Hypertension is a rising problem in the developed countries. Some rare familial hypertensive syndromes have been found to be caused by monogenic mutations. Essential hypertension is generally regarded as a complex genetic trait caused by multiple genes. The polygenic effects on blood pressure are modulated by gene-gene and gene-environment interactions. Most investigations for genetic causes of essential hypertension were approached with candidate genes, and a few candidate genes, such as angiotensinogen gene, were reported to be associated with essential hypertension, though the reports from different populations were still conflicting. Genome-wide scans with multiple markers have mapped several loci associated with blood pressure. The loci reported were on chromosomes 2p, 2q, 5q, 6q, 15q, 17, and 18q respectively. However, to date, no genotype had been conclusively linked to essential hypertension. It has long been hoped that genetic research on hypertensive animals, such as rats, will facilitate our understanding of the genetics of human essential hypertension. So far, more than twenty loci were reported to be associated with blood pressure in rats. More understanding of the genetic basis of essential hypertension should be possible in the future and would be expected to help earlier diagnosis, more effective risk factor assessment and more individualized treatment of hypertension. However, the difficulty of genetic analysis for essential hypertension and the possibility of inter-population differences in genetic factors should be kept in mind.

Key Words: Hypertension • Genetics • Linkage analysis • Association analysis • Candidate genes • Genetic markers

The first notion that blood pressure might be heritable was in the study conducted by Wilhelm Weitz in 1923. Studying 82 patients of hypertension and 267 normotensive patients, Weitz observed that 77% of the parents of hypertensive patients developed stroke/heart diseases, whereas only 30% of parents of normotensives had these diseases. Several other findings also support the hypothesis of genetic causes of hypertension. First, there is greater concordance of blood pressure between monozygotic twins than dizygotic twins. Second, similarity of blood pressure within family is greater than between families. Familial aggregation of blood pressure is not only caused by shared environmental factors because biological siblings had more concordance in blood pressure than adoptive siblings living in the same household. Third, several forms of hypertensive syndromes are caused by monogenic mutations. Current evidence supports that essential hypertension is caused by polygenic disorders. It is estimated that about 40% of variation in human blood pressure is determined by genetic variants. The interaction among multiple genes and with the environment results in widely quantitative variation of blood pressure in the population.

APPROACHES TO GENETIC MAPPING

Review of some basic terms and methods frequently used in genetic studies is presented below.
Phenotype and trait
A distinct character of a living organism constitutes a phenotype or trait, which may be heritable or environmentally determined or both. Examples of heritable traits include white and red eyes in *Drosophila*, sickle cell anemia in humans.

Candidate genes
Physiologic study has revealed the pathways implicated in the regulation of blood pressure, such as autonomic nerve activity, renin-angiotensin-aldosterone system, to mention only a few. Consequently we are likely to assume mutations in one or several of these genes, such as α-receptor gene, renin gene, angiotensinogen gene, etc., will lead to variation of blood pressure. Therefore, these genes are called candidate genes.

Markers and locus
A gene or a piece of DNA of known location on a chromosome can be used as a marker for genetic mapping. The position of a particular gene on a chromosome is called a locus. To be used as a marker, the DNA of a locus has to be polymorphic, that is, more than one form in DNA sequences. Candidate genes with polymorphism may also serve the roles of markers.

Independent assortment
The second of Mendel’s laws described the phenomenon that each gene would be inherited independent of another. This law has had to be modified by subsequent discovery of linkage, i.e. that allelesgenes on the same chromosome tend to be inherited together.

Linkage and recombination
In contrast to independent assortment, the phenomenon that alleles close on the same chromosome tend to be inherited together is called linkage. However, during meiosis, homologous chromosomes are paired and crossing-over events may occur between paired chromatids and pieces of DNA may be exchanged at some positions; this phenomenon is called recombination. Recombination between two loci is more likely to occur if these two loci are farther apart on the chromosome. On the contrary, the closer the two loci on the chromosome, the more rarely will recombination occur. In other words, the closer two genes are, the more likely they have linkage. In genetic study, the disease or trait-causing gene is usually unknown. The phenomenon of cosegregation of a phenotype and a marker would suggest linkage and thus might hint that the phenotype-causing gene is near the marker. If the evidence of cosegregation is significant, methods such as DNA sequencing can be used to investigate this smaller piece of DNA and try to locate the phenotype-causing gene.

