Molecular genetics of human hypertension

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ABSTRACT

EH (essential hypertension) is a major public health problem in many countries due to its high prevalence and its association with coronary heart disease, stroke, renal disease, peripheral vascular disease and other disorders. Epidemiological studies have demonstrated that EH is heritable. Owing to the fact that blood pressure is controlled by cardiac output and total peripheral resistance, many molecular pathways are believed to be involved in the disease. In this review, recent genetic studies investigating the molecular basis of EH, including different molecular pathways, will be highlighted.

INTRODUCTION

EH (essential hypertension) is a major public health problem in many countries due to its high prevalence and its association with coronary heart disease, stroke, renal disease, peripheral vascular disease and other disorders [1]. EH is regarded as a multifactorial condition, the onset and severity of which are influenced by both genetic and environmental factors. The role of genetic factors in the aetiology of hypertension is supported by cross-sectional studies that document familial aggregation of the disorder despite different environmental factors [2,3]. Twins and adoption studies have indicated a greater degree of trait concordance among identical compared with dizygotic twins [4] and among natural compared with adoptive siblings [5] respectively, which also stress the importance of genetic factors [6]. The exact form of the underlying genetic mechanism remains unanswered. Estimates of genetic variance range from 20–50% [6–8]. The identification of variant genes that contribute to the development of hypertension is complicated by the fact that the two entities that determine BP (blood pressure), namely cardiac output and total peripheral resistance, are themselves controlled by other intermediary phenotypes, including the autonomic nervous system, vasopressor/vasodepressor hormones, the structure of the cardiovascular system, body fluid volume, renal function and many others (Figure 1). Identification of genes underlying BP variation has the capacity to define primary physiological mechanisms causing this trait, thereby clarifying disease pathogenesis, establishing molecular diagnostics and developing a novel therapy for hypertension [9]. Substantial progress has been made in the last decade towards detection of genes underpinning several Mendelian forms of hypertension traits, which may present early in life with distinct phenotypes [9].

Key words: blood pressure, candidate gene, genetics, hypertension, molecular pathway, renin–angiotensin–aldosterone system (RAAS).

Abbreviations: ACE, angiotensin-converting enzyme; AGT, angiotensinogen; AME, apparent mineralocorticoid excess; Ang II, angiotensin II; AT1R, type 1 Ang II receptor; BMI, body mass index; BP, blood pressure; CI, confidence interval; CYP11B1, steroid 11β-hydroxylase; CYP11B2, aldosterone synthase; D1, dopamine 1; DD, deletion/deletion; EH, essential hypertension; ENaC, epithelial sodium channel; ER, oestrogen receptor; FHH, familial hyperkalaemia and hypertension; GRA, glucocorticoid remediable aldosteronism; GREB1, gene regulated by oestrogen in breast cancer 1; GRK4, G-protein-coupled-receptor kinase 4; GST, glutathione-S-transferase; GSTM1, glutathione S-transferase M1; HPCAL1, hippocalcin-like 1; 11β-HSD2, 11β-hydroxysteroid dehydrogenase 2; HTNB, hypertension and brachydactyly; ID, insertion/deletion; EH, essential hypertension; ENaC, epithelial sodium channel; ER, oestrogen receptor; FHH, familial hyperkalaemia and hypertension; GRA, glucocorticoid remediable aldosteronism; GREB1, gene regulated by oestrogen in breast cancer 1; GRK4, G-protein-coupled-receptor kinase 4; GST, glutathione-S-transferase; GSTM1, glutathione S-transferase M1; HPCAL1, hippocalcin-like 1; 11β-HSD2, 11β-hydroxysteroid dehydrogenase 2; HTNB, hypertension and brachydactyly; ID, insertion/deletion; JSNP®, Japanese Single Nucleotide Polymorphisms; LOD, logarithm of the odds; L-PGDS, lipocalin-type prostaglandin D synthase; mtDNA, mitochondrial DNA; NHE, sodium/hydrogen exchanger; OR, odds ratio; PTP1B, protein phosphatase 1B; RAAS, renin–angiotensin–aldosterone system; SBP, systolic BP; SHR, spontaneous hypertensive rat; SNP, single nucleotide polymorphism; UCP, uncoupling protein; WNK, with no K (lysine) protein kinase.

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Currently, most published data on human EH arises from association-based studies and genome-wide screens [10]. In this review, we focus on recent progress in the understanding of genetic basis of hypertension.

