Korean J Biol Sci 8: 41-47, 2004

Genetic Analysis of Kallikrein-Kinin System in the Korean Hypertensives

Byung Yong Kang[1], Joon Seol Bae[2] and Kang Oh Lee*

Department of Life Science, Sahmyook University, Seoul 139-742, Korea;
[1]Research Institute for Life Science, Sahmyook University, Seoul 139-742, Korea;
[2]Department of Biochemistry, College of Science, Yonsei University, Seoul 120-749, Korea

Key Words:

Hypertension
Kallikrein-Kinin system
Korean population

The kallikrein-kinin system affects regulation of blood pressure, and genes encoding for the components of this system have been considered as good candidates for hypertension. To evaluate the relationship between genetic polymorphisms of candidate genes involved in this system and hypertension, we performed case-control studies using genetic markers in Korean normotensives and hypertensives, respectively. By association study, there was a marginal association with hypertension in AA genotype distribution of A1789G polymorphism in the hKLK1 gene (P=0.0754). Thus, this genetic polymorphism may weakly contribute to the susceptibility to hypertension in Koreans. We also observed that significant linkage disequilibrium exists among three polymorphic sites in the hKLK1 gene studied, suggesting that the three genetic polymorphisms can be useful as genetic markers in clinical association studies. Further studies using larger sample sizes and more genetic markers will be needed to clarify genetic influence of kallikrein-kinin system for hypertension.

Blood pressure is a complex trait determined by an array of interlocking homeostatic systems with feedbacks that maintain homeostasis in the face of widely varying environmental factors. Hypertension is defined by arbitrary limits, but represents the upper end of this distribution of blood pressure determined by a combination of genetic and environmental factors (Kang et al., 2000). Candidate genes that determine blood pressure variation include those whose products have a direct role in blood pressure such as the components of kallikrein-kinin system.

The kallikrein-kinin system appears to be an important regulator of cardiovascular function. This phylogenetically ancient system of proteases, substrates, peptides, peptidases and inhibitors has some responsibility for the regulation of local and perhaps systemic hemodynamics, vascular permeability, inflammatory response, activation of neuronal pathway and the movement of electrolytes, water and metabolic substrates across epithelia and into other tissues (Bhoola et al., 1992).

Kallikreins are serine proteases involved in posttranslational processing of polypeptides (Chung et al., 1986). By generating hormone peptides, kallikreins regulate processes such as organ perfusion, systemic blood pressure, sodium and water homeostasis, and inflammation (Clements, 1994; Margolius, 1996). There are two groups of kallikreins: glandular or tissue kallikrein, and plasma kallikrein. Tissue kallikrein (hK1) is the major kinin-forming enzyme in the kidney (Meneton et al., 2001), synthesized in the distal tubules, and released into urine and the peritubular interstitium (Slim et al., 2002). The level of urinary kallikrein excretion reflects the renal synthesis of this enzyme, and is influenced by genetic factors and dietary sodium intake (Berry et al., 1989; Zinner et al., 1976). Urinary kallikrein excretion is reduced in hypertension as well as renal diseases (Margolius et al., 1974). An association between blood pressure and a kallikrein gene polymorphism has been observed in rat strains (Pravenec et al., 1991).

The tissue kallikrein (hKLK1) gene is located on chromosome 19 (19q13.2-q13.4), together with several homologous genes coding for non-kinin-forming serine proteases or unidentified protein products (Harvey et al., 2000). The hKLK1 gene contains five exons spanning over 5.2 kb, and codes for an inactive prokallikrein form that is activated by intracellular proteolysis of a short amino-terminal peptide (Evans et al., 1988). Several polymorphisms in the hKLK1 gene have been identified (Berge and Berg, 1993; Berge et al., 1997).

Kininogen is a substrate for kallikreins, and the liberated kinin has various pharmacological properties including effects on the cardiovascular system, smooth

*To whom correspondence should be addressed.
Tel: 82-2-3399-3561, Fax: 82-2-971-6812
E-mail: leeko@syu.ac.kr

muscle contraction, ion transport, pain production, capillary permeability, arachidonic acid metabolism, and cell proliferation. Thus, this may be involved in hypertensive, diabetic diseases or inflammatory disorders (Margolius, 1989; Proud and Kaplan, 1998).

