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A Meta-Analysis on the Associations of Gly460Trp Polymorphism of the α-Adducin Gene with Hypertension

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ABSTRACT : **Gly460Trp polymorphism of á-adducin gene has been reported to be associated with hypertension in some populations, and we, therefore, attempted to replicate this finding in Korean population. There were no significant differences in allele and genotype frequencies of Gly460Trp polymorphism in Korean normotensives and hypertensives, respectively. The meta-analysis revealed that the Mantel-Haenszel estimate of the odds ratio across the studies was 1.17 (95%CI 1.04-1.31), and that there was significant evidence against homogeneity of the odds ratio among the studies included (Breslow-Day test = 27.34, df = 9, P = 0.001). Although the meta-analysis appeared in favour of association between the Gly460Trp polymorphism of α-adducin gene and hypertension, there was the considerable heterogeneity among the studies and the evidence is also rather borderline. Further comprehensive approaches are needed to resolve this debatable issue.**

Key words: α-Adducin, Hypertension and Meta-Analysis

Introduction

Hypertension is known to be caused by polygenes, and its phenotypic expression is modulated by various environmental factors. Recent advances in molecular biology have allowed investigation of the role of candidate genes for hypertension. The affected sib-pair approach revealed that angiotensinogen, lipoprotein lipase and α-adducin gene polymorphisms are genetic risk factors for human essential hypertension (Cusi *et al.*, 1997; Jeunemaitre *et al.*, 1992; Wu *et al.*, 1996). Among them, it was observed that angiotensinogen and α-adducin genes predispose to hypertension both in human and animal model (Bianchi *et al.*, 1995; Kim *et al.*, 1995).

α-Adducin was first identified as a cytoskeletal protein in erythrocytes (Bianchi *et al.*, 1994) and may play a role in the formation of spectrin-actin complexes, which anchor some transmembrane proteins, such as the Na^+-K^+ ATPase (Tripodi *et al.*, 1996). Genetic variation of α-adducin has been shown to affect renal sodium transport at the cellular level in the Milano Hypertensive (MHS) rat strain (Tripodi *et al.*, 1996). This variation appears to account for the blood pressure differences between MHS rats and normotensive control rat strains (Bianchi *et al.*, 1994).

In human, the α-adducin gene locus was located on chromosome 4p16.3, and the significant linkage between microsatellite markers mapped close to the α-adducin gene locus and hypertension was detected by linkage analysis (Casari *et al.*, 1995). Also, Cusi *et al.*, (1997) reported that a molecular variant of α-adducin gene (with tryptophan instead of glycine at amino acid number 460) was associated with hypertension in Italian and French population, and this finding was also reproduced in 2 Japanese study groups (Iwai *et al.*, 1997; Tamaki *et al.*, 1998). However, other studies reported no association between this genetic variation and hypertension in Scottish (Kamitani *et al.*, 1998), Anglo-Australian (Wang *et al.*, 1999), African-American (Larson *et al.*, 2000) and 2 Japanese study groups (Ishikawa *et al.*, 1998; Kato *et al.*, 1998).

In view of the importance of the α-adducin gene as a genetic marker for hypertension, the present case-control study was designed to investigate the association between the Gly460Trp polymorphism of α-adducin gene and

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hypertension in Korean population. Also, we have carried out the meta-analysis of the current and previous results in order to summarize and interpret the accumulated data on the possible association between this polymorphism and hypertension. The application of the meta-analysis allows us to focus on the observed odds ratio and 95% confidence intervals as well as difference in proportions in each of the individual studies as an indication of the generalizability of differences between normotensives and hypertensives.

Materials and Methods

Subjects

We obtained 196 blood samples from the outpatients of Seoul Hygiene Hospital, Seoul, Korea. Out of these samples, 96 essential hypertensive Korean individuals were defined as having a blood pressure above 140/90 mmHg. Subjects with secondary forms of hypertension were excluded from the study.

