

Lack of Evidence for an Association Between α -Adducin and Blood Pressure Regulation in Asian Populations

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Recent studies have found the tryptophan allele of a glycine to tryptophan polymorphism at position 460 (G460W) of the α -adducin protein to be associated with essential hypertension in European populations. We examined whether the tryptophan allele is associated with hypertension in a different population, comprised of subjects of Chinese origin from Taiwan, and Chinese and Japanese origin from the San Francisco Bay area and Hawaii. We adapted the 5' allelic discrimination assay or TaqMan to type individuals for the G460W polymorphism, and using this method we typed more than 1000 individuals. The frequency of the W allele was slightly increased in the treated subjects in the Chinese population (0.458 *v* 0.423) but not the Japanese population (0.549 *v*

0.558). We considered dominant, recessive, and additive models in our analysis. There was a significant result for a recessive model for systolic blood pressure in the Chinese population (χ^2 6.84, *df* = 2, *P* < .05), but only suggestive evidence for diastolic blood pressure (χ^2 3.30). In contrast, in the Japanese population, there was no evidence for a positive association under any model. For the combined Chinese and Japanese samples, the evidence for association with α -adducin was not significant. *Am J Hypertens* 2000;13:704-709
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Hypertension affects 15% to 20% of the adult population, and is one of the major risk factors for stroke, myocardial infarction, and renal disease. Despite intense effort, our understanding of the pathogenesis of hypertension is rather poor. Twin, adoption, and epide-

miologic studies indicate that variation in blood pressure (BP), to some extent, is genetically determined (for a recent review, see Lifton).¹ One of the goals of the Stanford Asia and Pacific Program for Hypertension and Insulin Resistance (SAPPHIRE) is to identify genes for essential hypertension. To reduce genetic

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heterogeneity, a possible confounding factor in any attempt to map loci for hypertension, we have focused on the relatively homogeneous Chinese and Japanese populations.

Recently a G to T transversion, which causes a missense mutation—glycine (G) to tryptophan (W)—at codon 460 in the α -adducin gene was described. A case-control study suggested an association between the W allele and hypertension in the Italian and French populations.² Two smaller case-control studies in the Japanese also reported association between the W allele and essential hypertension.^{3,4} However, more recent studies using Scottish and Japanese populations have failed to find association between this polymorphism and hypertension.⁵⁻⁷

We developed a high-throughput method to detect the G460W polymorphism, and using this method, we genotyped more than 1000 subjects. Contrary to previous studies, we find little evidence for an association between this variant in the α -adducin gene and essential hypertension in the Chinese and Japanese populations.

METHODS

Subject Recruitment The SAPPHiRe Network consists of three field centers with the Stanford field center encompassing the greater San Francisco Bay Area; the Hawaii field center covering the island of Oahu and outer islands; and the Taiwan field center consisting of a consortium of the three major hospitals located in Taipei and one branch hospital in Taichung. Seventy-eight percent of subjects recruited at the Stanford field center are of Chinese descent, whereas the remaining are of Japanese descent. For the Hawaii field center, only 16% are of Chinese descent, whereas the majority, 84%, are of Japanese ancestry. For the Taiwan field center, all subjects are Chinese.

The Stanford and Hawaii field centers recruited the majority of their subjects through collaborative efforts with the Kaiser Permanente Group. Newspaper and television ads constitute other recruitment sources. The Taiwan field center is made up of the following major hospitals: National Taiwan University Hospital, the Veterans General Hospitals of Taipei and Taichung, and the Tri-Service General Hospital/National Defense Medical Center. The major sources of subject referrals are from the Cardiology and Endocrinology Clinics in these hospitals.

Inclusion Criteria The study design incorporated both concordant sibpairs (both sibs with hypertension) and discordant sibs (one hypertensive and one hypotensive sib). Subjects were included on the following criteria: 1) current age of subjects must be between 35 and 60 years. Subjects currently more than 60 years old may also be eligible provided that docu-

mentation of their hypertension status before age 60 is available. 2) Japanese or Chinese ancestry, that is, all four grandparents Japanese or all four grandparents Chinese. Hypertension is defined as follows: systolic BP (SBP) ≥ 160 mm Hg or diastolic BP (DBP) ≥ 95 mm Hg or taking two medications for high blood pressure (stage II hypertension). Alternatively, the subject could have uncontrolled hypertension, that is, taking one medication for high blood pressure and has either systolic BP ≥ 140 or diastolic ≥ 90 mm Hg.