The kininogen (KNG) gene is located on chromosome 3 proximal to two closely related genes, the α_2-HS glycoprotein and the histidine-rich glycoprotein (Fong et al., 1991; Cheung et al., 1992). This gene is 27 kb long, and has 11 exons (Kitamura et al., 1985).

Kinin receptors are presently characterized as B_1, B_2 and perhaps B_3 receptors. Bradykinin B_1 receptor is less prominent than B_2 (Schneck et al., 1994). Bradykinin B_2 receptor (BDKRB2) gene was cloned (Hess et al., 1992), and by homology search, this receptor was grouped to the superfamily of the G-protein-coupled seven transmembrane domain receptors. Studies on the genetic structure of this gene revealed that it consists of three exons (Kammerer et al., 1995). Three well-defined polymorphisms located in each of the three exons were characterized (Braun et al., 1995a, b).

To characterize the genetic susceptibility of kallikrein-kinin system for hypertension, studies in different populations must be conducted. The present case-control studies were designed to investigate association between polymorphisms of candidate genes encoding for the components of the kallikrein-kinin system and hypertension in the ethnically homogeneous Korean population.

Materials and Methods

Subjects

Total 236 Korean subjects were recruited from clinical pathology of the Seoul Hygiene hospital, Seoul, Korea. Of these individuals, 88 subjects were diagnosed with hypertension. Subjects were classified as having hypertension if they exhibited systolic blood pressure (SBP) > 140 mmHg and diastolic blood pressure > 90 mmHg and had no clinical signs, symptoms, and laboratory findings suggestive of secondary hypertension. Age-controlled normotensives consisted of 148 indivials having systolic blood pressure (SBP) < 140 mmHg and diastolic blood pressure < 90 mmHg. Male/female (M/F) ratio was not statistically different between two groups (For normotensives, the M/F ratio was 51.7%/48.3%; for hypertensives, the M/F ratio was 56.7%/43.3%; χ^2= 0.8822, df=1, P=0.3476). Due to varying sample quality and sample exhaustion, not all DNAs were successfully assayed for each polymorphism.

Genetic analysis

Total genomic DNA was isolated using a commercial genomic DNA purification kit (QIAamp; QIAGEN) (Kang and Lee, 2003). All 5 polymorphisms were assayed by means of PCR, either by direct size analysis or by analysis of the products of restriction enzyme digestion. Amplification reaction was performed in 50 ul containing 0.1 mM each of dCTP, dATP, dGTP and dTTP, 10 *p*mol of each oligonucleotide primer, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 1.25 units of *Taq* DNA polymerase (Perkin-Elmer/Cetus) and 100 ng genomic DNA. The primer sequences used are shown in Table 1. Amplification was carried out for 30 cycles of denaturation at 94°C for 1 min, annealing at 50~65°C for 1 min, and extension at 72°C for 1 min in Perkin-Elmer model 9700 thermal cycler. After PCR reaction, about 10 ul of each PCR product was digested by appropriate restriction enzymes, and genotyped in ethidium bromide-stained agarose gels or polyacrylamide gels after electrophoresis.

Statistical analysis

Genotypes were assigned to each subject, and allele frequencies were calculated from genotype frequencies in the hypertensive and normotensive groups, respectively. Differences in total alleles for candidate gene polymorphisms on all chromosomes for the two groups were tested using χ^2-analysis. Standardized pairwise linkage disequilibrium, D, was calculated. The degree of nonrandom association was determined by calculation of Ć (Hill and Robertson, 1968) and D' (Lewontin, 1964) between polymorphic sites. A Monte Carlo approach

Table 1. Polymorphic sites and primer sequences of each candidate gene in the kallikrein-kinin system