Genotyping

Genomic DNA was prepared from buffy coat of 5 ml blood after lysis of red blood cell (Sambrook *et al.*, 1989). The Gly460Trp polymorphism of α-adducin gene was detected by using polymerase chain reaction(PCR)-*Ban*II digestion(Wang *et al.*, 1999). The sequence of the sense primer was 5'-CTCAGACACAGTTTTCAGAAGCAGCAG-3', and antisense primer 5'-CGACTTGGGACTGCTTCC-ATTGGGCC-3'. PCR was performed in a final volume of 50 ul (100 ng of genomic DNA, 20 pmol of each primer, 200 uM each of the four dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-HCl, pH 8.4 and 2.5 unit of *Taq* DNA polymerase). After initial denaturation at 95°C for 3 min, there were 36 cycles of 94, 55 and 72°C for 1 min each, finishing with a step at 72°C for 30 min. An aliquot of 12 uL PCR product mixture was then incubated with the restriction enzyme *Ban*II, for 5 hr at 37°C in a total volume of 30 uL with buffer recommended by the supplier. Electrophoresis was performed on a 4% agarose gel.

Biochemical assays

Total blood cholesterol (TC), triglyceride (TG) and high-density lipoprotein (HDL)-cholesterol levels were determined enzymatically, and the LDL-cholesterol level was calculated by Friedewald's equation (Friedwald *et al.*, 1972). Plasma Lipoprotein (a) (Lp(a)) level was measured by the immuno-precipitation method, and plasma apolipoprotein AI (ApoAI) concentration was determined by immunoturbidimetric method.

Statistical analysis

Genotypes were assigned to each subject and from these values, allele numbers were calculated. The frequency of any prescribed allele in a sample is equal to twice the number of homozygotes for that allele (because each homozygote carries two copies of the allele) plus the number of heterozygotes for that allele (because each heterozygote carries one copy) divided by two times the number of individuals in the sample (because each individual carries two alleles at the locus). The significance of the difference between total observed genotypes or alleles for the normotensives and hypertensives was tested by χ^2-indepence analysis. The significant deviation from Hardy-Weinberg equilibrium (HWE) was analyzed by a χ^2-fitness analysis. The polymorphism information content (PIC) was estimated by the methods of Bostein *et al.*, (1980). The relative risk of hypertension associated with allelic variation is expressed in terms of an odds ratio (OR) with 95% confidence intervals (CI). Comparison of demographic parameters across genotypes was performed using a parametric one-way ANOVA test.

Meta-analysis

The common odds ratio estimator (fixed effect model) and the 95% confidence interval (CI) was calculated using the Mantel-Haenszel technique (Mantel and Haenszel, 1959). Heterogeneity was tested by Breslow-Day method (Breslow and Day, 1980) and publication bias was examined by plotting a funnel plot of reported effect, as assessed with the natural log of the odds ratio, against trial size (Dickersin and Berlin, 1992). All statistical test of the meta-analysis was performed by SAS version 6.12 statistical software (SAS Institute, Cary, North Carolina, USA).

Results

Association between Gly460Trp polymorphism of α-adducin gene and hypertension

Gly460Trp polymorphism of α-adducin gene was detected by digestion with restriction enzyme *Ban*II after PCR amplification (Fig. 1). Gly allele yielded bands of 84 bp and 24 bp, and Trp allele gave a 108 bp band. The genotype and allele frequencies of α-adducin gene are displayed in Table 1. The observed genotype distributions of the α-adducin gene were not different from those expected for HWE. The genotype frequencies of Gly/Gly, Gly/Trp and Trp/Trp were 18, 43 and 39% in normotensives, and 16, 40 and 40% in hypertensives, respectively. There were no significant differences in allele and genotype frequencies between normotensives and hypertensives. The heterozygosity and