**MENDELIAN FORMS OF HYPERTENSION**

Molecular genetic studies have identified mutations in eight nuclear genes and one mitochondrial gene that cause Mendelian forms of hypertension (Table 1). They include CYP11B1/CYP11B2 (genes encoding steroid 11β-hydroxylase/aldosterone synthase; on chromosome 8p) in GRA (glucocorticoid remediable aldosteronism) [11], SCNN1B and SCNN1G (genes encoding for β- and γ-subunits of ENaC (epithelial Na⁺ channel) respectively; on chromosome 16p) in Liddle’s syndrome [12–15], HSD11B2 (gene encoding 11β-HSD2 (11β-hydroxysteroid dehydrogenase 2); on chromosome 16q) in the syndrome of AME (apparent mineralocorticoid excess) [17,18], NR3C2 (gene encoding MR (mineralocorticoid receptor); on chromosome 4q) in hypertension exacerbated in pregnancy [19] and mutated genes of the serine/threonine protein kinases, WNK1 [with no K (lysine) protein kinase 1; on chromosome 12p] and WNK4...
(on chromosome 17q), cause pseudohypoaldosteronism type II [also known as FHH (familial hyperkalaemia and hypertension) or Gordon’s syndrome] [20]. The molecular basis of the former four syndromes has been reviewed in detail by Lifton et al. [9]. Extensive studies of FHH by Mayan et al. [21] have found that affected subjects with WNK4 Q565E mutations have hypercalciuria accompanied by lower serum calcium levels, supporting a mechanism of renal calcium wasting. Together with the observation that WNK4 regulates the renal outer medullary potassium channel, as well as epithelial chloride/base exchange and the sodium/potassium/chloride co-transporter, the interaction between WNK4 and a calcium channel or transport was suggested [21]. Most of these disorders are due to defective genes acting in the same physiological pathway in the kidney, altering net renal salt reabsorption [9].

Hypertension and dyslipidaemia cluster more often than expected for the risk of many common cardiovascular diseases, i.e. myocardial infarction, congestive heart failure and stroke [22]. A cluster of metabolic defects caused by mutation in a mitochondrial tRNA was identified in one large Caucasian kindred by Wilson et al. [23]. The kindred features a cluster of hypertension, hypercholesterolaemia and hypomagnesaemia. Direct sequencing and SSCP (single-strand conformational polymorphism) analysis of the entire mitochondrial genome were performed and a novel mutation conferring a uridine → cytidine transition was identified at nucleotide 4291 of the MT-T1 gene (mitochondrial tRNA11e). The mutation occurs immediately 5′ to the tRNA11e anticodon. Uridine at this position is one of the most conserved bases. Biochemical studies with anticodon stem–loop analogues of tRNA have been performed and indicate that substitution of cytidine for uridine at this position markedly impairs ribosome binding [24]. Thus the authors [23] speculated that the complexity can arise from a single mutation because of the combined effects of reduced penetrance and pleiotropy and underlines the value of studying very large kindreds. One further Mendelian form of hypertension – HTNB (hypertension and brachydactyly) – has been mapped to a defined chromosomal region 12p12 [25], but the molecular basis of the underlying gene still awaits identification. This genetic region nearly overlaps with a later whole-genome-scan linkage analysis for EH in a large Chinese pedigree [26], which indicates the susceptibility gene(s) for EH may reside on chromosome 12p12.

Even though these rare syndromes with Mendelian inheritance only account for a small fraction of the pathological BP variation in the general population, they provide new insight into the pathophysiology of hypertension. The identification of the molecular mechanisms of BP variation also has implications for the development and use of antihypertensive treatments that need not be restricted only to individuals with Mendelian forms of hypertension [9].

**EH**

**Association studies in hypertension**

**RAAS (renin–angiotensin–aldosterone system)**

The human AGT gene (coding for angiotensinogen), an AGT precursor of the system, encodes a hepatic protein cleaved by renin and digested further by ACE (angiotensin-converting enzyme) to generate the physiologically active Ang II (angiotensin II). Ang II, via presynaptic AT1Rs (type 1 Ang II receptors), potentiates the release of noradrenaline (norepinephrine). This peptide, together with aldosterone, which is generated in the adrenal zona glomerulosa by CYP11B2, maintains the circulating plasma volume that, in turn, through stimulation of cardiopulmonary and arterial mechanoreceptors, may influence sympathetic tone and increase heart rate variability [27]. Due to the important role of RAAS in the regulation of water and sodium balance as well as BP [28], numerous studies have investigated the relationship between RAAS and EH [29–35]. Two genes of the renin–angiotensin system have provided evidence for association. The AGT gene variant M235T is associated with higher circulating AGT levels and EH in several, but not all, populations [31,33,35,36]. The ACE gene was also implicated in the aetiology of hypertension. The gene-coding area carries an ID (insertion/deletion) polymorphism within intron 16. Some studies have shown that this polymorphism is strongly associated with increased BP in males [37–39]; however, a negative association was also detected in some linkage and association studies [29,40]. The relationship between EH and the genes encoding AGT, ACE as well as AT1R was studied in 173 hypertensive individuals and 193 normotensive Chinese Tibetans individuals. The AGT M235T and the promoter G – 6A polymorphisms showed association with EH in Tibetan women. No association could be detected for polymorphisms in ACE and AT1R with EH [41]. The AGT M235T allele was demonstrated to be in linkage disequilibrium with allelic variants in the AGT promoter region (G – 6A and A – 20C), which may affect the basal rate of AGT transcription and could account for phenotypic variation in plasma AGT concentrations [42–45]. The relationship between ACE and environmental factors predisposing to EH has been investigated in 1099 subjects from one Mongolian population. The study claimed evidence for an interaction between the ACE DD (deletion/deletion) and ID polymorphism and cigarette smoking, alcohol drinking and BMI (body mass index) [46]. As RAAS plays important roles in the regulation of water and sodium balance, the α-adducin Gly460Trp variation is also believed to induce significant differences in the activity of the Na+/K+ ATPase which, in the renal proximal tubule, affects sodium reabsorption [47]. One study in a Chinese Han population (479 subjects from 125 nuclear families) revealed that ACE ID, α-adducin Gly460Trp
and CYP11B2 − 344C/T polymorphisms interact to influence SBP (systolic BP; \( P < 0.05 \)), suggesting these genes might indeed predispose to hypertension, especially in an ecogenetic context characterized by high salt intake \cite{48}. The ACE2 gene, a homologue of ACE, has been recently discovered \cite{49}. ACE2 appears to be a negative regulator of ACE in the heart \cite{50}. A case-control study investigating four SNPs (single nucleotide polymorphisms) of ACE2 and EH provided no evidence for an association in an Anglo–Celtic Australian population \cite{51}. In this study, the 152 hypertensive subjects studied were the offspring of parents who both had hypertension, and similarly the 193 normotensive subjects were from normotensive parents over the age of 50 years, which could have high inherently biological power. However, the data indicate little support for ACE2 in genetic predisposition to EH \cite{51}.

