Genetics of essential hypertension

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Essential hypertension affects 1 billion people worldwide and its genetic basis is well established. For this review we surveyed the literature on the genetics of hypertension during the past 18 months and we now report the highlights. There has been publication of the two largest genome scans for blood pressure and new loci including significant linkage to chromosome 6q have been reported. The molecular basis of Gordon’s syndrome has been partially unravelled with a dual function for WNK4 in ion transport regulation being discovered. There has also been progress in narrowing rodent quantitative trait loci using congenic approaches and several linkage peaks have now been demonstrated to have more than one loci. We also report some of the initial findings from pharmacogenetic studies.

INTRODUCTION

Human hypertension affects 1 billion people worldwide and is implicated in 7.1 million deaths each year from ischaemic heart disease and stroke (www.who.int/en/index.html). Recent alarming data suggests that by applying modern diagnostic thresholds of 140/90 mmHg, a 55-year-old with normal blood pressure (BP) has a 90% lifetime prospect of developing hypertension (1). It is clear from family and epidemiological studies that hypertension arises from a complex interplay between genetic and environmental lifestyle exposures including dietary sodium intake, excess alcohol consumption and body weight (2). In addition, twin studies and segregation analyses have shown that between one-third and one-half of the inter-individual variation of BP is heritable (2,3).

The last decade has seen substantial progress towards detection of genes underpinning several Mendelian hypertensive traits, which may present early in life with distinct phenotypes (4,5). At the same time mapping strategies and genomic screening with experimental models of hypertension have offered insights into the possible genetic architecture of the trait in humans (6). This has generated opportunities for comparative mapping of syntenic regions and loci from rodent to man (6–9). At present most published data on human essential hypertension (EH) arises from candidate gene studies and preliminary reports from several genome screens on a variety of hypertensive phenotypes (10,11). In this review we focus upon progress toward understanding the genetic basis of hypertension during the last 18 months.

GENOME SCREENS FOR BLOOD PRESSURE TRAITS IN MAN

There are now many publications describing the results of genome-wide screens for genes controlling BP. The majority have reported numerous chromosomal regions with suggestive evidence of linkage (reviewed in 10,12).

In the past 12 months the results of two of the largest genome scans completed to date have been reported: the US National Institute of Health funded Family Blood Pressure Program (FBPP) (13–16) and the Medical Research Council funded British Genetics of Hypertension (BRIGHT) study (11). The FBPP comprises four multi-centre networks (GENOA, GenNet, HyperGEN and SAPHIRe); each centre has recruited participants with different selection criteria from multiple ethnic groups. None of the four networks found any chromosomal region with genome-wide significant evidence of linkage. The highest LOD score, 2.96 was linked to diastolic BP on chromosome 1q in Caucasians using variance components analysis (15). The authors reported that there were no compelling candidate genes in this chromosomal region.

In contrast the BRIGHT study reported a principle locus for hypertension on chromosome 6q (LOD score of 3.21) that attained genome-wide significance using a locus counting method and three further loci with suggestive evidence of linkage on chromosomes 2q, 5q and 9q (11). The BRIGHT study represents the largest homogenous Caucasian resource that has been published to date (1599 families, comprising 2010 sibling-pairs). Comparison of results with the FBPP do not reveal any overlap between the regions found. In one way

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this is surprising, as we would expect some overlap within the same ethnic group. It is feasible, however, that differences in the results are attributable to subtle differences in the phenotypes that have been studied or differences in statistical power. It is important to note that only half of the FBPP recruits have been studied at this point.

In addition to the FBPP and the BRIGHT study, three other smaller genome screens were published recently. Von Wowern et al. (17) performed a genome-scan of 91 Scandinavian families with early onset hypertension. They found that one region on chromosome 14 attained genome-wide significance and a locus on chromosome 2 with suggestive linkage. A genome-scan for pulse pressure was also reported (18). In this study 26 large Utah pedigrees were studied and two regions were found to contain loci with genome-wide suggestive evidence of linkage, chromosomes 8p and 12q. This is the second genome screen reported for pulse pressure, but the results are not concordant with a smaller study in Mexican Americans (19). Once again this demonstrates the difficulties being encountered in replicating genome scan results, even with apparently the same phenotype, albeit from a different ethnic group.

