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Review

Genetic Determination of Human Essential Hypertension

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MATSUBARA, M. *Genetic Determination of Human Essential Hypertension.* Tohoku J. Exp. Med., 2000, **192** (1), 19-33 —— Recent advances in genetic determination of human essential hypertension (EHT) are discussed by reviewing the candidate genes. Candidate genes have been selected based on genetic information from classical linkage analysis (affected sib-pair analysis) or mendelian hypertension (autosomal dominant inheritance of hypertension). Most of these genes are, directly or indirectly, coupled to salt handling of the kidney, being included in the renin-angiotensin system (RAS), steroid-hormone metabolism, and renal sodium transporters. Angiotensinogen (AGT) gene in RAS was first described as a strong candidate associated with the onset of hypertension, since sib-pair linkage analysis has demonstrated the trait loci for hypertension which includes the coding region for AGT. M235T polymorphism of AGT has been studied extensively in many populations including Japanese, and the results suggest a weak, but significant linkage with hypertension. The presence (insertion [I]) or absence (deletion [D]) of 287 bp in intron 16 of angiotensin converting enzyme gene has also been examined in RAS, and the results suggest D polymorphism as a risk factor for hypertension in men. Other components in RAS, such as renin, angiotensinogen II type I receptor, or kallikrein have also been studied, but the available information is still incomplete. Genetic investigations of mendelian hypertension has identified the genetic mechanisms for glucocorticoid remediable aldosteronism, apparent mineral corticoid excess, and Liddle's syndrome as chimeric gene duplications of CYP11B1 (aldosterone synthase gene) and CYP11B2 (11β-hydroxylase gene), mutations in the gene of 11β-hydroxysteroid dehydrogenase type 2 that catalyzes the conversion of cortisol to cortisone, and mutations in β or γ subunit of epithelial sodium channel (ENaC), respectively. Subsequently, genetic variants of CYP11B2 and β or γ subunit of ENaC have been found, suggesting the -344C polymorphism of CYP11B2, 594S variant of βENaC, and two rare variants of γENaC as risk factors for EHT. In spite of the

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extensive research, haplotypes in individual populations remain to be elucidcated in most candidate genes. Even casual conclusions of possible linkage with EHT need to be further examined with better determinations of phenotypes, such as ambulatory and home blood pressure monitoring or identification of onset of hypertension in cohort studies. ———— blood pressure; genetic polymorphisms; renin- angiotensin system; steroid hormone metabolism; renal sodium transporters

In the 1990's, considerable efforts have been made worldwide to identify the genes responsible for the development of essential hypertension (EHT). Identification of genetic determinants of human hypertension will provide key leads in unveiling the pathogenesis of EHT, which will permit classification of hypertensive individuals based on underlying abnormalities, and ultimately permit optimal management. "Hypertension," however, is a clinical manifestation that appears relatively late in life, and around 30% of blood pressure variance is considered to be due to genetic factors (Hanis et al. 1983). In addition, the number of genes involved in hypertension is still unknown. Therefore, the genetic approach taken to date has been mainly to study candidate genes, and, unfortunately, no clear picture as to the genetic determination of EHT has emerged so far, in part, due to divergent results even in the same gene polymorphism.

In spite of these difficulties, recent investigations unveiled the molecular mechanisms of three mendelian hypertensions, glucocorticoid-remediable aldosteronism (GRA) (Lifton et al. 1992), apparent mineral corticoid excess syndrome (AME) (Mune et al. 1995), and Liddle's syndrome (Shimkets et al. 1994). Classical genetic linkage study of hypertensive families has demonstrated the trait locus including the coding region for angiotensinogen (AGT) (Jeunemaitre et al. 1992a). All of these genes directly or indirectly influence renal sodium handling (Lifton 1995). Based on the implications of these findings, the candidates genes have been selected for association studies with hypertension. By classifying the pathway for enhanced renal sodium absorption into three major systems, renin-angiotensin system (RAS), steroid hormone metabolism, and sodium transporters, as indicated in Fig. 1, recent progress is discussed.