Bozec et al. \cite{52} studied mechanical properties of the carotid artery according to their AGT M235T genotype in 98 never-treated hypertensive patients (aged 24–80) and in Agt mutant mice. Few studies have investigated the effect of candidate genes of RAAS on large artery stiffness in hypertensive patients. This was the first study that has included only never-treated hypertensive patients. It is very important to exclude patients treated previously, because antihypertensive drugs may affect the arterial wall components and thus arterial stiffness. The study found that patients homozygous for T allele had a reduced carotid distensibility and an increased stiffness of the carotid wall independent of BP compared with patients homozygous for the M allele. The carotid distensibility in Agt1/2 mice, however, was not significantly different from that of Agt1/1 (wild-type). Stiffness of the arterial wall was lower in the Agt1/2 mice compared with wild-type mice. The model of mutant mice for Agt gene has been developed by Smithies and Kim \cite{53} and Kim et al. \cite{54}. Their genotypes are Agt1/1 (wide-type with two singleton copies of the gene) and Agt1/2 (one wild-type and one duplicated copy). These mice are characterized by increased BP and plasma AGT concentrations as the number of Agt gene copies increases (plasma concentrations of AGT is 24% greater in Agt1/2 than in wild-type mice) \cite{53,54}. Hence Agt1/2 mice have a genetically determined increase in plasma AGT concentrations similar to that of TT homologous patients. The greater BP was not associated with arterial hypertrophy, resulting in a greater circumferential wall stress in Agt1/2 mice \cite{52}. The in vivo and in vitro pressure responses to Ang II were reduced in Agt1/2 mice, whereas the contractile response to phenylephrine was not significantly different between Agt1/1 and Agt1/2 mice, indicating the integrity of the contractile apparatus and suggesting a dysfunction of the AT1R signalling pathways in Agt1/2 mice. In hypertensive patients in whom treatment was stopped at least 3 weeks before investigation, Benetos et al. \cite{55} found a positive association between the wave velocity, a marker of aortic stiffness, and both the AT1R A1166C and ACE II/DD polymorphisms. These results suggest that the AGT 235TT genotype could be a genetic marker for arterial stiffness in never-treated hypertensive patients; the arterial wall hypertrophy and stiffening in AGT 235TT patients are probably mediated by an increased stimulation of AT1R, whereas the opposite carotid phenotype of Agt1/2 mice is probably the result of a dysfunction on the AT1R pathways \cite{52}.