Gong et al. (20) studied a large Chinese hypertensive kindred (n = 387 individuals) and a locus on chromosome 12p was discovered. This region overlaps with the region containing the gene for autosomal dominant hypertension with type E brachydactyly, a condition observed a few years ago in a Turkish kindred (21). Indeed, a recent paper has just described the genetic basis of this condition in the Turkish family; affected individuals have a complex chromosomal rearrangement involving a deletion, a reinsertion and an inversion (22).

Three candidate genes were assessed (ATP-dependent potassium channel KIR6.1, a sulfonyl urea receptor SUR2 and the phosphodiesterase PDE3A) and some functional studies were performed, but no mutations or functional effects were found in the six patients studied. The authors report that the candidate region is gene-poor, however there are some expressed sequence tags that need to be fully characterized. The cloning of this gene will be of major interest as it is possible that it will also be involved in EH.

Each genome scan reported differs in numbers, ethnicity, family types, design and the phenotyping strategy (there are studies with both hypertension as a discrete trait and those that have taken BP as a continuous variable). We have summarized the main findings of all human genome scans undertaken thus far, including pre-eclampsia to ascertain if there are any chromosomal regions that have been found in more than one study (Fig. 1). We observed all human chromosomes except 13 and 20 to have BP, hypertension or pre-eclampsia loci. Indeed chromosomes 1, 2, 8, 11, 12, 15, 16, 18 and 19 have regions that have been found in more than one study, yielding some encouraging consistent support for BP loci. Chromosome 2 has three cytogenetic intervals, 2p25.3–p16.3, 2p16.1–p12 and 2q23.3–q24.3, where more than one study has mapped a blood pressure locus. The regions found by Laivuori et al. (23) and Angius et al. (24) directly overlap, similarly further along the chromosome the regions delineated by Caulfield et al. (11) and Zhu et al. (25) completely overlap. There are also three chromosomal regions with partially overlapping data. In these intervals both BP and pre-eclampsia loci have been mapped. The use of different markers, study designs and poor precision with linkage data make it difficult to know if the studies have found the same gene. The challenge is to identify which of these loci are genuine and it will be the results of fine mapping or grid tightening in the first instance that will yield this information. The results in the main have been disappointing as very few studies reported genome-wide significant loci. However, some of the loci are likely to survive grid tightening, albeit not necessarily with augmented LOD scores; this will serve to inform direct and indirect linkage disequilibrium analyses.

THE WNK KINASES—NEW INSIGHTS INTO BLOOD PRESSURE REGULATING MECHANISMS

Mutations in the genes encoding the WNK kinases 1 and 4 cause pseudohyposaldosteronism type II or Gordon’s syndrome, a rare autosomal dominant condition that has hypertension as a cardinal phenotypic feature (5). It has been shown that deletions within intron 1 of the WNK1 gene and missense mutations in WNK4 are responsible for the condition (5). The WNK kinases are both expressed in the kidney within the distal convoluted tubule and the collecting ducts and have been implicated in net renal re-absorption, as well as net potassium and hydrogen excretion (5). Over the past year there has been a series of papers that have shed light on the mechanisms by which mutations in the WNK kinases lead to the distinct phenotype observed in Gordon’s syndrome patients (hypertension, hyperkalaemia and metabolic acidosis) and the regulation of ion transport in the distal nephron of the kidney.

The clinical features of Gordon’s syndrome can be corrected with thiazide diuretics; these drugs are specific inhibitors of the sodium chloride co-transporter (NCCT). This observation fuelled speculation that the defect in Gordon’s is due to increased activity of NCCT. Recent exciting data from Wilson et al. (26) and Yang et al. (27) have now shown that WNK 4 directly interacts with the NCCT in vitro and inhibits its activity by reducing the numbers of the receptors on the cell surface. Importantly constructs with the Gordon’s specific missense mutations lose their ability to suppress NCCT which would lead to net sodium retention. This direct interaction provides an explanation for the hypertension, the biochemical phenotype and the sodium retention observed in Gordon’s syndrome.