RAS and Kallikrein-Kinin system

Epidemiological, clinical, and experimental evidence has shown that the RAS plays an important role in blood pressure regulation. The final product of this system, angiotensin II (AT II), stimulates renal sodium absorption and aldosterone synthesis (Figs. 1 and 2), and enhances vascular tone through type 1 receptor of AT II (AT1). All of these actions favor a rise in blood pressure. Two steps of enzymatic reactions are included for AT II formation as indicated in Fig. 2. The cleavage of AGT by renin is the rate-limiting step in the cascade of enzymatic events. In human, angiotensin converting enzyme (ACE) inactivates brady-

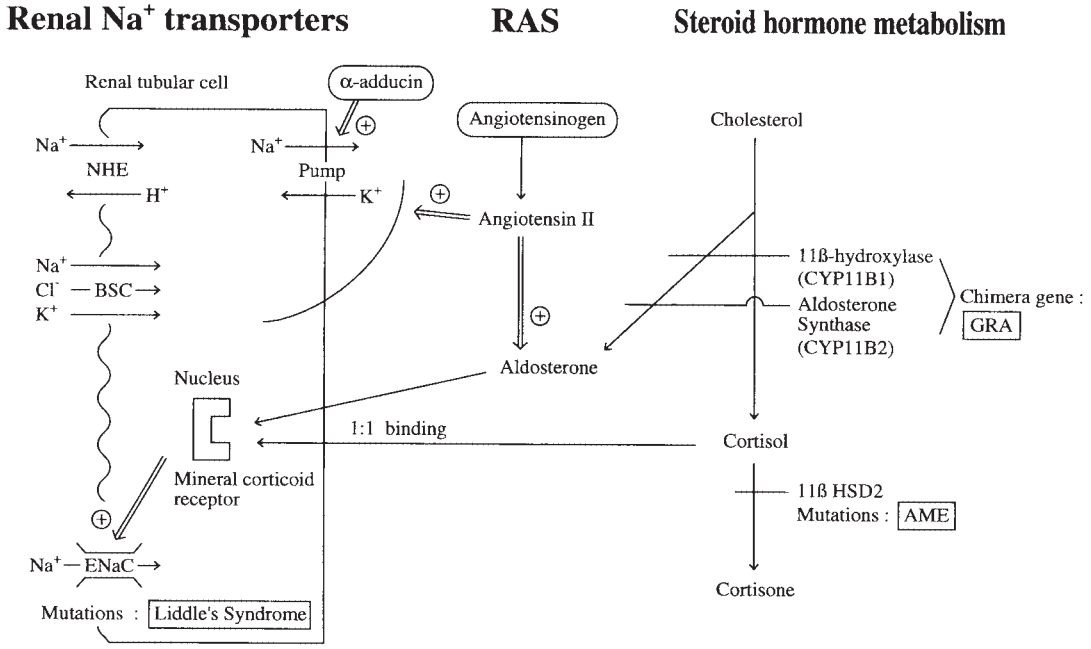

Fig. 1. Sodium handling systems associated with the regulation of blood pressure. Genetic abnormalities found in these systems, which affect blood pressure level, are indicated. ▯, included in trait locus for human hypertension. ○, genetic abnormalities induce mendelian hypertension; RAS, renin-angiotensin system; NHE, Na^+-H^+ exchanger; BSC, bumetanide sensitive cotransporter. ENaC, epithelial sodium channel; GRA, glucocorticoid-remediable aldosteronism; AME, apparent mineralcorticoid excess.

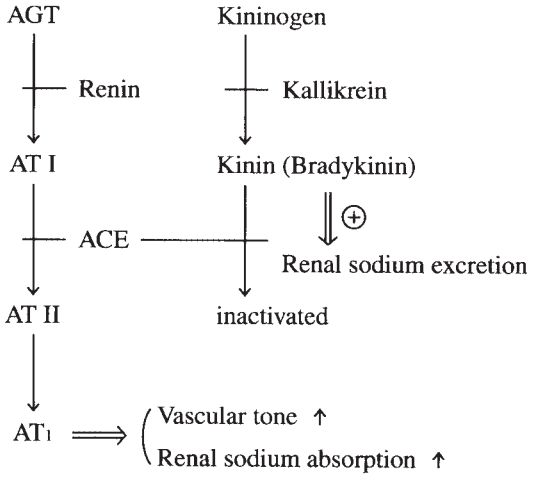

Fig. 2. Renin-angiotensin system and kinin-kallikrein system. AGT, angiotensinogen; AT I, angiotenin I; ACE, angiotensin converting enzyme; AT II, angiotensin II; AT_1, angiotensin II type 1 receptor.

kinin, which induces sodium excretion in the kidney, as well as produces AT II, which enhance renal sodium reabsorption. Therefore, all of the proteins included in RAS are theoretically good candidates for genetic analysis.