**Genes acting on pathways for BP regulation outside the RAAS**

An ever-expanding repertoire of genes outside of the RAAS has been tested for involvement in the genetic basis of EH (Table 2). A number of studies have shown a correlation between hyperinsulinaemia, insulin resistance and hypertension \cite{56}. Speirs et al. \cite{57} tested several novel potential candidates, namely, GRK4 (gene encoding G-protein-coupled-receptor kinase 4), HSD3B1 (gene encoding 3β-hydroxysteroid dehydrogenase/isomerase 1) and PTP1B (gene encoding protein phosphatase 1B) genes in 168 Caucasian EH patients and 312 normotensive controls. The regulation of sodium excretion by the kidney is of paramount importance for homoeostasis of the extracellular fluid volume and thereby of arterial BP. GRK4 was implicated in human hypertension by desensitization of G-protein-coupled receptors, including the D1 (dopamine 1) receptor \cite{58–60}. In humans with EH, there is a decrease in the responsiveness of the D1 receptor in proximal tubules due to the uncoupling of the D1 receptor from its G-protein–effector enzyme complex \cite{59,61}. 3β-Hydroxysteroid dehydrogenase/isomerase 1 plays a role in the biosynthesis of steroid hormones, including aldosterone \cite{62}. It has been proposed that allelic variations in the HSD3B1 gene could lead to elevated plasma aldosterone, resulting in an increased intra-vascular volume and hypertension \cite{63}. PTP1B negatively regulates insulin signalling via receptor dephosphorylation \cite{64}. However, no association between variants in HSD3B1 and PTP1B genes and hypertension could be detected \cite{57}. In contrast, the V allele of the A486V variant of GRK4 \( \gamma \) showed association with elevated BP \( (P = 0.02 \) for EH). Zhu et al. \cite{65} studied the relationship between the SLC9A3 gene [coding for NHE (sodium/hydrogen exchanger) 3] and EH in 399 subjects of African or Afro–Caribbean origin (68% with EH) and 292 subjects Caucasian origin (50% with EH), trying to examine the relationship with hypertension and biochemical indices of sodium balance. Six variants were identified in total. NHE3 is a member of an increasing number of NHEs responsible for transport of sodium and hydrogen ions across the proximal tubule \cite{66}. Moreover, animal studies highlight that this class of genes has potential importance in the control of BP \cite{67–69}; however, no association between the variants was detected in EH patients from either African and Afro–Caribbean origin or Caucasian origin \cite{65}. Gain-of-function mutations in the \( \beta \)- and
$\gamma$-subunits of ENaC cause the monogenic form of hypertension known as Liddle’s syndrome [12–15]. One recent investigation in a Finnish population [70] has shown a higher prevalence of three ENaC variants ($\beta$ENaC G589S, $\beta$ENaC i12–17CT and $\gamma$ENaC V546I) in 347 hypertensive patients compared with 175 normotensive individuals and 301 randomly chosen blood donors ($P < 0.01$). When frequencies of the individual gene variants in the hypertensive patients were compared with those in the other two groups combined, only the frequency of the $\beta$ENaC i12–17CT variant was significantly higher among the hypertensive patients than in the other two groups ($P = 0.001$), whereas there was no significant difference in the prevalence of $\beta$ENaC G589S and $\gamma$ENaC V546I variants between the hypertensive and control groups. Patients carrying the three variant alleles also had an increased urinary potassium excretion rate in relation to their renin levels ($P = 0.034$). However, no change in activity of the two ENaC amino acid variants was detected when they were expressed in Xenopus oocytes compared with wild-type ENaC [70].

Chromosome 2p24–p25 has been shown to be linked with hypertension in several studies [71–73]. A large study was thus carried out in a Japanese general population investigating the association of polymorphisms in this region with BP. Forty-seven polymorphisms in 14 genes in the region between D2S2278 and D2S168 and in the region just outside of these two markers (between nucleotides 8845292–11946689) were genotyped in 1880 individuals, 796 of whom were hypertensive and 1084 normotensive [74]. Multivariate logistic regression analysis with adjustment for age, BMI, hyperlipidaemia, diabetes mellitus, smoking, drinking and antihypertensive medication identified 11 SNPs in three genes that were associated with hypertension using a dominant or recessive model ($P < 0.05$). From them, only one SNP in the HPCAL1 gene (coding for hippocalcin-like 1) in women ($P = 0.003$) and two SNPs in the GREB1 gene (coding for gene regulated by oestrogen in breast cancer) in men ($P = 0.008$) had a significant association with susceptibility to hypertension and BP modulation [74]. SBP in women with the AA + AC genotype of the positively associated SNP IMS-JST 126186 [JSNP® (Japanese Single Nucleotide Polymorphisms) database] in the HPCAL1 gene was 16.7 mmHg higher than that with the CC genotype ($P = 0.003$). HPCAL1 shares 94% amino acid identity with hippocalcin, which functions as a neuronal calcium sensor and possesses a Ca$^{2+}$/myristoyl switch allowing it to translocate to the membrane [75]. The SBP in men with GG + GC genotypes of IMS-JST 149391 in the GREB1 gene was 9.2 mmHg higher than in those with the GG genotype ($P = 0.008$) and was 9.2 mmHg higher in men with the AA + AG genotype of IMS-JST 149390 in the GREB1 gene than in those with the GG genotype ($P = 0.008$). The two SNPs in the GREB1 gene were in tight linkage disequilibrium. GREB1 was identified as a direct target gene of ER (oestrogen receptor) $\alpha$ and is evolutionarily conserved compared with mouse genome [76,77]. Oestrogen has depression effects through the improvement of endothelial dysfunction [78] and modulation of sympathetic nerve activation [79] in animal experiments, and oestrogen