Gene	RFLP site	Primer sequence	Reference
hKLK1	G812C	5'-GTCTACAGCTCTGGGGATCGGACG-3' 5'-CTCCCACATCCCCCCACTGTCTCAC-3'	Berge et al., 1997
	A1789G	5'-ATCCTGCCTAATGATGAGCGC-3' 5'-GGCTGCCTCACCACACAGGTGTCT-3'	Berge et al., 1997
	A2233C	5'-ATCGGGGCCACCCCAGCTGTGTTA-3' 5'-CTGTTCTGGCGTCCCTGACTCGCC-3'	Berge et al., 1997
KNG	C587T	5'-CTTGGGCACTTATTATATTGCCACAC-3' 5'-AACTTTATCATTAAATTTTTGCCTACTTAC-3'	Cheung et al., 1993
BDKRB2	R48/R35	5'-GAAGGTGGCCCAGTATGAGC-3' 5'-GATTGGTCAGGATTTATGG-3'	Braun et al., 1995b

Table 2. Genotype and allele frequencies of the three candidate gene polymorphisms encoding for the components of the kallikrein-kinin system in normotensive and hypertensive groups

Gene	Genotype Frequency			Allele Frequency	
	n (%)	n (%)	n (%)	n (%)	n (%)
hKLK1 G812C	GG	GC	CC	G	C
normotensive	120(81.1)	27(18.2)	1(0.7)	0.90	0.10
hypertensive	73(83.9)	13(14.9)	1(1.2)	0.91	0.09
hKLK1 A1789G	AA	AG	GG	A	G
normotensive	23(15.9)	84(57.9)	38(26.2)	0.45	0.55
hypertensive	19(21.8)	37(42.5)	31(35.6)	0.43	0.57
hKLK1 A2233C[1]	AA	AC	CC	A	C
normotensive	23(15.5)	66(44.6)	59(39.9)	0.38	0.62
hypertensive	14(15.9)	31(35.2)	43(48.9)	0.34	0.66
KNG C587T	CC	TC	TT	C	T
normotensive	132(100.0)	0(0.0)	0(0.0)	1.00	0.00
hypertensive	80(100.0)	0(0.0)	0(0.0)	1.00	0.00
BDKRB2 R48/R35	R48/R48	R48/R35	R35/R35	R48	R35
normotensive	83(100.0)	0(0.0)	0(0.0)	1.00	0.00
hypertensive	85(98.8)	1(1.2)	0(0.0)	0.99	0.01

was used to overcome the difficulties of the statistical analysis in the highly polymorphic markers (extended haplotype) in association studies (Sham and Curtis, 1995). P-values less than 0.05 were considered as significant. Statistical analysis was performed using SPSS for Windows V11.0 (SPSS Inc, Chicago).

Results

Gene frequency

PCR analysis revealed polymorphic patterns in the candidate genes encoding components of kallikrein-kinin system (Table 2). Fig. 1 shows the electrophoretic patterns of three genetic polymorphisms in the hKLK1 gene. G812C polymorphism in exon 1 of the hKLK1 gene was detected by the restriction enzyme *Bst* UI. G allele produced a band of 262 bp, while C allele yielded bands of 238 bp and 24 bp. The genotype frequencies of GG, GC, and CC were 81.1%, 18.2% and 0.7% in normotensives, and 83.9%, 14.9%, and 1.2% in