Hyperkalaemia is also observed in Gordon’s syndrome and in a set of elegant experiments by Kahle and colleagues it has recently been demonstrated that WNK4 also directly interacts with the potassium channel ROMK demonstrating a dual role for WNK4 in ion transport regulation (28). Using Xenopus laevis oocytes it was found that the potassium current exuded by expression of ROMK2 was inhibited by WNK4, this interaction was not kinase-dependent, and that the mechanism is via inhibition of endocytosis of ROMK. Constructs with mutations causing Gordon’s syndrome (Q562E and E559K) were found to increase inhibition of ROMK. The clinical picture of Gordon’s patients with mutations in WNK4 now has an explanation at the molecular level. Although further work is required to determine other regulators of WNK4 and its molecular interactions, this illustrates how the combination of genetics and functional genomics can offer access to unexpected biochemical interactions.
The exact mechanism by which mutations in WNK1 cause Gordon’s syndrome has yet to be fully elucidated. This protein is expressed in several epithelia and in vitro it has been observed that it prevents WNK4 interacting with the NCCT (27,29). In the past year the genomic structure of WNK1, its promoters and enhancers have been published and it has been demonstrated that there are several isoforms (30). WNK1 knockout mice have also been generated via a functional screening programme for genes regulating BP. Heterozygous mice were found to be viable with low BP and with no other notable phenotype; homozygous mice died during embryonic development at or around day 13 (31). Using a Taqman assay it was demonstrated that WNK1 mRNA expression was reduced by 49 and 50.1% in the kidney and the thymus, respectively, in the heterozygote. The creation of this WNK1 knockout model will undoubtedly prove to be extremely useful for helping to elucidate mechanisms by which mutations in WNK1 lead to hypertension.

**LINKAGE SCREENS IN RODENT MODELS OF HYPERTENSION**

During the 1990s crosses between hypertensive strains including the Dahl salt-sensitive rat and the spontaneously hypertensive rat, and inbred non-hypertensive strains like the Wistar Kyoto rat, Lewis and Milan normotensive strain, showed...
CONGENIC STUDIES IDENTIFY SEVERAL QTL PER LINKAGE

Linkage studies have identified many QTL, each tens of centi-morgans in length, on most rat chromosomes (6). The challenge to identify susceptible variants within these large QTL has seen attention shift to congenic mapping (35). Recently published work has focused on dissecting linked regions on RNO1 (36–43), RNO2 (44–47), RNO3 (48), RNO5 (49,50), RNO7 (51), RNO8 (52), RNO9 (53) and RNO16 (54). We will focus on the recent work on RNO10 as an illustration (41,43,55–57).

In a series of congenic lines with DNA from either the Milan normotensive strain or the Lewis rat introgressed onto the genetic background of the Dahl salt-sensitive rat, Garrett et al. (43) have identified blood pressure QTL of 2.6 and 3.2 cM separated by 24 cM on RNO10. The more terminal of these regions is only seen in congenics derived from one of the normotensive strains (Milan), implying that the other (Lewis) has a BP raising allele at this position (43). These data are supported by congenics reported by Sivo et al. (56) and later by Palijan et al. (43,57). Interestingly these congenic lines are not consistent with a role for the potential candidate genes: inducible nitric oxide synthase (Nos2), angiotensin 1-converting enzyme 1 (Ace) gene and protein kinase lysine deficient (Prkwnk4) in the hypertensive phenotype of the Dahl salt sensitive rat. As corroborative evidence, Monti et al. (58) found no difference in sequence or expression levels of Prkwnk4 between the stroke prone hypertensive or the Wistar Kyoto rat.

It is important to note that using congenic analysis several BP QTL are also seen for single linkage peaks on RNO1 (36,37,42,59,60), RNO2 (44–47), RNO3 (61) and RNO5 (49). This parallels observations made in other complex diseases such as type 1 diabetes (62,63). There are two implications for human genetics: firstly the number of hypertension loci may be more than many researchers had anticipated and secondly, in order to detect these genes, analytical techniques that can tease out closely linked, or interacting loci must be developed.

IMPLICATING INDIVIDUAL GENES AT RODENT QTL

While congenic mapping is a powerful approach it is unlikely to narrow QTLs to a region much smaller than 200 kb in size. For example, the 177 kb segment of chromosome 7 that was identified by Garrett and Rapp (51), although relatively small, contained eight positional candidate genes, one of which was aldosterone synthase Cyp11b1, the gene involved in glucocorticoid-remediable aldosteronism and human hypertension (51,64). In order to implicate one of these genes, other technologies such as transcription profiling, knockout, knockin, transgenic models and small interfering RNA (siRNA) must be used.

A combination of congenic mapping and transcription profiling was successfully used to implicate CD36 in insulin resistance (65). Recently a similar approach has been applied by McBride et al. (66), who compared the transcriptional profile in kidneys from the SHR-stroke prone rat and a congenic strain with a 22 cM region of WKY chromosome 2. They identified a single transcript, glutathione S-transferase μ-type 2 (Gstm2) up-regulated in the normotensive lines (66). It remains to be shown if this effect contributes to the hypertensive phenotype or is a secondary phenotype (66).