### Table 2: Summary of studies of candidate genes acting on pathways for BP regulation outside the RAAS

<table>
<thead>
<tr>
<th>Candidate genes</th>
<th>Molecular basis in EH</th>
<th>Polymorphisms</th>
<th>Association with EH</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRK4 [57]</td>
<td>Desensitization of G-protein-coupled receptors, including the $D_1$ receptor in proximal tubules</td>
<td>RA65L and A142V, A486V</td>
<td>No</td>
</tr>
<tr>
<td>HSD3B1 [57]</td>
<td>The biosynthesis of steroid hormones, including aldosterone</td>
<td>Leu$^{308}$ T $\Rightarrow$ C</td>
<td>No</td>
</tr>
<tr>
<td>PTP1B [57]</td>
<td>Regulating insulin signalling via receptor dephosphorylation</td>
<td>1A0BinsG</td>
<td>No</td>
</tr>
<tr>
<td>SCN1B [70]</td>
<td>A key determinant of sodium homeostasis</td>
<td>$\beta$ENaC G589S and $\beta$ENaC i12–17CT</td>
<td>Yes</td>
</tr>
<tr>
<td>SCN1G [70]</td>
<td>A key determinant of sodium homeostasis</td>
<td>$\gamma$ENaC V546I</td>
<td>No</td>
</tr>
<tr>
<td>GREB1 [74]</td>
<td>Depressor effect through the improvement of endothelial dysfunction and modulation of sympathetic nerve activation</td>
<td>$\rightarrow$ 13945A $\Rightarrow$ T and 45718A $\Rightarrow$ G</td>
<td>Yes in men</td>
</tr>
<tr>
<td>HPCAL1 [74]</td>
<td>Protection of neurons against calcium-induced death stimuli in co-operation with neuronal apoptosis inhibitor protein</td>
<td>IMS-JST 126186 A $\rightarrow$ C</td>
<td>Yes in women</td>
</tr>
<tr>
<td>BDNR1 [89]</td>
<td>Activate the arachidonic acid nitric oxide cascade</td>
<td>SNPs in NT 026437, 76646507, 76623594 and 76647595</td>
<td>Yes in AC</td>
</tr>
<tr>
<td>BDNR2 [89]</td>
<td>Activate the arachidonic acid nitric oxide cascade and affect the insulin-dependent glucose transport/utilization</td>
<td>$\rightarrow$ 5BC/T</td>
<td>Yes in AA</td>
</tr>
<tr>
<td>CAT [84]</td>
<td>Reduce smooth muscle cell contraction and proliferation induced by endothelia, Ang II and $\alpha$-adrenoreceptor agonists</td>
<td>$\rightarrow$ B44 A/G and C/T</td>
<td>Yes in GC</td>
</tr>
</tbody>
</table>
insufficiency may be related to postmenopausal hypertension [80]. Genetic variation in ERα has been associated with coronary artery wall atherosclerosis and stroke [81,82]. ERs are required for normal vascular physiology in males [83] and oestrogen has direct vasodilator properties in men, as it does in women, but the relevance of this remains to be understood. Thus the authors concluded that GREB1 might play a role in BP regulation [74].

Genetic heterogeneity may exist in different populations for the genesis of hypertension. One association was assessed between the SNPs in the promoter region of the CAT gene (coding for catalase) and EH in Greek Caucasians and African-Americans. An association was found with the specific genotype combination of CAT−844 homozygous AA together with CAT−262 CT or TT in Caucasians only (100 hypertensive and 93 normotensive subjects; \( P = 0.0339 \)), whereas no association was observed in African–Americans (129 hypertensive and 98 normotensive subjects) [84]. The role of oxidative stress in hypertension has been tested in a number of studies [85]. Catalase, a protein converting H₂O₂ into water and oxygen, has been shown to reduce smooth muscle cell contraction and proliferation induced by endothelia, Ang II and α-adrenoreceptor agonists [86]. Experimental studies have shown a protective role of higher catalase expression levels in hypertensive animal models [87,88]. Similarly, one SNP in the \( BDKRB2 \) gene (coding for bradykinin receptor B₂) and three SNPs in the \( BDKRB1 \) gene (coding for bradykinin receptor B₁) were associated with hypertension in American Caucasians (\( n = 220; \) \( P \) values were between 0.026 and 0.0004). One SNP in the promoter region of \( BDKRB2 \) gene was associated with hypertension in African–Americans (\( n = 218; \) \( P = 0.044 \)) [89]. Bradykinin has a variety of vasoactive and metabolic effects, including vasodilation via interaction with components of the arachidonic acid cascade [90] and enhancing insulin-independent glucose transport [91] through B₁ and B₂ receptors. Genetic variations of the receptors may alter the function capacity of bradykinin, which may thus alter an individual susceptibility to hypertension.

The results described above suggest that individual SNPs may not be as important as the interaction among several SNPs. The genetic factors that contribute to hypertension are likely to be different among different ethnic populations. Further studies of association in a large number of genes in different pathways will be required to identify the possible interaction among genes and the full array of genetic factors causing hypertension.

Genetic variants underlying hypertension may represent an independent risk factor for development of hypertension-developed end-organ damage

Patients with hypertension are at increased risk of cardiovascular diseases. Epidemiological studies show that the risk of cardiovascular disease mortality and morbidity is much higher in hypertensive patients compared with normotensive people. Whether the genetic variants causing hypertension have an additive effect on pathways related to the risk for developing end-organ damage has been studied recently. Fabris et al. [92] studied variants from AGT M235T, ACE ID, AT;R A1166C and CYP11B2 −344C/T in 86 hypertension patients with renal insufficiency and 172 hypertensive patients without renal damage matched for age and hypertension. The study was followed for 2 years and investigated whether these variants may act synergistically and confer an increased risk for renal failure in hypertensive patients. AGT TT/AT;R AC (\( P = 0.0018 \)) and CYP11B2 CC/ACE DD (\( P = 0.0012 \)) showed a positive interaction in the development of renal insufficiency among hypertensive patients, and the association of AGT MM/AT;R AA (\( P = 0.04 \)) and AGT MM/AT;R AA/CYP11B2 TT (\( P = 0.04 \)) or AGT MM/AT;R AA/CYP11B2 TC (\( P = 0.03 \)) combinations were associated with a reduced risk of renal failure. The authors [92] concluded that, in patients with EH, an unfavourable allelic pattern of components of the RAAS may contribute to the increase risk for the development of renal failure.