AGT gene was the first gene identified to be linked with essential hypertension (Jeunemaitre et al. 1992a). Sib-pair linkage analysis of two large popula-

tions of hypertensive sibships demonstrated the trait locus including code for AGT. Sequencing has identified many genetic variants, including frequent alleles, T174M and M235T (Jeunemaitre et al. 1992a, 1997). The polymorphisms which are located in coding region changing amino acid sequence or those located in uncoding region possibly influencing transcription or translation are indicated in Fig. 3.

Subsequently many association and case-control studies have been performed on M235T variants with divergent results. Meta-analysis studies (Kunz et al. 1997; Staessen et al. 1999) concluded that the 235T variant behaves as a marker

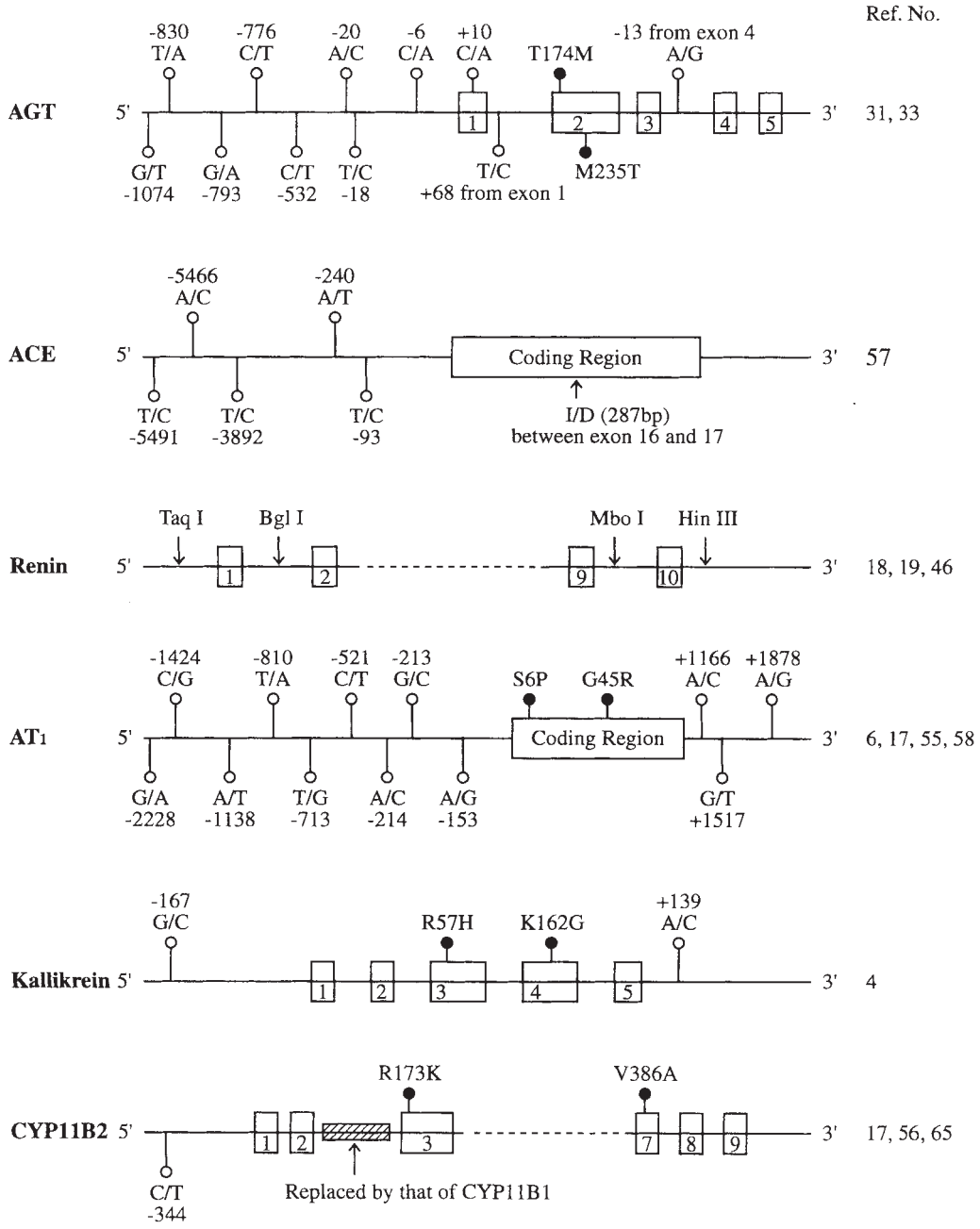

Fig. 3. Polymorphic substitutions of candidate genes for essential hypertension. Synonymous base substitutions in the coding regions are excluded.