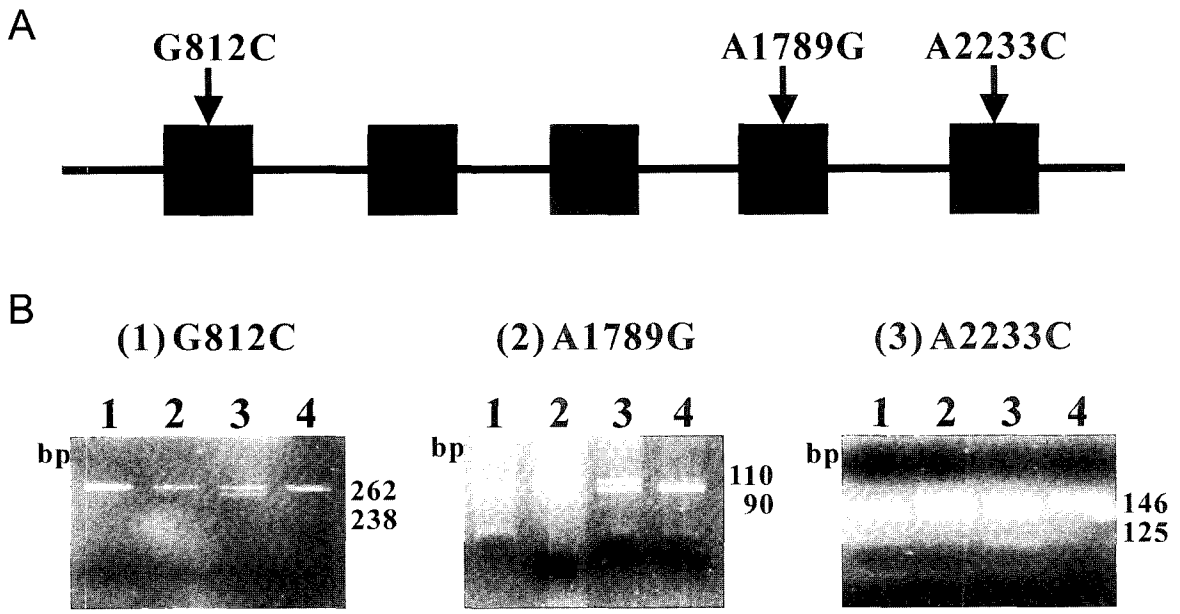

Fig. 1. A polymorphic map and electrophoretic patterns of the hKLK1 gene. A, A polymorphic map of the hKLK1 gene. Shaded box, exon; dash, intron. B, Electrophoretic patterns of the hKLK1 gene. (1) G812C polymorphism. Lane 1, 2 and 4, GG genotypes; lane 3, GC genotype. (2) A1789G polymorphism. Lane 1 and 3, AG genotypes; lane 2, AA genotype; lane 4, GG genotype. (3) A2233C polymorphism. Lane 1, CC genotype; lane 2 and 3, AC genotype; Lane 4, AA genotype.

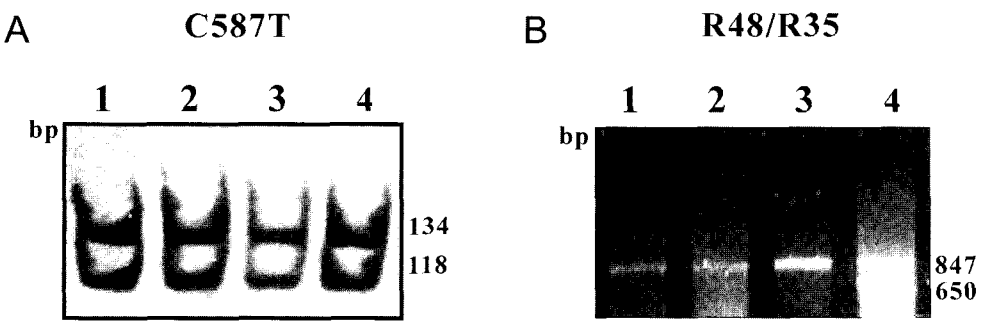

Fig. 2. Electrophoretic patterns of the *KNG* and *BDKRB2* genes. A, Electrophoretic patterns of the *KNG* gene. Lane 1~4, CC genotypes. B, Electrophoretic patterns of the *BDKRB2* gene. Lane 1~3, R48/R48 genotypes; lane 4, R48/R35 genotype.

hypertensives. The heterozygosity and PIC values of G812C polymorphism were 0.18 and 0.16 for normotensives, and 0.16 and 0.15 for hypertensives, respectively. According to heterozygosity and PIC values, the G812C polymorphism showed a relatively low degree of polymorphism in both groups. There were no significant differences in genotype and allele frequencies between the two groups by case-control comparison ($P > 0.05$).