Linkage analysis, association analysis and linkage disequilibrium
The approach to search whether there is cosegregation of a phenotype and a marker in families is called linkage analysis. If the frequency of cosegregation is beyond what is expected by chance, there is suggestion of linkage. To investigate the relationship between a phenotype and a marker between unrelated case group and control group is called association analysis. The phenomenon that the frequencies of a particular marker between case group and control group are different than expected by chance is called linkage disequilibrium. It implies that the phenotype-related locus is probably nonrandomly associated with the marker locus, either because of close physical proximity between them or because the combination is under some form of selection pressure.

HYPERTENSION CAUSED BY MONOGENIC MUTATION
The two well-studied examples of hypertension caused by monogenic mutations are Liddle’s syndrome and glucocorticoid-remediable aldosteronism (GRA). Liddle’s syndrome was first reported in 1963. In the characterized family, multiple siblings had early onset of hypertension. Some of the patients had hypokalemia. Laboratory examinations of these patients revealed low levels of aldosterone and low plasma renin activity. Hypertension and hypokalemia could be improved by triamterene, an inhibitor of the distal renal epithelial sodium channel (ENaC). These characteristics hinted that epithelial sodium channel genes were the candidate genes of Liddle’s syndrome. Linkage analysis performed nearly thirty years after the first report of this syndrome revealed complete linkage of this syndrome with mutations in the β subunit of the epithelial sodium channel.
Thereafter, Liddle’s syndrome caused by mutations in γ subunit of the epithelial sodium channel was also reported. Glucocorticoid-remediable aldosteronism was reported in 1966. Patients with this syndrome were characterized with hypertension, increased aldosterone secretion and low plasma renin activity, all of which could be relieved by dexamethasone. Studies later revealed increased urinary excretion of 18-oxocortisol and 18-hydroxycortisol. With these pathophysiologic findings, it was rational to take genes of aldosterone synthase and steroid 11β-hydroxylase as candidate genes for GRA. Genes of aldosterone synthase and steroid 11β-hydroxylase are 95% identical in sequences and all on chromosome 8q. In 1992 Lifton et al. reported complete linkage of GRA in a large kindred to gene duplication due to unequal crossing-over and recombination. This mutation leads to fusion of the 5’ regulatory region of 11β-hydroxylase type 2 gene to the coding sequences of aldosterone synthase gene and causes the transcription of aldosterone synthase gene under the regulation of adrenocorticotropic hormone (ACTH). The chimaeric gene results in increased production of aldosterone with hypertension due to increased salt and water retention. Dexamethasone can suppress the secretion of ACTH and results in decreased production of aldosterone synthase and blood pressure.

Other forms of hypertension so far reported which were caused by monogenic mutations include syndrome of apparent mineralocorticoid excess (AME), hypertension exacerbated in pregnancy, etc. In vitro study revealed that both aldosterone and cortisol could activate mineralocorticoid receptor (MR) with nearly the same potency. In vivo cortisol was efficiently metabolized by 11β-hydroxysteroid dehydrogenase (11βHSD) to cortisone, which cannot activate MR. The metabolism of cortisol to cortisone protects MR from being activated by cortisol in vivo. Mune et al. reported mutations leading to homozygous loss of function of 11β-hydroxysteroid dehydrogenase in patients with AME. Because the function of 11βHSD is lost, cortisol cannot be effectively metabolized to cortisone. Consequently, MR of AME patients can be activated by cortisol, which circulates at concentrations hundreds-fold higher than those of aldosterone, and results in hypertension. Hypertension exacerbated in pregnancy is caused by a missense mutation, MR S810L (substitution of leucine for serine at codon 810), of mineralocorticoid receptor. Besides aldosterone and cortisol, normal MR can also bind progesterone and spironolactone. However, progesterone and spironolactone normally cannot activate MR. On the contrary, MR with S810L mutation can be activated by both progesterone and spironolactone. During pregnancy, high level of progesterone activates MR and results in hypertension.

GENETIC STUDIES IN ESSENTIAL HYPERTENSION

Most studies searching for genetic causes of essential hypertension have been conducted with association analysis of candidate genes. The candidate genes investigated include renin gene, angiotensin-converting enzyme (ACE) gene, angiotensinogen gene, angiotensin II type 1 receptor (AT1) gene, ENaC genes, 11βHSD gene, sympathetic α-receptor genes, β-receptor genes, endothelial nitric oxide synthase (eNOS) gene, atrial natriuretic peptide (ANP) gene, adducin gene and others.