for EHT in Caucasians, though it is a weak marker. The odd ratio increased moderately when patients with a positive family history or severe hypertension were considered (Kunz et al. 1997). Although functional analyses have been used to confirm the statistical evidences incriminating AGT in EHT, the 235T variant does not affect the Km of renin, and its secretion and metabolism are unaltered in 235 M AGT (Corvol et al. 1999). Jeunemaitre and coworkers (1997), however, found that G→A substitution at position -6 upstream of the initial transcription site was present in almost complete linkage disequilibrium with 235 T allele. This nucleotide substitution has a modest, but significant, effect on the basal rate of AGT transcription (Inoue et al. 1997), which could account for the increase in plasma AGT in some subjects with 235T AGT.

Since Rigat et al. (1990) identified a polymorphism based on the presence (insertion [I]) or absence (deletion [D]) within intron of 287-bp nonsense DNA domain, which is partially associated with plasma ACE level, the ACE I/D polymorphism has been a major molecular target for linkage analysis of EHT in many populations, though early human studies (Jeunemaitre et al. 1992b) failed to identify the linkage between the human ACE locus (in chromosome 17) and ETH. A major locus for high blood pressure in the rat is located on rat chromosome 10, which contains the rat ACE locus (Hilbert et al. 1991; Jacob et al. 1991). Furthermore, recent study (O'Donnell et al. 1998) described a positive linkage between ACE locus and hypertension in hypertensive sibs. The haplotypes of ACE gene known to date is shown in Fig. 3. Compared to the number of studies on I/D allele, little is known about other polymorphisms, and haplotype determination is even considered to be incomplete probably due to the scale of the ACE molecule (26 exons) and too much interest in I/D polymorphism. Recently, studies with large number of subjects of different populations, Caucasians (O'Donnell et al. 1998) and Japanese (Higaki et al. 2000) have been described. Table 1 summarizes the results of these studies, demonstrating the involvement of D allele in EHT of men, but not of women. On the contrary, Giner et al. (2000) recently suggested that salt-sensitivity is associated with II and ID genotypes, but not with DD. Thus, further investigations are still required to determine the implication of D/I polymorphisms on human blood pressure.

Although conversion of AGT to AT I by renin is the rate-limiting step of the RAS, the number of genetic studies of renin published to date is small. Naftilan et al. (1989) first described the polymorphisms of renin gene determined by restriction fragment length polymorphism (RFLP) without finding any linkage with ETH. The same RFLP method has repeatedly demonstrated four variants in uncoding regions (Fig. 3) with divergent results (Frossard et al. 1998, 1999). Thus, renin variants are less likely to be associated with EHT.

Angiotensin II receptors, which mediate the actions of AT II, also represent interesting candidate genes for EHT. Humans type 1 receptor (AT_1) is present predominantly in vascular cells and in both kidney and adrenal gland mediating

TABLE 1. *Association between DD polymorphism of ACE and EHT in different populations*

Population	Sex (number)	Genotype (number)	Odd ratio (CI)	Reference
Caucasians	Male	DD (437)	1.59 (1.13-2.23)	O'Donnell et al.
	(1445)	DI (719)	1.19 (0.88-1.61)	(1998)
		II (288)	1.00	
	Female	DD (492)	1.01 (0.70-1.44)	
	(1650)	DI (845)	0.80 (0.59-1.09)	
		II (313)	1.00	
Japanese	Male	DD (307)	1.80 (1.21-2.53)	Higaki et al.
	(2340)	DI (1096)	1.14 (0.87-1.51)	(2000)
		II (937)	1.00	
	Female	DD (352)	1.17 (0.79-1.72)	
	(2674)	DI (1141)	0.87 (0.65-1.17)	
		II (1181)	1.00	

