium. Thus, eight SNPs were confirmed. Of these SNPs, the minor allele frequency was 9% or higher, the effect size per allele was up to 1.16 mmHg and \( r^2 \) ranged from 0.03 to 0.09. The aggregate effect accounted for \( 0.8 \)–\( 1.5 \) mmHg systolic blood pressure (SBP) and 0.3–0.8 mmHg diastolic blood pressure (DBP), and odds ratios for hypertension of 0.85 and 0.90, respectively.

CYP1A2 (cytochrome P450), FGF5 (fibroblast growth factor; cell growth and proliferation), SH2B3 (possibly: inflammatory signalling in endothelial cells), MTHFR (homocysteine metabolism), C10orf107 (unknown), ZNF652 (zinc finger protein) and PCLCD3 (vascular smooth muscle signalling), as well as the above natriuretic peptide genes.

Brief review of the field

Genetic factors account for 30–60% of the variability in blood pressure [3]. Candidate gene studies identified ~20 rare mutations (minor allele frequency <0.1%) with a strong effect on blood pressure in recent years [4]. These typically affect renal sodium handling and cause rare familial hypertensive syndromes, or hypotension. These mutations also affect blood pressure in heterozygous carriers, but by their rarity are not main determinants of blood pressure in the population [5]. Despite many candidate gene studies, most of the heritable variability in blood pressure in the population remains unexplained. Therefore, the combined effects of many common genetic variants with small effects per gene were proposed to be involved. Obviously, very large studies are needed to substantiate this hypothesis.

GWAS have become feasible by major recent advances in genetic technology, in particular the mapping of the variability of the human genome in the HapMap project (http://www.hapmap.org), and chip technology enabling genotyping of \( >100 \) 000 SNPs per individual on a single chip. GWAS provides a hypothesis-free discovery tool for the dissection of common complex traits, like blood pressure. It requires rigorous statistics and/or independent replication in different populations to preclude false-positive findings, given the enormous number of tests. GWAS successfully identified loci and candidate genes for several common traits including length, height, body mass index, diabetes, obesity and myocardial infarction [6]. Discovery of blood pressure genes still lagged behind, allegedly due to the high moment-to-moment variability (measurement error) relative to the small effects per gene, and according-

Advance Access publication 25 February 2010
ly required huge sample sizes. By virtue of unequalled sample sizes, the Global BPgen and CHARGE consortia [7] now succeeded in identifying the first common genetic variants associated with blood pressure. This generated a large number of new candidate genes that will require functional studies to support a pathophysiologic role.

Currently, the ongoing globe-wide collaboration between hundreds of researchers in even larger GWAS meta-analyses will allow the detection of additional loci, with even smaller effects. The rapid developments in technology, including high-density chips, high-density imputation and improved sequencing techniques, will allow better resolution of markers associated with the disease locus in the near future. This will help prioritization of candidate genes. Prioritization is crucial given the expanding number of candidate genes with however small effects and the only modest overlap between results of different GWAS [2,7,8].

The natriuretic peptide study illustrates the effort required to properly follow up on just a single hit, even when an intermediate phenotype, i.e. natriuretic peptide level, is available to facilitate the hypothesis-driven analysis. For many candidate genes, such an intermediate phenotype is not available, and practical and financial hurdles will likely preclude follow-up of many current and future GWAS hits. Pathway analysis may provide an alternative strategy to follow up on GWAS data [9].

GWAS data from population isolates are of interest for their greater genetic and lifestyle homogeneity. A GWAS study in Amish identified a novel candidate gene for blood pressure, STK39, that replicated in non-Amish as well. This serine/threonine kinase regulates the Na+/Cl cotransporter NCCT in the distal convoluted tubule and is a phosphorylation target for upstream WNK kinases [10]. Rare mutations in WNK kinases are associated with pseudohyoparathyroidism type II, an autosomal dominant form of sodium-sensitive hypertension and hyperkalaemia, and common mutations contribute to blood pressure variability in the population [11]. So, rare and common mutations share common pathways here, and modulation of WNK kinases may hold promise for antihypertensive intervention. Of note, the association between STK39 and blood pressure was stronger when sodium intake was taken into account [10].