L-PGDS (lipocalin-type prostaglandin D synthase) is a secretory protein of the lipocalin superfamily, which synthesizes PGD₂ (prostaglandin D₂) from PGH₂ (prostaglandin H₂) [93]. Patients with hypertension exhibited a higher level of L-PGDS in serum and urine [94]. Thus one Japanese group investigated the association between its variants and the severity of carotid arteriosclerosis in 782 EH patients. The study [94] suggested that the 4111A → C polymorphism in the \( PTGDS \) gene (coding for L-PGDS) is associated with the severity of carotid arteriosclerosis (\( P = 0.002 \)) and inversely correlated with increased HDL (high-density lipoprotein)-cholesterol (\( P < 0.001 \)) in Japanese hypertensive patients. However, the other variants had no relationship with the phenotype studied. The functional mechanism of the 4111A/C in L-PGDS remains unknown [95].

A small cross-sectional study in 140 normotensive subjects was carried out to ascertain the relationship between the polymorphism of the \( GSTM1 \) gene [coding for GST (glutathione S-transferase) M1], BP level and exposure to cigarette smoking. For analysis, the combination of genotypes, sex and smoking behaviour were used as qualitative variables, and age, BMI and heart rate were used as covariates. The combination ‘present-GSTT1 (GST theta 1), null-GSTM1’ genotypes [OR (odds ratio), 0.001; 95 % CI (confidence interval), 0.00–0.439; \( P = 0.025 \)], heart rate (OR, 1.065; 95 % CI, 1.018–1.114; \( P = 0.006 \)) and interaction between BMI and combination of ‘present-GSTT1, null-GSTM1’ genotypes (OR, 1.319; 95 % CI, 1.058–1.644; \( P = 0.014 \)) was detected to be associated with SBP. The results suggested
that the GSTM1 gene is one of the candidate genes altering baseline SBP in normotensive individuals when the age, sex and smoking behaviour etc. were considered [96]. The GSTs are involved in the detoxification of many toxic compounds of different chemical structures in cigarette smoke [97], whereas cigarette smoking is one of the major risk factors to cardiovascular diseases [98].

Mitochondrial genome mutations and EH

DeStefano et al. [99] studied maternal and paternal effects in the development of human EH in American Caucasians, Greek Caucasians and African–Americans. They found that, among parents with known hypertensive status, the proportion of affected mothers was significantly higher than the proportion of affected fathers in all three ethnic groups [99]. The fraction of patients with EH potentially due to mtDNA (mitochondrial DNA) mutation involvement is estimated at 55 % (95 % CI, 45–65 %) [100]. A complete sequencing of the mitochondrial genome from 20 hypertensive probands in African–American (n = 10) and Caucasian families (n = 10) was carried out. A total of 297 bp exchanges were identified, including 24 in rRNA genes, 15 in tRNA genes and 46 amino acid substitutions, with the remainder involving the non-coding regions or synonymous changes [101]. Several of these have been associated with cardiovascular and renal pathologies in previous studies [102–104]. Among them, an A10398G mutation in the MT-ND3 gene (coding for mitochondrially encoded NADH dehydrogenase 3), identified in 12 hypertensive individuals of both ethnic groups, has been shown to occur with increased frequency in African–Americans with EH associated with end-stage renal disease [102]. These mitochondrial mutation data thus can serve as a starting point for case-control association studies.

UCPs (uncoupling proteins) are inner mitochondrial membrane-associated proteins and act as proton channels or transporters. Although mitochondria use energy derived from fuel combustion to create a proton electrochemical gradient across the mitochondrial inner membrane, UCPs uncouple proton entry in the mitochondrial matrix from ATP synthesis [105]. A functional polymorphism (−866 G/A) in the UCP2 promoter has been reported to be associated with obesity in an analysis of 340 obese and 256 never-obese middle-aged Caucasian subjects (P = 0.007) [106]. Another association study between this polymorphism and obesity, hypertension as well as Type II diabetes mellitus was carried out in a Japanese population with 342 Type II diabetic patients (among them 158 patients complicated with hypertension), 156 hypertensive patients without diabetes mellitus and 134 control subjects. The polymorphism was detected to be significantly associated with hypertension (frequency of A allele, 51.8 % in hypertensives compared with 46.6 % in normotensives; P < 0.05), but was not associated with obesity in the Japanese population, which is in contrast with the significant association with obesity in Caucasians [107].