Like G812C polymorphism, A1789G polymorphism in exon 4 of the hKLK1 gene was also detected by the restriction enzyme *Bst* UI. When an A allele of A1789G polymorphism was present, the 110 bp PCR fragment was not digested by *Bst* UI, whereas was reduced to bands a 90 bp and 10 bp when G allele of A1789G polymorphism was present. The genotype frequencies of AA, AG and GG were 15.9%, 57.9% and 26.2% in normotensives, and 21.8%, 42.5% and 35.6% in hypertensives, respectively. The heterozygosity and PIC values of G812C polymorphism were about 0.49 and 0.37 for both groups, respectively. According to the heterozygosity and PIC values, the A1789G polymorphism indicated the highest genetic diversity in both groups among the genetic markers tested in this study. There were no significant differences in genotype and allele frequencies between the two groups, but AA genotype was more frequent in hypertensives compared to normotensives ($\chi^2 = 5.1699$, df = 2, $P = 0.0754$).

A2233C polymorphism in the exon 5 was detected by the restriction enzyme *Mse* I. The A allele produced bands of 125 bp and 21 bp, whereas C allele yielded a band of 146 bp. The genotype frequencies of AA, AC and CC were 15.5%, 44.6% and 39.9% in normotensives, and 15.9%, 35.2% and 48.9% in hypertensives, respectively. The heterozygosity and PIC values of the A2233C polymorphism were 0.47 and 0.36 for normotensives, and 0.45 and 0.35 for hypertensives, respectively. According to the heterozygosity and PIC values, the A2233C polymorphism indicated reasonably high degree of gene diversity in both groups. The genotype and allele frequencies of this polymorphism were not significantly different between normotensives and hypertensives ($P > 0.05$).

C587T polymorphism in the exon 5 of the KNG gene was detected by the presence or absence of the cutting site for restriction enzyme *Csp45* I. The C allele produced bands of 134 and 118 bp, while the T allele yielded a band of 252 bp, (Fig. 2A). Only the C allele was detected in our subjects, and thus, showed a monomorphic pattern in Koreans.

The R48/R35 polymorphism in the BDKRB2 gene is located in the 3' untranslated region of this gene, and two common alleles consisted of 48 or 35 repeat units (named R48 or 35), respectively (Fig. 2B). In our study, the R48 allele was common in Korean subjects, while the R35 allele was observed only in one hypertensive subject.

Haptotype frequency and linkage disequilibrium of the hKLK1 gene

Linkage disequilibrium between the three polymorphic sites in the hKLK1 gene was assessed by a pair-wise analysis of the linkage disequilibrium statistics Ć and D' (Table 3). Significant linkage disequilibrium was observed between the three polymorphic sites studied ($P < 0.05$).

Distribution of extended haplotype in the hKLK gene is displayed in Table 4. Because the existence of double heterozygotes disturbed the construction of extended haplotype, we performed the maximum likelihood estimation of haplotype distribution with an EH program. The G-G-C haplotype was found to be the most common in normotensive and hypertensive groups, respectively. By Monte Carlo simulation, no significant difference was

Table 3. Pair-wise linkage disequilibrium statistics (Δ, D') among polymorphic sites in the hKLK1 gene

D'	Δ		
	G812C	A1789G	A2233C
G812C		0.3301	0.3069
A1789G	0.8906		0.7702
A2233C	0.7141	0.9156	

Table 4. Estimated haplotype distribution of three DNA polymorphisms in the hKLK1 gene

Polymorphic site			Subject	
G812C	A1789G	A2233C	Normotensive	Hypertensive
G	A	A	0.278643	0.281408
G	A	C	0.079993	0.118877
G	G	A	0.007631	0.023134
G	G	C	0.544078	0.508088
C	A	A	0.082692	0.037924
C	A	C	0.006948	0.013846
C	G	A	0.000000	0.000000
C	G	C	0.000015	0.016723
	P[1]		0.619131	

[1]The significant difference between normotensives and hypertensives was not observed in haplotype frequencies (T_2=1.780896, df=3, P> 0.05, simulation number=10,000).

observed in extended haplotype frequency between two groups (P > 0.05).