CI, 95% confidence interval.

physiological actions of AT II as shown in Fig. 1. AT_1 is composed of 5 exons and the entire coding region is harbored only on exon 5. This gene was first screened by Bonnardeaux and coworkers (1994), who found a significant increase in allelic frequency of +1166A/C polymorphism (in 3' untranslated region) in hypertensive subjects. To date, 16 polymorphisms have been described, and most of them are located in the uncoding region or a synonymous base substitution (not shown) in the coding region as in Fig. 3 (Bonnardeaux et al. 1994; Poirier et al. 1998; Erdmann et al. 1999). Two polymorphisms which lead to an amino acid change were described only in one report without any association study with EHT (Rolfs et al. 1994). Most of the studies of AT_1 variants have been conducted on the association with cardiovascular events as well as hypertension. Best evaluated is the +1166 A/C polymorphism, though it is unclear whether this polymorphism in the 3' untranslated region of AT_1 gene has functional implications. At present, the linkage between AT_1 variants and EHT is still to be determined (Poirier et al. 1998; Zang et al. 2000), but the polymorphisms in AT_1 are considered to be important in cardiac accidents (Erdmann et al. 1999).

The kallikrein-kinin system includes four major components, kallikrein, kininogen, kinin, and kininase (ACE) (Fig. 2). Kallikrein is a protease which synthesizes kinin from kininogen. There is sufficient evidence indicating that kinin regulates renal sodium and water excretion, and, thus, genes for kininogen and kallikrein could be candidates for genetic approaches in human hypertension. However, only kallikrein gene has been investigated to date, probably due to the evidence that urinary kallikrein excretion is altered in individuals (Margolium et al. 1971; Lieberthol et al. 1983) or families (Berry et al. 1989) with EHT. Berge

and Berg (1993) first examined the association between the polymorphism of kallikrein gene detected by RFLP (Taq I polymorphism) without finding association with hypertension. The same group then screened the gene by single-stranded conformation polymorphisms (SSCP) method and identified four polymorphisms shown in Fig. 3 (Berge et al. 1997), but they failed to find any association with hypertension.

Synthesis of aldosterone and metabolism of cortisol

Both mineralocorticoid and glucocorticoid are synthesized from cholesterol though several steps of enzymatic reactions. Genetic investigations on hypertensive patients with autosomal dominant inheritance have found genetic abnormalities of mineralocorticoid synthase enzyme (GRA) (Lifton et al. 1992) and 11βhydroxysteroid-dehydrogenase (11βHSD2) which converts cortisol to cortisone (AME) (Mune et al. 1995). Both of these genetic abnormalities elevate blood pressure by excess aldosterone or aldosterone-like effect of cortisol as indicated in Fig. 1.

GRA is one specific form of primary aldosteronism. A hybrid gene originating from previous recombination between the CYP11B1 (gene coding enzyme for cortisol formation) and CYP11B2 (gene coding aldosterone synthase) genes produces extra aldosterone independent of serum potassium or AT II concentration in the zona fasciculata where glucocorticoid is normally produced. In addition, mutations in CYP11B1 lead to a hypertensive form of congenital adrenal hyperplasia (White et al. 1991; Helmberg et al. 1992; Curnow et al. 1993) due to the accumulation of 11-deoxycorticosterone and its metabolites. In contrast, mutations in CYP11B2 lead to various forms of aldosterone deficiency, characterized by salt wasting and hypotension (Pascoe et al. 1992; Mitsuuchi et al. 1993), suggesting that abnormalities in either of CYP11B1 or CYP11B2 can lead to important changes in arterial pressure and could be responsible for some forms of EHT. Since idiopathic hyperaldosteronism is sometimes considered a variant of EHT (Ganguly 1998), several groups (Fardella et al. 1996; Brand et al. 1998a; Davies et al. 1999; Mulatero et al. 2000) have examined CYP11B2 polymorphisms in association with hypertension or plasma aldosterone concentration based on haplotypes of CYP11B2 determined by White and Slutsker (1995) (Fig. 4). Polymorphism at -344C/T, which has strong linkage disequilibirium with V386A mutation in exon 7 (Pojoga et al. 1998) or with R173K mutation in exon 3 (Komiya et al. 2000), has been vigorously examined due to the location of -344 position in promoter of the gene. Although -344 T allele is now considered to be observed more frequently in hypertensive individuals with low renin- high aldosterone plasma concentration or increased urinary aldosterone excretion, Brand et al. (1999) failed to find salt-sensitivity in young normotensive subjects with -344 T polymorphism. Therefore, like other candidate genes, further investigations are still required for the final conclusion.