Angiotensin-converting enzyme (ACE) gene

The ACE gene contains an insertion/deletion polymorphism (I/D) of a 287 bp Alu repeat in intron 16. Several reports showed correlation of this polymorphism with plasma ACE activity that is higher in those individuals with the deletion (D/D) allele. Qu et al. reported DD genotype was associated with essential hypertension in Chinese population. However, reports of the association between ACE genotype and essential hypertension were conflicting. Some reported significant association between ACE insertion (I/I) allele and hypertension. No association has been reported in other investigations, including Chuang’s report on Taiwanese.

Angiotensinogen gene

The first exciting evidence of association between essential hypertension and an angiotensinogen gene variant M235T (substitution of threonine for methionine at codon 235) was reported by Jeunemaitre et al. in sib pairs study and later supported by other studies. However, studies performed in different regions, including in Japanese, have not confirmed the same results.
ported linkage disequilibrium of 235T variant with an adenine, instead of a guanine, at six residues upstream from the initiation site for transcription (-6A instead of -6G). They also reported that promoter with the -6A variant had higher transcription rate.25 Wang et al. reported association of 235T and -6A with hypertension in Amis tribes of eastern Taiwan.26 Chiang et al. also reported association of 235T with hypertension in Taiwanese population.27 However, Wu et al. did not find association between -6A and hypertension; instead, a G-217A variant was associated with hypertension in Taiwanese population.28

**Angiotensin II type 1 receptor**

The hemodynamic effects of angiotensin II are mediated through angiotensin II type 1 (AT1) receptor. A silent polymorphism with adenine changed to cytosine at position 1166 (A1166C) was reported to associate with severe essential hypertension.15,29 Jin et al. reported an A-810T polymorphism in the promoter region of AT1 was associated with hypertension in a Chinese population.30

**Epithelial sodium channel genes**

A variant with threonine shifted to methionine at codon 594 (T594M) in the β subunit of ENaC was associated with hypertension in a single study.31 Another study showed that a variant with substitution of valine for glycine (G442V) in the β subunit was more common in blacks than in whites (16% vs. 1%). However, this variant was not associated with hypertension.32 Wong et al. found significant linkage between systolic blood pressure and chromosome 16p12 that encodes the β and γ subunits of epithelial sodium channel.33

**11β-hydroxysteroid dehydrogenase gene**

Lovati et al. reported a marker of polymorphic microsatellite in 11βHSD type 2 gene was associated with salt-sensitive hypertension.34 A polymorphism G534A (Glu178Glu) was identified by Melander et al. in exon 3 of the HSD11B2 gene. The frequency of G534G homozygotes was higher in patients with primary hypertension than in normotensive control subjects, suggesting that a mutation in linkage disequilibrium with the G534A polymorphism could increase susceptibility to primary hypertension.35

**Sympathetic α-receptor genes and β-receptor genes**

A polymorphic glutamic acid stretch containing either 9 or 12 glutamic acids in the α2B-adrenergic receptor was found by Baldwin et al., who reported lack of association with essential hypertension.36 A variant with substitution of glycine for arginine (R16G) of β2 adrenergic receptor variant was found associated with hypertension.37 However, several studies have failed to confirm this finding.38,39

**Endothelial nitric oxide synthase (eNOS) gene**

Nitric oxide (NO) is a potent vasodilator and is produced from L-arginine by NO synthase (NOS). The NOS expressed in endothelium (eNOS) has a Glu298Asp variant. Shoji et al. reported association of this variant with hypertension.40 However, other researches using either Glu298Asp variant or microsatellite markers did not reveal association with hypertension.41,42

**α-Adducin gene**

Adducin is a cell membrane protein and is involved in cell signal transduction, regulation of actin cytoskeleton, and ion transport. A missense mutation (Gly460Trp) in human α-adducin was reported to be associated with primary hypertension.43 However, conflicting results were reported both in Japanese and Chinese.44,45

**Atrial natriuretic peptide (ANP)**

Atrial natriuretic peptide is produced mainly in cardiac atria; it plays a role in the regulation of blood pressure by modulating sodium-water homeostasis. The reports about association of polymorphisms within the ANP gene with essential hypertension are very conflicting. Positive association was reported in research in African Americans.46 Negative results were reported in Taiwanese, Japanese and a Hong Kong population.47-49