Discussion

Because many biochemical and physiological systems impact on blood pressure regulation and hypertension susceptibility, many of these identified genes and polymorphisms are candidates for population-level association studies involving blood pressure levels or hypertension status. The Kallikrein-kinin system has been postulated to play a role in the homeostasis of normal blood pressure, and defects in this system could contribute to the pathogenesis of clinical hypertension (Margolius, 1995). Previous studies have shown that the tissue kallikrein-kinin system is involved in many physiological and pathological processes such as blood pressure homeostasis, renal sodium excretion, allergy, and inflammatory disorders (Bhoola et al., 1992; Margolius, 1989).

In this study, we evaluated the association of hypertension with three polymorphic markers of the hKLK1 gene in Korean population. Among the polymorphisms studied, G812C and A2233C polymorphisms were not significantly associated with hypertension in our subjects. Thus, these two polymorphisms might not be good predictors for risk of hypertension in the Korean population. However, we found a marginal association of the A1789G polymorphism in the hKLK1 gene with hypertension. The AA genotype frequency of the A1789G polymorphism was 21.8% in hypertensive group compared with 15.9% in normotensive individuals (P=0.0754). Whether the AA genotype directly accounts for a physiological effect or acts, as a marker for a causative mutation, is as yet unclear. Because the A1789G polymorphism does not cause an alteration in amino acid sequence, it is likely to be in linkage disequilibrium with a "causative" variant nearby in the genome. The AA genotype was present at a low frequency in our subjects. Since the incidence of hypertension is 20~30% (Friend et al., 1996; Kurtz and Spence, 1993) and the causative loci for this condition may contribute to the disease even in a small proportion of the population, the AA genotype would be expected to be on the same chromosome as the putative "causative" allele. Nevertheless, because our result was statistically negative, further studies will be required to clarify the relationship between the A1789G polymorphism and hypertension.

Linkage relationships among DNA markers in any genetic loci may influence the choice of polymorphisms used in linkage or association studies. An inverse relationship between linkage disequilibrium and physical distance between loci is of course expected. Our results revealed that significant linkage disequilibrium exists among the three polymorphic sites in the hKLK1 gene. The three sites are within a relatively small genetic distance in the hKLK1 gene (Berge et al., 1997) and exihibit strong non-random associations among them. Thus, in the case of the hKLK1 gene, population studies probably would not require a large sample size to detect association.

In analysis of genes with single nucleotide polymorphisms (SNPs), extended haplotype distribution is important in discovering genetic background of hypertension. Distribution of extended haplotype of the hKLK1 gene in our subjects revealed predominance of the G-G-C haplotype in both groups. However, there were no significant differences between the two groups, suggesting that any haplotype with the three polymorphisms may not be useful as a genetic marker for hypertension.

Kininogen is a single chain glycoprotein and constitutes a member of the cystatin superfamily with stefin and cystatin (Fong et al., 1991). Kinin liberated from kininogen by proteolysis can affect many physiological functions including blood pressure homeostasis, but its role in human homeostatic mechanisms and disease are just beginning to be understood (Margolius, 1995). Cheung et al. (1993) have described a C587T polymorphism in the exon 5 of the KNG gene. The identification of SNP in this candidate gene provides a powerful tool to understand the role of the KNG gene associated with hypertension. However, none of the Korean subjects in this study had the T allele in the KNG gene.

The BDKRB2 gene has been proposed as one of candidate genes to be involved in the complex genetic underpinnings of common chronic disorders such as hypertension and ischemic heart disease (Gainer et al., 2000). The exon 3 in the BDKRB2 gene include polymorphism that consists of multiple repeat units of 15 or 16 bp in length and that are characterized by the following consensus sequence: TGGA (A) GGGCTAGAACC

(Braun et al., 1995b). This polymorphism located in the 3'-untranslated region of this exon has two common alleles consisting of 48 or 35 repeat alleles, respectively. The frequency of the R48 allele in the BDKRB2 gene is the most frequent in the Korean population. The R48 allele frequency (0.99) of the BDKRB2 gene found in the Korean population was higher than that in the South German population (0.84) (Braun et al., 1995b). The R35 allele in the Korean population was detected only in one hypertensive subject, reflecting very low informativeness as a genetic marker.