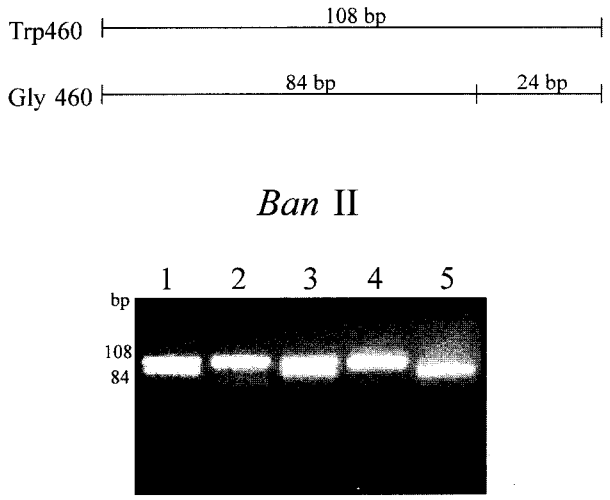

Fig. 1. Gly60Trp polymorphism of a-adducin gene. Lane 1 and 3, G/y/Trp heterozygotes; lane 2 and 4, Trp/Trp homozygotes; lane 5, Gly/Gly homozygote.

PIC values of Gly460Trp polymorphism represented the values of 0.4779 and 0.3637 for normotensives and 0.4688 and 0.3589 for hypertensives, respectively. According to the PIC value, Gly460Trp polymorphism of α-adducin gene showed relatively high PIC values in the both groups.

The comparison of clinical parameters among genotypes of Gly460Trp polymorphism in the α-adducin gene

Table 2 represents the comparison of clinical parameters across the genotypes of the α-adducin gene. Any parameters was not significantly associated with genotypes of Gly460Trp polymorphism in our study group.

Discussion

To identify the causative gene of hypertension, a large number of case-control studies and rat cross experiments have been performed. Using genetically hypertensive rats, many quantitative trait loci responsible for blood pressure have been mapped on the rat genome, but few loci, only on chromosome 1, 4 and 8 were confirmed to cosegregate with hypertension in human by the affected sib-pair method (Cusi *et al.*, 1997; Jeunemaitre *et al.*, 1992; Wu *et al.*, 1996). One of these candidate genes is α-adducin gene, and a report which showed a significant association between a newly identified mutation (Gly460Trp polymorphism) in human α-adducin gene and hypertension in some populations (Cusi *et al.*, 1997; Iwai *et al.*, 1997; Tamaki *et al.*, 1998) emphasized the clinical importance of this gene.

In the present study, however, we could not detect any

Table 1. Genotype and allele frequencies of Gly460Trp polymorphism of the α-adducin gene in normotensives and hypertensives

	Genotype No. (%)			Allele No. (%)		H[1]	PIC[2]
	Gly/Gly	Gly/Trp	Trp/Trp	Gly	Trp		
Normotensives	18(18)	43(43)	39(39)	79(40)	121(60)	0.4779	0.3637
Hypertensives	16(17)	40(42)	40(42)	72(38)	120(62)	0.4688	0.3589
^{2}		0.1570			0.1650		
P		0.9240			0.6840		
Odds ratio(CI)[3]			1.09(0.72-1.63)				

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.
The observed genotype distribution was not in Hardy-weinberg equilibrium (For normotensives, ^{2}=1.006, df=1, P=0.3157; for essential hypertensives, ^{2}=1.1850, df=1, P=0.2763; for essential hypertensives).

Table 2. Clinical characteristics according to genotypes of Gly460Trp polymorphism in the á-adducin gene

Variables	Genotypes		
	Gly/Gly (No.)[8]	Gly/Trp (No.)	Trp/Trp (No.)
Age (year)	59.9 ± 8.7 (34)[9]	59.6 ± 12.7 (80)	59.44 ± 11.0 (78)
BMI (kg/m^2)[1]	23.7 ± 2.1 (33)	23.4 ± 2.2 (67)	23.3 ± 2.7 (77)
Tg (mg/dl)[2]	141.7 ± 85.4 (26)	132.2 ± 67.0 (58)	126.6 ± 85.3 (66)
TC (mg/dl)[3]	164.2 ± 41.8 (26)	149.7 ± 35.2 (58)	151.4 ± 36.6 (66)
LDL-chol (mg/dl)[4]	106.8 ± 39.5 (26)	95.9 ± 38.4 (58)	98.8 ± 33.7 (66)
HDL-chol (mg/dl)[5]	29.0 ± 11.2 (26)	27.4 ± 8.4 (58)	27.7 ± 9.2 (66)
Lp(a) (mg/dl)[6]	19.5 ± 18.1 (30)	15.1 ± 11.4 (62)	16.1 ± 11.9 (63)
Apo AI (mg/dl)[7]	102.42 ± 41.1 (8)	99.4 ± 33.7 (25)	93.5 ± 30.1 (13)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol, [6]lipoprotein (a), [7]apolipoprotein AI and [8]Number.
[9]Values are mean ± SD (Standard Deviation).