For several traits, including blood pressure, quality of phenotyping poses a limitation to GWAS, as the sheer sample size needed for GWAS usually does not allow detailed clinical phenotyping. In most available studies, blood pressure is biased by antihypertensive treatment in subjects with high blood pressure. This is accounted for by adding 10 or 15 mmHg to the observed BP as the least of several poor solutions such as ignoring treatment or excluding treated subjects [12]. Data from longitudinal cohorts starting in childhood [11] or newly discovered untreated hypertensives [13] can circumvent this bias, but are sparse. Data on modifiable environmental factors such as sodium intake are usually lacking.

Thus, while proving impetus to analyses of common complex traits, GWAS have distinct limitations that may considerably hamper the eventual clinical relevance of their outcomes. These relate to quality of the phenotypes as well as limitations inherent to complex trait genetics. GWAS have only limited power to detect rare variants and recent mutations that may account for a considerable part of the heritable fraction. In fact, the discovery of more and more loci with smaller and smaller effects—the current trend—only modestly decreased the unexplained part of the heritable fraction of blood pressure variability, and it is unlikely that just increasing the sample size for GWAS will substantially reduce this unexplained part. The remainder may be due to a variety of recent, and accordingly, (very) rare mutations, or other genetic variation, such as copy number variation, or epigenetic variation. For the moment, the impact of these factors is poorly understood.

What is in it for the practising nephrologist?

The current data support a role for combined small effects of many common genes in the genetic component of blood pressure. Obviously, such small effects are of little relevance to the individual patient. As common variants affect many individuals however, their aggregate effects may nevertheless contribute to the overall risk in the population and public health. The discovery of novel candidate genes can furthermore serve better unravelling of the pathophysiology of hypertension.

For the individual patient, the potential of genetic studies for individual risk profiling and treatment may become relevant in the near future. It has been argued that the predictive potential of well-established clinical parameters still exceeds that of genetic markers, as shown for diabetes [14], but it is probably fair to say that genetic markers are still on the verge of their potential impact.

The association of natriuretic peptide genes (and STK39) with blood pressure is in line with those of rare mutations affecting blood pressure, being all involved in renal sodium handling one way or another. This underlines the predominant role of sodium status for blood pressure and of gene–environment interaction with sodium intake in the eventual effects of risk alleles. An intervention in sodium status can prove this assumption, as previously shown for the angiotensin-converting enzyme (ACE) (I/D) genotype [15]. From a therapeutic perspective, the intervention in sodium status by diet or diuretic can be anticipated to be particularly effective to reduce the genetically conferred risk for higher blood pressure, as already shown for genetic variants in α-adducin and WNK-1 pathways and their interaction [13]. In line with this assumption, in the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) study, the effect of chlorthalidone on event rate was related to an NPPA variant, albeit not the current ones [16]. Considering the many genes involved and their interactions within and between pathways, it will probably be fruitful to address multiple genes and pathways simultaneously [13] and thus construct specific profiles associated with natural course and therapy response. Pharmacogenomic studies, analysing outcome of available clinical trials in relation to genotypes, are currently going on and may prove useful to forward the concept of personalized medicine, hopefully to improve therapeutic efficacy and reduce adverse effects and costs [17].
Sodium-dependent hypertension is a common feature of chronic kidney disease. It would be particularly important to know whether the risk alleles for hypertension identified in the general population confer a similar or increased effect in renal patients, all the more so because sodium intake in renal patients is usually too high [18]. Alternatively, gene effects may be overruled by the effect of renal disease as such. Specific studies in renal patients are therefore warranted. Natriuretic peptide markers, in particular NT-proBNP, are widely used as markers for cardiovascular prognosis, with particular prognostic potency in renal patients [19]. It would be of interest to test whether the prognostic impact of NT-proBNP is modified by genetic variation in NPPA/NPPB.

What are the lessons for future research in nephrology?

The above studies demonstrate that genetic epidemiology has advanced to the point where dissection of complex traits, such as hypertension and renal disease and its complications, is feasible. The available studies also illustrate the challenges. GWAS as well as candidate gene studies for complex traits require not only state-of-the-art expertise in genetic epidemiology, molecular genetics and statistics but also sample sizes unheard of in nephrology, and independent replication of results. This requires new ways of organizing research and research infrastructure in large network-style collaborations. This should allow for harmonization and exchange of data to meet adequate sample sizes and to keep up with the rapid developments in expertise and technology. Accurate phenotyping and harmonization of phenotypes across different cohorts will be a critical success factor. Large-scale phenotyping of renal function in general population cohorts is not trivial, as obvious from the debates on performance on estimated glomerular filtration rate (eGFR). Longitudinal data on renal function may provide a clinically relevant way out here, by better identification of subjects with progressive renal function loss [20].