**GENOME-WIDE MAPPING FOR LOCI ASSOCIATED WITH HYPERTENSION**

In contrast to qualitative traits such as white and red eyes in *Drosophila*, blood pressure is a continuously quantitative trait variant. It is generally regarded that genetic determinants of blood pressure are multiple; each
related gene contributes variable quantities of blood pressure, resulting in different levels of blood pressure distributed in the population. Because the loci of most of the genes involved in determination of quantitative variation of blood pressure are still unknown, they are named quantitative trait loci (QTL). Several genome-wide linkage analyses have been reported. Baima et al. reported evidence of linkage between essential hypertension and a locus on human chromosome 17q.\(^{50}\) Xu et al. performed extreme-sib-pair scan with 367 markers in Chinese and could not find evidence of linkage with significance.\(^{51}\) Nevertheless, Krushkal et al. reported 4 regions, which were on chromosomes 2p22.1 to 2p21, 5q33.3 to 5q34, 6q23.1 to 6q24.1, and 15q25.1 to 15q26.1, related to systolic blood pressure.\(^ {52}\) Hsueh et al. mapped a blood pressure-related QTL on chromosome 2q31-34 in Amish.\(^ {53}\) Levy et al. reported a gene influencing blood pressure on chromosome 17 in subjects from the Framingham Heart Study.\(^ {54}\) Kristkansson et al. reported a locus on chromosome 18q was linked with essential hypertension in a genome-wide scan with 904 microsatellite markers using 120 Icelandic families with 490 hypertensive patients.\(^ {55}\) Wu et al. reported association between markers in chromosome 17q23 and young-onset hypertension in Taiwan.\(^ {56}\) Ge et al. reported linkage of 2q14-23 and 5q32 with hypertension in Chinese sib pairs.\(^ {57}\)

**GENETIC STUDIES IN HYPERTENSIVE ANIMAL MODELS**

Starting from a colony of animals, animals with elevated blood pressure are selected for inbreeding; at each consecutive generation, those animals still with elevated blood pressure are again selected for inbreeding until hypertension is present in 100% of the offsprings. Thereafter, mating among brother-sister at each generation is continued for about 20 generations to achieve a state of homozygous at all genomic loci of these animals. Several strains of rat with genetic hypertension have thus been generated. It has long been expected that investigation on the genetic causes of hypertension in animals, mainly rats and mice, can help in answering the questions of genetic causes of essential hypertension in humans. Most genetic researches conducted one or two decades ago on hypertension in rats used the case-control design. The spontaneously hypertensive rats (SHR) were often used as case group, while the Wistar-Kyoto rats (WKY) served as control group. However, these two strains were later found to differ not only in some blood-pressure-related alleles but also in multiple blood pressure-unrelated loci. Therefore many of the genes reported different between both strains were irrelevant to hypertension and the chances of identifying hypertensive genes were low in simple comparative genetic studies.

To overcome the shortcomings of comparative genetic studies between hypertensive rat strain and normotensive strain, crosses between hypertensive strain and normotensive strain were conducted and the second filial generation (F2) used for genetic studies. With this approach, some of the QTL can possibly be mapped to certain chromosome segments, though most of the chromosome segments are likely too large for more precise positional mapping. To confirm the existence of a blood pressure QTL and to narrow the chromosome region containing the QTL, congenic rats were bred.\(^ {58}\) Procedures to breed congenic strains are begun with crossing a donor strain with a recipient strain. The rats of F1 generation are screened and those heterozygous at the target genomic segment are selected. These selected rats are backcrossed with the recipient strain and their progenies heterozygous at the target genomic region selected. After backcrossing of eight or more generations, it is estimated that 99% of the genome is from the recipient. However the target genomic segment is always kept heterozygous by screening. Thereafter, brother-sister mating of these rats is conducted and those progenies with homozygous target genomic segment from the donor are selected. Consequently, most of the genome of congenic rats is from the recipient strain, except the target region that is from the donor strain. Therefore, if a congenic strain retains the blood pressure-related QTL from the donor and still has elevated blood pressure, physiologic evidence for the existence of a blood pressure related gene in the QTL is obtained. In 1991, Iwai and Inagami isolated a gene (Sa gene) preferentially expressed in the kidneys of hypertensive rats.\(^ {59}\) However, further study on Sa gene with development of congenic strains excluded Sa gene as a candidate locus.\(^ {60}\) By linkage study
in SHR, Aitman mapped a QTL related to hypertension, hypertriglyceridemia, and metabolic syndromes to a region of chromosome 4. A congenic strain of SHR was bred by replacing this region of chromosome 4 in the SHR with the corresponding region from the Brown Norway (BN) rat. Hypertension metabolic syndrome was ameliorated in this congenic strain. Further differential cDNA microarray analysis of fat tissues between original SHR progenitor and the SHR chromosome 4 congenic strain isolated a strongly differentially expressed gene, Cd36. Cd36 had been discovered as a platelet cell surface protein. It is proposed to be a transmembrane transporter of long fatty acids and is highly expressed in adipose tissue, cardiac muscle, monocytes, macrophages, dendritic cells and vascular endothelium. Deletional mutation was found and proposed to be responsible for insulin resistance and causing defective fatty acid and glucose metabolism in spontaneously hypertensive rats. However, transgenic rats of Cd36 in the SHR could only ameliorate metabolic disturbance but not hypertension.