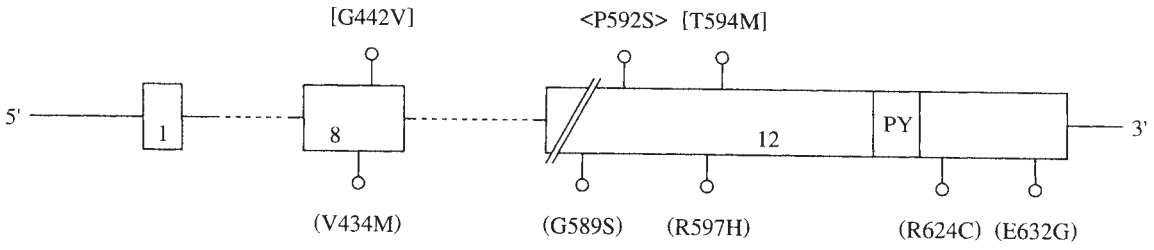

Fig. 4. Polymorphisms in β subunit of epithelial sodium channel. ( ), Caucasian population; [ ], black population; < >, Japanese.

In addition to the implication of genetic mechanism for AME, a recent case report of a girl with AME, who presented with low-renin hypertension without hypokalemia or other phenotypic features (Wilson et al. 1998), strongly suggest that a subset of patients among EHT population may suffer from a very discrete form of AME. Thus, 11βHSD2 gene was examined by Brand et al. (1998b) using both affected sib pair method and SSCP screening. The results, however, suggested that 11β-HSD2 gene does not contribute substantially to EHT in Caucasians, though Watson et al. (1996) suggested a genetic association of 11β-HSD2-flanking microsatellite and hypertension in African Americans with end-stage renal disease. The full spectrum of clinical manifestations of AME patients is currently being defined (Morineau et al. 1999; Nunez et al. 1999), and recently Paolo et al. (2000) reviewed the role of 11β-HSD2 in human hypertension, showing haplotypes of 11β-HSD2 gene, which had been found in AME patients.

Renal sodium transporters

In mid 1970's, abnormalities of ion transport in the cell were detected in patients with EHT (Orlov et al. 1999). Increased activities of both ubiquitous forms of the Na^+/H^+ exchanger (NHE) or Na^+-K^+-Cl^- cotransporter (bumetanide sensitive cotransporter [BSC]) have been described in patients with EHT (Orlov et al. 1999), though genetic approaches on these transporters are still to be performed. Renal cell specific isoforms of NHE or BSC are distributed in the proximal tubules or in the thick ascending limb of Henle, respectively, where renal sodium absorption is enhanced by direct stimulation with AT II (Harris and Young 1977; Amlal et al. 1998), as indicated in Fig. 1. Recently, single base substitution of subunit of G-protein β3 subunit gene, which results in the loss of 41 amino acids, has been described as a genetic risk for EHT in Caucasian population probably through functional alteration of NHE (Siffert et al. 1998). In Japanese population, however, such association between the same genetic variant of G-protein and EHT is absent (Ishikawa et al. 2000).

Although alteration of Na^+-K^+ ATPase activity in human hypertension has not been documented to date, an increase in the maximal activity of Na^+-K^+ ATPase has been reported in membrane fractions from the renal cortex of hypertensive rats (Milan hypertensive strain) (Meizi et al. 1989; Parenti et al. 1991).

Na^+-K^+ ATPase provides ouabain-sensitive hydrolysis of ATP coupled to the inward movement of K^+ and outward movement of Na^+, supplying a fundamental source for transcellular sodium reabsorption in renal tubules. Though molecular based approaches on Na^+-K^+ ATPase genes have not been yet reported, affected sib-pair analysis of hypertensive families identified the trait locus which includes coding region of α-adducin (Casari et al. 1995; Cusi et al. 1997), one of the proteins that regulates Na^+-K^+ ATPase activity. Adducin is a cytoskeletal protein present in many tissues, including the kidney (Hughes and Bennett 1995). Alterations in adducin by genetic mutations in α- and β- subunits have been shown to influence the surface expression and maximum velocity of Na^+-K^+ ATPase (Tripodi et al. 1996), further implicating adducin as a candidate for genetic investigations. Subsequently, G460T polymorphism of α-adducin was identified (Cusi et al. 1997) and analyzed in association with hypertension, although the results were divergent (Cusi et al. 1997; Busjahn et al. 1999). However, all studies of α-adducin locus demonstrated the involvement of this locus in hypertension (Casari et al. 1995; Cusi et al. 1997) or regulation of blood pressure (Busjahn et al. 1999), and, thus, further investigations including the determination of the haplotypes of adducin genes are still required for the final conclusion.