In conclusion, the present study showed that the AA genotype of A2233C polymorphism in the hKLK1 gene might be associated with hypertension in our subjects of Korean origin. However, the risk of incurring false-positive or false-negative results in a case-control study due to chance effect is always possible. This should be mainly due to the relative heterogeneity of the populations screened, devived from mixing of groups from different geographic regions. Of course, there was little possibility of ethnic heterogeneity because all subjects participating in our study were ethnically homogeneous Koreans. Nevertheless, our data need to be verified in a larger population or another ethnic group in the future.

References

Berge KE, Bakken A, Bøhn M, Erikssen J, and Berg K (1997) Analyses of mutations in the human renal kallikrein (hKLK1) gene and their possible relevance to blood pressure regulation and risk of myocardial infarction. *Clin Genet* 52: 86-95.

Berge KE and Berg K (1993) No effect of *Taq* I polymorphism at the human renal kallikrein (KLK1) locus on normal blood pressure level or variability. *Clin Genet* 44: 196-202.

Berry TD, Hasstedt SJ, Hunt SC, Wu LL, Smith JB, Ash KO, Kuida H, and Williams RR (1989) a gene for high urinary kallikrein may protect against hypertension in Utah kindreds. *Hypertension* 13: 3-8.

Bhoola KD, Figueroa CD, and Worthy K (1992) Bioregulation of kinins: kallikreins, kininogens, and kininases. *Pharmacol Rev* 44: 1-80.

Braun A, Kammerer S, Böhme E, Müller B, and Roscher AA (1995a) Identification of polymorphic sites of the human bradykinin B_2 receptor gene: full length cDNA, genomic organization and identification of the regulatory region. *Biochem Biophys Res Commun* 211: 226-233.

Braun A, Kammerer S, Böhme E, Müller B, and Roscher AA (1995b) Identification of polymorphic sites of the human bradykinin B_2 receptor gene. *Biochem Biophys Res Commun* 211: 234-240.

Cheung PP, Cannizzaro LA, and Colman RW (1992) Chromosomal mapping of human kininogen gene (KNG) to 3q26 to qter. *Cytogenet Cell Genet* 59: 24-26.

Cheung PP, Kunapuli SP, Scott CF, Wachtfogel YT, and Colman RW (1993) Genetic basis of total kininogen deficiency in Williams' trait. *J Biol Chem* 268: 23361-23365.

Chung DW, Fujikawa K, McMullen BA, and Davie EW (1986) Human plasma prekallikrein, a zymogen to a serine protease that contains four tandem repeats. *Biochemistry* 25: 2410-2417.

Clements JA (1994) The human kallikrein gene family: a diversity of expression and function. *Mol Cell Endocrinol* 99: C1-C6.

Evans BA, Yun ZY, Close JA, Tregear GW, Kitamura N, Nakanishi C, Callen DF, Baker E, Hyland VJ, Sutherland GR, and Richards RI (1988) Structure and chromosomal localization of the human renal kallikrein gene. *Biochemistry* 27: 3124-3129.

Fong D, Smith DI, and Hsieh WT (1991) The human kininogen gene (KNG) mapped to chromosome 3q26 → qter by analysis of somatic cell hybrids using the polymerase chain reaction. *Hum Genet* 87: 189-192.

Friend LR, Morris BJ, Gaffney PT, and Griffiths LR (1996) Examination of the role of nitric oxide synthase and renal kallikrein as candidate genes for essential hypertension. *Clin Exp Pharmacol Physiol* 23: 564-566,

Gainer JV, Brown NJ, Bachvarova M, Bastien L, Maltais I, Marceau F, and Bachvarov DR (2000) Altered frequency of a promoter polymorphism of the kinin B_2 receptor gene in hypertensive African-Americans. *Am J Hypertens* 13: 1268-1273.

Harvey TJ, Hooper JD, Myers SA, Stephenson SA, Ashworth LK, and Clements JA (2000) Tissue-specific expression patterns and fine mapping of the human kallikrein (KLK) locus on proximal 19q13.4. *J Biol Chem* 275: 37397-37406.