Hilbert et al. performed genome-wide linkage studies with microsatellite markers in crosses between the stroke-prone spontaneously hypertensive rat strain (SHRSP) and the normotensive Wistar-Kyoto strain and localized two genetic loci, BP/SP-1 and BP/SP-2, that contributed significantly to blood pressure variation in the F2 population. BP/SP-1 was assigned to rat chromosome 10, and BP/SP-2 to rat chromosome X. Jacob et al. also mapped in a cross between SHRSP and WKY strain a gene, Bp1, having a major effect on blood pressure. Bp1 was also on rat chromosome 10. Another locus, Bp2, with weaker linkage to blood pressure was on rat chromosome 18. To date, genome-wide scans have identified more than 20 blood-pressure QTL distributed on every rat chromosome. However, the positions of the QTL have not been precise enough to allow the genes controlling blood pressure to be identified.

CURRENT STATUS OF GENETIC STUDIES OF HYPERTENSION IN TAIWAN

Most of the genetic studies of hypertension in Taiwan were association analysis with candidate genes. The ACE gene and angiotensinogen gene have been the targets of intensive research. Chuang et al. reported no association of ACE gene with hypertension. Chiang et al. reported association of 235T variant of angiotensinogen gene with hypertension in Taiwanese. Though Wang also reported association of 235T variant of angiotensinogen gene with hypertension in Amis tribes, the results of studies on –6A of angiotensinogen gene were different in Amis tribes and in Taiwanese. Wang reported association in Amis tribes, while Wu et al. reported no association in Taiwanese. Instead, Wu reported association of hypertension with -217A of angiotensinogen gene in Taiwanese. To the best of our knowledge, only another Wu et al. reported results of genome-wide scan for hypertensive loci, which was on chromosome 17q23 in young-onset hypertension in Taiwan. Recently, an exciting report by Tsai et al. showed that angiotensinogen haplotypes were associated with hypertension and might act synergistically with I allele of the ACE gene.

SUMMARY AND FUTURE PERSPECTIVES

This article was written as an introductory review rather than a comprehensive summary. The number of genes related to essential hypertension is estimated in the range of thirties to forties or even more. After decades of intensive research, the diagnosis and treatment of essential hypertension is still completely clinically oriented. Currently, no options of diagnosis, preventive measures or treatment of essential hypertension can be made based on the large amount of research results published. Genome-wide searches for hypertensive genes are not only expensive but also labor-intensive. However, most of the studies were conducted in non-oriental populations, except a few by Chinese or Japanese. In the hope that understanding genetic causes of essential hypertension will lead to earlier diagnosis, earlier implication of preventive measures, and more effective and specific treatment, those studies conducted in Caucasians have to be verified in Taiwanese before the information is applied to our population. It is also imperative to begin studies on monogenic hypertension in Taiwan so that this group of patients will receive treatment based on their pathophysiologic changes and the better understanding of essential hypertension would also be facilitated.
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高血壓的基因研究

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隨著社會的發展，高血壓的盛行率也跟著升高。數種家族性高血壓已被確定由單基因突變所造成。這些研究也為高血壓與基因遺傳有關奠下了證據。本態性高血壓被認為是由多種基因的變異與環境的互動所造成。由於生理與血壓調控有關的基因稱為候選基因，大多數本態性高血壓的基因研究在於尋找本態性高血壓和候選基因的相關性。已有報告本態性高血壓與少數候選基因，例如血管張力素原基因，具有有意義的相關；然而其相關性仍因種族而有差別。也有利用多個標幟 (markers) 進行全基因體掃瞄，並發現數個與本態性高血壓相關的局部區域 (locus)：它們分佈於染色體 2p，2q，5q，6q，15q，17，和 18q 等。一般預期高血壓動物之基因研究將有助於尋找本態性高血壓的致病基因，目前已有約二十個大鼠之高血壓相關的局部區域被定位。高血壓的基因研究將有助於高血壓的早期診斷、高血壓及心臟血管危險因子的分析，以及未來更個人化的治療。然而我們也要瞭解高血壓基因研究的複雜性而且高血壓的基因變異可能因種族而有差異。

關鍵詞：高血壓、基因、相連性分析、相關性分析、候選基因、基因標幟。