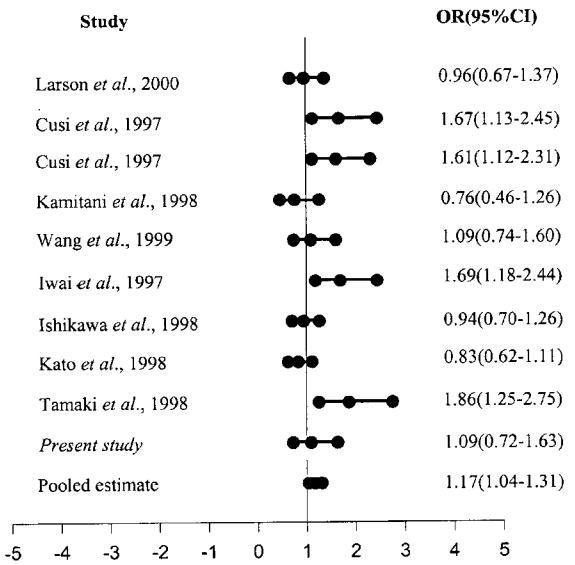

Fig. 2. Odds ratios and 95% confidence intervals for the effect of Typ allele of α-adducin gene from 10 studies.

association between the Gly460Trp polymorphism of the α-adducin gene and hypertension or other cardiovascular risk factors in Korean population. Therefore, it is unlikely that this polymorphism may contribute to the pathogenesis of hypertension or other cardiovascular diseases in our subjects.

To further assess the association of the Gly460Trp polymorphism in the α-adducin gene with hypertension, we performed the meta-analysis from 10 studies (Fig. 2). In total, the studies contain 1,622 normotensives and 1946 hypertensive subjects. The overall allele frequencies of Gly and Trp were 0.69 and 0.31 in normotensives, and 0.67 and 0.33 in hypertensives, respectively. The Mantel-Haenszel OR and 95% CI across all studies is 1.17 (1.04-1.31), suggesting the 17% increase of Trp allele.

There was, however, significant evidence against the homogeneity assumption of the odds ratio (Breslow-Day test = 27.34, df = 9, P = 0.001). There are several possible reasons for the discrepancy among studies. First, ethnic difference in genetic and/or environmental backgrounds may have contributed to the different results. In healthy subjects, the Trp allele frequencies of Korean (0.60) and Japaneses (0.51~0.60) populations were significantly higher than those of Caucasians (0.14~0.31) and African-American (0.07) populations (Table 3). Differences of these allele frequencies may partly result from genetic drift during the history of individual populations. When included studies were stratified by ethnic origin, the Mantel-Haenszel OR of the subgroup of Caucasian origin is slightly higher than that of subgroup of Asian origin (For 4 Caucasian groups, Mantel-Haenszel OR and 95% CI is 1.23 (1.06-1.58); for 5 Asian groups, Mantel-Haenszel OR and 95% CI is 1.14 (0.98-1.32)). In other word, it is likely that the Gly460Trp polymorphism

Table 3. Comparison of allele frequencies of Gly460Trp polymorphism in the α-adducin gene from various ethnic groups