Hess JF, Borkowski JA, Young GS, Strader CD, and Ransom RW (1992) Cloning and pharmacological characterization of a human bradykinin (BK-2) receptor. *Biochem Biophys Res Commun* 184: 260-268.

Hill WG and Robertson A (1968) Linkage disequilibrium of finite populations. *Theor Appl Genet* 38: 226-231.

Kammerer S, Broun A, Arnold N, and Roscher AA (1995) The human bradykinin B_2 receptor gene. *Biochem Biophys Res Commun* 21: 234-240.

Kang BY, Kim KT, Eo HS, Lee KH, Hong SS, Shin JH, and Lee CC (2000) Association between genetic variation of the insulin receptor gene and essential hypertension in the Korean population. *Korean J Biol Sci* 4: 87-90.

Kang BY and Lee KO (2003) Genetic polymorphisms of t-PA and PAI-1 genes in the Korean population. *Korean J Biol Sci* 7: 249-253.

Kitamura N, Kitagawa H, Fukushima D, Takagaki Y, Miyata T, and Nakaniski S (1985) Structural organization of the human kininogen gene and a model for its evolution. *J Biol Chem* 260: 8610-8617.

Kurtz TW and Spence MA (1993) Genetics of essential hypertension. *Am J Med* 94: 77-84.

Lewontin RC (1964) The interaction of selection and linkage. I. General considerations: heterotic models. *Genetics* 49: 49-67.

Margolius HS (1989) Tissue kallikreins and kinins: regulation and roles in hypertensive and diabetic diseases. *Annu Rev Pharmacol Toxicol* 29: 343-364.

Margolius HS (1995) Kallikreins and kinins: some unanswered questions about system characteristics and role in human disease. *Hypertension* 26: 221-229.

Margolius HS (1996) Kallikreins and kinins: molecular characteristics and cellular and tissue responses. *Diabetes* 45 Suppl 1: S14-S19.

Margolius HS, Horwitz D, Pisano JJ, and Keiser HR (1974) Urinary kallikrein excretion in hypertensive man: relationships to sodium intake and sodium-retaining steroids. *Circ Res* 35:

820-825.

Meneton P, Bloch-Faure M, Hagege A, Ruetten H, Huang W, Bergaya S, Ceiler D, Gerhing D, Martins I, Salmon G, Boulanger CM, Nussberger J, Crozatier B, Gasc JM, Heudes D, Bruneval P, Doetschman T, Menard J, and Alhenc-Gelas F (2001) Cardiovascular abnormalities with normal blood pressure in tissue kallikrein deficient mice. *Proc Natl Acad Sci USA* 98: 2634-2639.

Pravenec M, Kren V, Kunes J, Scicli AG, Carretero OA, Simonet L, and Kurtz TW (1991) Cosegregation of blood pressure with a kallikrein gene family polymorphism. *Hypertension* 17: 242-246.

Proud D and Kaplan AP (1988) Kinin formation mechanisms and role in inflammatory disorders. *Annu Rev Immunol* 6: 49-83.

Sham PC and Curtis D (1995) Monte Carlo tests for associations between diseases and alleles at highly polymorphic loci. *Ann Hum Genet* 59: 97-105.

Schneck KA, Hess JF, Stonesifer GY, and Ransom RW (1994) Bradykinin B_1 receptors in rabbit aorta smooth muscle cells in culture. *Eur J Pharmacol* 266: 277-282.

Slim R, Torremocha F, Moreau T, Pizard A, Hunt SC, Vuagnat A, Williams GH, Gauthier F, Jeunemaitre X, and Alhenc-Gelas F (2002) Loss-of-function polymorphism of the human kallikrein gene with reduced urinary kallikrein activity. *J Am Soc Nephrol* 13: 968-976.

Zinner SH, Margolius HS, Rosner B, Keiser HR, and Kass EH (1976) Familial aggregation of urinary kallikrein concentration in childhood: relation to blood pressure, race and urinary electrolytes. *Am J Epidemiol* 104: 124-132.

[Received October 19, 2003; accepted March 5, 2004]