The Genomic Strategies for Treatment and Prevention of Cardiovascular Death in Uraemia and End-stage Renal Disease (GENECURE) consortium (www.genecure.eu), funded by the European Union (EU) under FP6, is currently establishing a network to facilitate genetic studies in nephrology in a collaboration of nine leading European nephrology centres. To this purpose, firstly, a data catalogue on renal cohorts that have DNA available was established along with a DNA bank. Cohort descriptions are available at http://www.genecure.eu/xcms/text/id/323. Moreover, a tool for data harmonization (denoted 'renal data-shaper') is being developed for prospective use in collaboration with Public Population Project in Genomics (P3G) (www.p3gobservatory.org), the world's leading group on description and harmonization of biobanks. Together, this will serve to provide an infrastructure for collaborative studies at the scale required for genetic studies on complex traits. GENECURE operates under the umbrella of Renal Genome Network (REGENET) (www.regenet.eu), a European network of renal investigators devoted to genetic studies [21] that will ensure the long-term management of the project. Renal investigators that have patient data and DNA available are welcome to join.

Take-home message

The major advances in genetic technology have brought the unravelling of the genetic basis of common complex traits such as hypertension and renal disease within reach, but considerable hurdles are still to be taken. The challenge for the years to come is to translate this into progress for the nephrology field with the final aim of clinical benefit for the renal patient.

Conflict of interest statement. None declared.

References

6. The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007; 447: 661–678
Forewarned is forearmed: arm with HIF activation*

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Summary of key findings

Patients suffering from end-stage kidney disease (ESKD) have three treatment options: haemodialysis, peritoneal dialysis and kidney transplantation. While a successful kidney transplant provides a good quality of life, the supply of organs for donation is always less than needed, prompting major efforts to improve early and late graft function to ensure the best use of donated organs. Hypoxia-inducible factor (HIF) is a master gene switch of a number of adaptive responses against ischaemia [1]. Kai-Uwe Eckardt’s group opened a new avenue in this field through their success in inducing an array of endogenous protective mechanisms regulated by HIF before initiation of the acute injury associated with transplantation [2].

Review of the field

Using an allogenic Fisher-Lewis rat kidney transplant model, Eckardt’s group investigated the effect of a small molecule, prolyl hydroxylase domain-containing protein (PHD) inhibitor, in organ donors. In normoxia, HIF-α undergoes proteasomal degradation, which is triggered by hydroxylation at one or two conserved proline residues. Inhibition of the initial hydroxylation by PHDs therefore leads to normoxic expression and activation of HIF. The HIF stabilizer was given to donor animals 6 h before kidney transplantation.

In the first set of experiments in the acute model (short-term follow-up of 10 days), pretreatment with the HIF stabilizer protected the donated organ and allowed better survival of the recipient animals. Because a reagent was given prior to kidney transplantation, this method may be considered ‘preconditioning’. The original preconditioning was the phenomenon that brief ischaemia treatment before the subsequent insult induced a state of resistance by initiating a cascade of biochemical events, which allowed for the upregulation of the cellular protective genes in the tissue [3]. Dating back to 2003, we reported that pharmacological preconditioning to activate HIF was effective in an ischaemia-reperfusion model of acute kidney injury [4]. Since then, we and others have confirmed successful preconditioning against ischaemia-reperfusion injury using various modalities to activate HIF [5,6]. Carbon monoxide (CO) is a powerful activator of HIF, and previous reports have also shown a protective effect of continuous CO exposure [7] or administration of a CO donor at the time of reperfusion [8] in an acute model of kidney transplantation. In their recent study, Bernhardt and colleagues showed the preconditioning effects of pharmaceutical HIF activation in an acute model of kidney transplantation.

In the case of ischaemic preconditioning, a biphasic temporal relationship exists between organ (cardiac) protection and the duration of reflow. Maximum protection...