Several mutations have been described in Liddle's syndrome pedigrees, most of them in the β subunit and some in γ subunit (Lifton 1995; Corvol et al. 1999). All these mutations either abolish or modify a highly conserved PY motif present in their intracellular carboxyl terminus region, which in turn alters the binding of ENaC with Nedd4, a ubiquitin-like protein that contains a WW domain, which can interact with the PY motif of ENaC. This leads to a low intracellular turnover of the channel and to an increase in the number of active channels exposed at apical membrane. The hormonal features of Liddle's syndrome; low plasma renin and low normal aldosterone have also been described in a subset of hypertensive patients especially those of African origin. Because of the well documented role of ENaC in sodium reabsorption and its implication in EHT, it was logical to consider ENaC as good candidate for EHT and to screen hypertensive patients for mutations in any one of three subunits. Subsequently, the most frequent variant in the C-terminus of the βENaC subunit (T594M) was found to be four times more frequent in hypertensive residents of African origin in London than in their normotensive congeners (Baker et al. 1998), but the linkage of ENaC to hypertension was not found in another black population (Persu et al. 1998). Since the frequency of polymorphisms are completely different between black and white populations, we (Matsubara et al. 2000) screened the last 2/3rd of the exon 12 of βENaC, and identified the absence of T594M mutation but presence of novel mutation of P592S without linkage with EHT (Fig. 4), suggesting the importance of screening of individual populations. In addition to β subunit, other groups screened for γENaC in Caucasians and African Caribbeans (Persu et al. 1999).

Although four relatively frequent polymorphisms were noted at the same frequency in hypertensive and normotensive subjects, two rare mutations that affect the protein sequence were considered to be involved in EHT. The same group also screened the αENaC, and found W493R polymorphism without any linkage with EHT (Corvol et al. 1999).

Conclusions

Putative identification of quantitative trait loci (QTLs) implicated in hypertension suggests the AGT as a candidate gene, and 235 M variant as a weak but significant risk factor for hypertension. Extensive studies of RAS have demonstrated that D/I polymorphism of ACE gene is probably involved in EHT. Implications from the molecular mechanisms for mendelian hypertension suggest the CYP11B2 and ENaC gene as strong candidates. Polymorphism at -334C in promoter of CYP11B2 is thought to be associated with idiopathic hyperaldosteronism or low renin hypertension. 594 M variant in βENaC or two rare variants in γENaC are also suggested to be involved in EHT.

As discussed by a study group working most vigorously in this field (Corvol et al. 1999), these casual conclusions may vary by re-examinations due to several problems. Even in the lines of investigations of AGT gene, in which large number of subjects have been included in different populations, the results have been divergent and, thus, meta-analyses are required to arrive to a conclusion in a single population only, Caucasian. In other candidates genes, the number of subjects, as well as population, is limited, and genetic findings of polymorphisms in individual populations are incomplete. Therefore, further studies are required particularly in the following areas: 1) Determination of genotypes in each population is necessary to establish a database for examination. 2) Better phenotyping of hypertensive subjects by ambulatory and home blood pressure measurements or by identifying onset of hypertension in cohort study will enhance the statistical power. 3) Detailed classification of EHT by clinical factors, such as plasma values for renin, aldosterone, cholesterol, etc., or presence of obesity, smoking, high salt diet, etc., will help detect the linkage between specific types of ETH and genetic variants. 4) Comprehensive analysis of several candidate genes in each individual will compensate a low attributable risk of a single gene.

Although it is difficult at present to propose a specific medication according to the genotype of a particular patient, cohort-study based genetic analyses in individual populations should provide information that should allow optimal management of some hypertensive patients based on underlying abnormalities.

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