Populations	Sample Number	Allele frequencies		P[1]	Reference
		Gly	Trp		
(Normotensives)					
African-American	432	0.93	0.07	<0.05	Larson *et al.*, 2000
Italian	151	0.86	0.14	<0.05	Cusi *et al.*, 1997
French	181	0.84	0.16	<0.05	Cusi *et al.*, 1997
Scottish	77	0.69	0.31	<0.05	Kamitani *et al.*, 1998
Anglo-Australian	112	0.77	0.23	<0.05	Wang *et al.*, 1999
Japanese	118	0.47	0.53	NS[2]	Iwai *et al.*, 1997
Japanese	194	0.43	0.57	NS	Ishikawa *et al.*, 1998
Japanese	159	0.40	0.60	NS	Kato *et al.*, 1998
Japanese	98	0.49	0.51	NS	Tamaki *et al.*, 1998
Korean	100	0.40	0.60		Present study
(Hypertensives)					
African-American	472	0.93	0.07	<0.05	Larson *et al.*, 2000
Italian	282	0.79	0.21	<0.05	Cusi *et al.*, 1997
French	195	0.76	0.24	<0.05	Cusi *et al.*, 1997
Scottish	74	0.75	0.25	<0.05	Kamitani *et al.*, 1998
Anglo-Australian	196	0.76	0.24	<0.05	Wang *et al.*, 1999
Japanese	125	0.34	0.66	NS	Iwai *et al.*, 1997
Japanese	170	0.44	0.56	NS	Ishikawa *et al.*, 1998
Japanese	223	0.44	0.56	NS	Kato *et al.*, 1998
Japanese	113	0.34	0.66	NS	Takami *et al.*, 1998
Korean	96	0.38	0.62		Present study

[1]Probability, [2]Not significant.

of α-adducin gene is weakly associated with increased risk for hypertension in Caucasian population, but not in Asian population including Korean population. Nevertheless, it could completely not explained by ethnic background because these heterogeneities were detected in both subgroups (For 4 Caucasian groups, Breslow-Day test = 9.06, df = 3, P = 0.028; for 5 Asian groups, Breslow-Day test = 16.84, df = 4, P = 0.002). Second, it might be due to sampling bias. When the difference in frequency of Trp allele in normotensives and hypertensives is about 2%, and the increase in risk of hypertension with the Trp allele is about 17%, then a study of 5,211 cases and a similar number of control subjects is required to have 80% statistical power to detect a difference at a probability of 0.05 between Gly and Trp allele. So far, none of the studies have been anywhere near this size. Thus, our result waits for the precise confirmation from large-scale prospective cohort study.

A major threat to the validity of meta-analysis is the existence of publication bias when studies with positive results get published, but studies with negative results disappear in the investigators drawer (Dickersin, 1990). In the absence of publication bias, should have the shape of a pyramid with a tapering funnel-like peak, because smaller studies tend to have larger standard errors. Publication bias does not seem to be a major factor in our meta-analysis owing to considerable horizontal funnel distribution (Fig. 3).

In conclusion, the current meta-analysis revealed a weak association between the Gly460trp polymorphism of α-adducin gene and hypertension However, further studies with a larger sample size are clearly warranted to conclusively establish an association between the Gly460Trp polymorphism of α-adduin gene and hypertension because the included studies have proven to be heterogeneous in the conventional test of homogeneity.

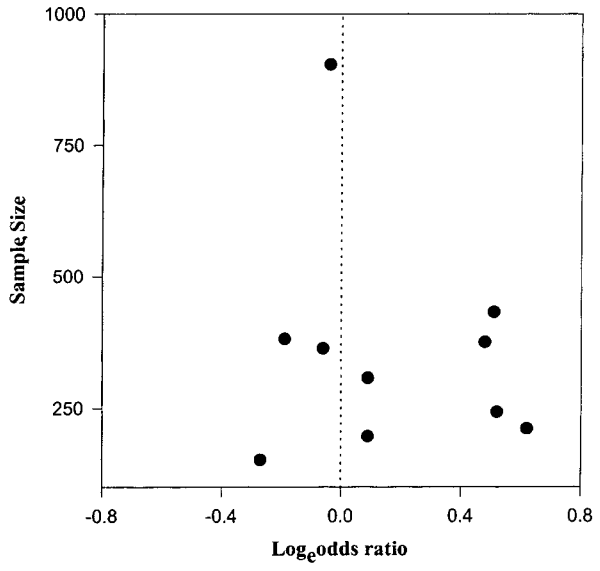

Fig. 3. Funnel graph to look for publication bias.

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