Knockout, knockin and transgenic models have yet to be used to help resolve the contribution of individual genes within a congenic region to hypertension, although there has been much interest in their use in understanding genes from known pathways particularly the renin–angiotensin system. Likewise siRNA (67) has yet to be used systematically to target gene expression from a congenic region. However, the first reported use of this technology in hypertension suggests it may be a powerful new tool for unravelling hypertension genetics. Snyder et al. (68,69) used siRNA to block expression of Nedd4 and Nedd4-2, both regulators of the renal epithelial sodium channel (ENaC), the gene mutated in Liddle’s syndrome. It was shown that Nedd4-2 regulates ENaC levels in a steroid hormone-dependent fashion (69). Moreover, this effect was ameliorated in cell lines expressing a variant allele of ENaC from an individual with Liddle’s syndrome (69).

Development of these approaches and others in a targeted systematic way will be required to understand the role of individual genes within congenic intervals.

PHARMACOGENETICS IN HYPERTENSION

The latest studies of non-pharmacological approaches and meta-analysis of drug-based intervention trials indicate that there are several effective strategies that can be deployed to reduce BP (70,71). In spite of guidelines encouraging the pursuit of good blood pressure control by use of several concurrent medications, there is strong evidence that BP control is sub-optimal in two-thirds of hypertensives (www.nhlbi.nih.gov/guidelines/hypertension/, www.hyp.ac.uk/bhs/) (72). One of the problems we face is our limited ability to tailor selection of medication to maximize efficacy and minimize adverse reactions in an individual.
The available evidence on the potential of pharmacogenetics to enable prediction of therapeutic effect on BP has recently been evaluated (73). Many of the initial studies have included small numbers, relied on single dosing rather than chronic dosing, used single nucleotide polymorphisms (SNPs), instead of haplotype tag SNPs and have not controlled for exposures, such as dietary sodium, which could attenuate or enhance responsiveness (73).

Pharmacogenetic studies in hypertension have begun to report larger sample sizes. Recently BP response to a thiazide diuretics was evaluated in 291 unrelated Hispanic men and women using SNPs which may be of functional importance within four common cardiovascular candidate genes (74). After adjustment for covariates only the endothelial nitric oxide synthase GLU298ASP variant was associated with a small BP response to hydrochlorothiazide (74). In a separate study the prediction of BP response to irbesartan or atenolol in reduction of left ventricular hypertrophy was tested by simultaneously genotyping 74 SNPs drawn from multiple candidate genes, which might influence target response, using a minisequencing microarray approach in the 97 hypertensive participants (75). The authors acknowledge the difficulty of appropriate adjustment of statistical analysis to account for multiple hypothesis testing and have accordingly accepted a greater stringency for statistical significance (75). The results indicate that plausible candidate gene SNPs from the alpha 2 adrenoceptor and beta 2 adrenoceptor SNPs predicted response to atenolol, whereas angiotensinogen, angiotensin-converting enzyme and aldosterone synthase SNPs were related to irbesartan response (75). This type of high-throughput approach incorporating comprehensive analysis of haplotype tagging SNPs in multiple candidates that might affect target response or metabolism of antihypertensives is beginning to be employed in extensive repositories of hypertensives associated with large-scale endpoint trials.

CONCLUDING REMARKS

At this time it is of note that all of the genes responsible for a hypertensive phenotype have come from studies of monogenic diseases, and all are kidney-specific, operating by affecting sodium balance. There are no positionally cloned genes identified to date in either rodent models or the human hypertension phenotype. There can be no doubt that linkage data published thus far in both human and animal models of hypertension attest to the complex polygenic nature of the disorder. In addition, there is still no evidence that BP loci identified in rodents and humans will be the same, but it seems plausible that each linkage peak in the rodent may comprise several QTL and this may also hold true for the human condition. This coupled with the lower power of linkage screens means that the number of human hypertension loci is most probably in the order of tens of genes. Recently reported sub-pair genome screens suggest that there are no genes with a major effect. Accordingly, it is likely that we are looking for many genes with a genotype relative risk of 1.2–1.5. The current challenge is to confirm linkage peaks and identify disease predisposing variants using new resources that are becoming available, for example the Hapmap project (76) and new genotyping technologies. We also need to gear up for genome-wide association studies with appropriate power to detect genes with a lower relative risk, this will ultimately enable comprehensive pharmacogenetic studies.

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