Mitochondrial coupling factor 6, an essential component of mitochondrial ATP synthase, suppresses the synthesis of prostacyclin in vascular endothelial cells [108]. The role of the gene was studied in SHRs (spontaneous hypertensive rats) [109]. In vivo, the peptide circulates in the rat vascular system, and its gene expression and plasma concentration are higher in SHRs than in normotensive controls. Functional analysis suggests it acts as a potent endogenous vasoconstrictor in the fashion of a circulating hormone [109]. Circulating coupling factor 6 is elevated in human hypertensive patients (n = 30) compared with normotensive subjects (n = 27; P < 0.01) and was increased after salt loading in hypertensive patients. The percentage changes in plasma coupling factor 6 level after salt restriction and loading were positively correlated with those in mean BP (r = 0.57; P < 0.01) and negatively correlated with those in plasma nitric oxide level (r = −0.51; P < 0.05) [109,110]. The elevated circulating coupling factor 6 in SHRs and human hypertension patients indicates that it is involved in the regulation of arterial BP in physiological and pathological conditions [109,110]. All of the above studies suggest that EH may not be only polygenic, but also a ‘polygenomic’ disorder. Further investigation of the genetic causes of hypertension should consider dysfunction not only in the nuclear genome, but also in the mitochondrial genome. Comprehensive analysis of the genetic cause for EH in both genomes will need to be appreciated in the future.

Genome-wide linkage analysis

Genome-wide linkage analysis predicts that multiple chromosomal regions may play a role in the development of human EH; however, lack of consistency across studies makes it difficult to draw any general conclusion for the genetic cause of human EH [26,72,111,112] (Table 3). An investigations focusing on only SBP and DBP (diastolic BP) in 1109 white female dizygotic twin pairs has been carried out [113]. No significant linkage with BP could be detected in this study, but several suggestive linkage regions were replicated and one novel suggestive linkage region for SBP on chromosome 11p was detected [113]. Significant linkage for longitudinal SBP from the Framingham Heart study was detected on chromosome 1q. In this study [114], the SBP for each individual was modelled as a function of age using a mixed modelling methodology; it was thus the best linear unbiased predictor of the individual’s deviation from the population rate of change in SBP for each year of age whilst controlling for gender, BMI and hypertension treatment. Two previous linkage studies of hypertension had found peak LOD (logarithm of the odds) scores in the same region [115,116]. Similarly, linkage on chromosomes 12q, 15q and 17q for mean SBP and linkage for both SBP
Table 3  Genome-wide scans of human EH and BP

<table>
<thead>
<tr>
<th>Study design</th>
<th>Population</th>
<th>Chromosomal location</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear mixed model for longitudinal SBP [114]</td>
<td>Framingham cohort</td>
<td>1q32-44</td>
<td>$2 \times 10^{-5}$ for SBP</td>
</tr>
<tr>
<td>Sib-pair analysis for longitudinal SBP covariates</td>
<td>Framingham cohort</td>
<td>1q32</td>
<td>$2.9 \times 10^{-1}$ for mean SBP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2q6</td>
<td>$2.1 \times 10^{-1}$ for mean SBP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17q24</td>
<td>$4.8 \times 10^{-4}$ for mean SBP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20q13</td>
<td>$4.2 \times 10^{-5}$ for slope SBP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20q13</td>
<td>$2.8 \times 10^{-4}$ for curvature SBP</td>
</tr>
<tr>
<td>Family-based linkage analysis [26]</td>
<td>Chinese</td>
<td>12p11</td>
<td>LOD score of 3.44 for EH</td>
</tr>
<tr>
<td>Dizygotic twin pairs [113]</td>
<td>Caucasian</td>
<td>11p12</td>
<td>LOD score of 2.28 for SBP</td>
</tr>
<tr>
<td>Family-based linkage analysis for age at hypertension diagnosis [118]</td>
<td>White</td>
<td>4q25</td>
<td>LOD score of 2.44 for EH in AA</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>4q32</td>
<td>LOD score of 2.05 for early-onset EH in AA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15q21-22</td>
<td>LOD score of 2.31 for EH in AA</td>
</tr>
<tr>
<td>Meta-analysis [119]</td>
<td>Mixed populations</td>
<td>5q11-14</td>
<td>$P_{\text{weighted}} = 0.0288$ for EH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5q23-34</td>
<td>$P_{\text{weighted}} = 0.0251$ for EH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6q25-qter</td>
<td>$P_{\text{weighted}} = 0.0315$ for EH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11q22-24</td>
<td>$P_{\text{weighted}} = 0.0084$ for EH</td>
</tr>
<tr>
<td>Meta-analysis [120]</td>
<td>Caucasian</td>
<td>2p12-q22.1</td>
<td>$P_{\text{weighted}} &lt; 10^{-4}$ for EH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3p14.1-q12.3</td>
<td>$P_{\text{weighted}} &lt; 10^{-4}$ for EH</td>
</tr>
<tr>
<td>Admixture mapping [129]</td>
<td>AA</td>
<td>6q24</td>
<td>$P &lt; 0.05$ for EH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21q21</td>
<td>$P &lt; 0.05$ for EH</td>
</tr>
</tbody>
</table>

Slope and curvature on chromosome 20q were detected in the other study of the data from the Framingham Heart study [117]. The linkage analysis for age at diagnosis of hypertension and early-onset hypertension in the HyperGEN (Hypertension Genetic Epidemiology Network) cohort of different populations was carried out [118]. Several suggestive linkage loci were detected and some of them have been reported to be linked to hypertension and BP in previous studies. These are encouraging results suggesting that linkage can be replicated from other studies and, therefore, new genetic factors with moderate-to-large effects can potentially be discovered. Considering the power of the individual studies, two genome-scan meta-analysis for hypertension were carried out individually [119,120]. Interestingly, the previous meta-analysis with different populations [111,115,121–123] failed to detect significant linkage to hypertension, only several regions with suggestive linkage were identified, including chromosomes 2p, 5q, 6q, 8p, 9p, 9q and 11q. From them, only regions on chromosomes 5q, 6q and 11q had $P < 0.05$ [119]. Controversially, meta-analysis of genome-wide scans for hypertension and BP in Caucasians [115,116,122,124–128] had significant linkage on chromosomes 2p12-q22 and 3p14-q12 [120]. The results strongly suggest a population difference in the common phenotype. The mixed populations probably have a considerable degree of genetic heterogeneity, which is one of the main reasons why pooling of the results in different populations in the meta-analysis did not enhance the signals. However, pooling of the results in Caucasians possesses a smaller degree of genetic heterogeneity. One admixture mapping for hypertension loci with genome-scan markers was carried out in African–Americans [129] using individuals from Nigeria as African ancestral population and European–Americans for the estimates of allele frequencies for European ancestors. The distribution of marker-location-specific African ancestry was shifted upwards in hypertensive cases compared with normotensive controls, and the markers were located on chromosome 6q24 and 21q21.

Even though numerous whole-genome screens have been carried out in different populations, no positionally cloned genes that are associated with EH have been identified to date. This confirms the complex polygenic nature of the disorder. Different hypertension genes might play role in different ethnic groups or even different subsets of large families, thus consistent linkage could be difficult to detect in different studies.

**PERSPECTIVE AND FUTURE STUDIES**

Future analysis of complex diseases such as EH will benefit from the development and application of analytical methods that have the ability to systematically evaluate the contribution of genes operating in heterogeneous environments. Comprehensive analysis of the genes lying in different pathway(s) that are essential...
for the development of hypertension will be a necessary tool, since it seems very likely from our present knowledge that many molecular variants acting in concert may be required to alter BP homeostasis.

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REFERENCES

16 Melander, O., Oro, M., Fagerudd, J. et al. (1998) Mutations and variants of the epithelial sodium channel gene in Liddle’s syndrome and primary hypertension. Hypertension 31, 1118–1124
70 Hantila-Handelberg, T., Kontula, K., Tikkkanen, I. et al. (2005) Common variants of the β and γ subunits of the epithelial sodium channel and their relation to plasma renin and aldosterone levels in essential hypertension. BMC Med. Genet. 6, 4
89 Cui, J., Melista, E., Chazaro, I. et al. (2005) Sequence variation of bradykinin receptors B1 and B2 and association with hypertension. J. Hypertens. 23, 55–62
92 Fabris, B., Bortoletto, M., Candido, R. et al. (2005) Genetic polymorphisms of the renin-angiotensin-aldosterone system and renal insufficiency in essential hypertension. J. Hypertens. 23, 309–316
98 Cui, J., Melista, E., Chazaro, I. et al. (2005) Smoking and hypertension as predictors of cardiovascular risk in population studies. J. Hypertens. Suppl. 8, S3–S8
114 James, K., Weitzel, L. R., Engelman, C. D., Zerbe, G. and Norris, J. M. (2003) Genome scan linkage results for longitudinal systolic blood pressure phenotypes in subjects from the Framingham Heart Study. BMC Genet. 4 (Suppl. 1), S83
115 Perola, M., Kainulainen, K., Pajukanta, P. et al. (2000) Genome-wide scan of predisposing loci for increased diastolic blood pressure in Finnish siblings. J. Hypertens. 18, 1579–1585

© 2006 The Biochemical Society
Meta-analysis of genome-wide scans for hypertension and
blood pressure in Caucasians shows evidence of
susceptibility regions on chromosomes 2 and 3.
Hum. Mol. Genet. 13, 2325–2332
A genome scan for hypertension susceptibility loci in
populations of Chinese and Japanese origins.
Am. J. Hypertens. 16, 158–162
A genome-wide affected sibpair linkage analysis of
hypertension: the HyperGEN network.
Am. J. Hypertens. 16, 148–150
123 Caulfield, M., Munroe, P., Pembroke, J. et al. (2003)
Genome-wide mapping of human loci for essential
hypertension. Lancet 361, 2118–2123
Genome-wide linkage analyses for hypertension genes in
two ethnically and geographically diverse populations.
Am. J. Hypertens. 16, 154–157
125 Von Wowern, F., Bengtsson, K., Lindgren, C. M. et al.
(2003) A genome wide scan for early onset primary
hypertension in Scandinavians. Hum. Mol. Genet. 12,
2077–2081
Evidence for a gene influencing blood pressure on
chromosome 17. Genome scan linkage results for
longitudinal blood pressure phenotypes in subjects from
the Framingham heart study. Hypertension 36, 477–483
127 Rice, T., Rankinen, T., Province, M. A. et al. (2000)
Genome-wide linkage analysis of systolic and diastolic
blood pressure: the Quebec Family Study. Circulation
102, 1956–1963
128 Thiel, B. A., Chakravarti, A., Cooper, R. S. et al. (2003)
A genome-wide linkage analysis investigating the
determinants of blood pressure in whites and African
Americans. Am. J. Hypertens. 16, 151–153
mapping for hypertension loci with genome-scan

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