Discordant sibpairs where one sibling is hypertensive (as above) and the other sibling is hypotensive were also included in the study. Hypotension is defined as BP in the bottom 30% of the age- and sex-adjusted BP distribution, which in our population translates into the following BP values: for men less than 45 years, SBP ≤ 115 mm Hg and diastolic BP ≤ 76 . For men more than 45 years, SBP ≤ 122 mm Hg and DBP ≤ 78 mm Hg were used. For women younger than 45 years, hypotension was defined as SBP ≤ 107 mm Hg and DBP ≤ 70 mm Hg. For those more than 45 years, the cut-off was SBP ≤ 118 mm Hg and DBP ≤ 75 mm Hg. There is no upper age cut-off for hypotensive sibs as long as both SBP and DBP readings are below the limit. However, the hypotensive sib must meet the lower age cut-off of 35 years or less.

Exclusion Criteria Families were excluded from the study if they met any of the following criteria: 1) one of the affected sibs is adopted (ie, no parent in common) or if the sibs have only one parent in common; 2) both parents have been treated for hypertension before the age of 60 years. If offspring reports about their parents' hypertension status are conflicting, then a single reliable report of hypertension in both parents before aged 60 years is cause for exclusion. This exclusion criterion, however, does not apply to discordant sibpairs. 3) Diabetic individuals were excluded. Diabetes uncovered as a result of SAPPHiRe laboratory work does not lead to exclusion. 4) Severe kidney disease (except stones and remote infections) of creatinine >1.5 mg/dL, unless documented proof that the subject met inclusion criteria before the increase in creatinine levels. 5) A body mass index greater than 35. 6) In addition, the following conditions are considered as cause for exclusion: ongoing (or within the past 6 months) treatment for cancer; terminal illness (life expectancy <6 months); liver cirrhosis or any other chronic illness; pregnancy or <6 months postpartum.

For sibpairs meeting the entry criteria, additional sibs meeting the same criteria (either hypertensive or hypotensive) were also recruited. In some families, sib recruitment is not yet complete, or some sibs were found upon examination not to meet criteria for hypertension or hypotension, but still had blood drawn.

These subjects were also included in the present analysis. However, most families have at least two hypertensive or hypotensive sibs.

In total, 411 families were genotyped, of which 295 are Chinese and 116 are Japanese. These included 48 families with one sib, 179 with two, 117 with three, 43 with four, 16 with five, 5 with six, 2 with seven, and 1 with eight sibs, or a total of 1061 subjects. Among these, 641 were hypertensive and taking at least one antihypertensive medication, 91 were hypertensive but unmedicated, 253 were hypotensive (unmedicated), and 76 were normotensive (unmedicated).

Blood Pressure Measurements In all field centers, blood pressures were measured according to a common protocol using the Dinamap automated blood pressure reading device. Three separate readings were taken. The present analysis is based on the average of the second and third readings for SBP and DBP.

Genotyping We adapted the 5' nuclease detection assay or TaqMan⁷⁻⁹ to detect the G460W polymorphism. To monitor the quality of genotypes, more than 500 individuals were typed in duplicate, and in every case the duplicates were concordant. The sequences of the primers and probes used in the TaqMan assay are given; in the probes, the polymorphic nucleotides are underlined. Note that the probes are antisense to the α -adducin coding sequence: AAD forward primer, 5'-GGCTGAACTCTGGCCGG; AAD reverse primer, 5'-ACACCTTAGTCTTCGACTTGGGAC; AADG460W-G probe, 5'-FAM-TCCATTCTGCCCTTCCTCGGA-TAMRA; AADG460W-W probe, 5'-TET-TTCCATTCTGCCATTCCTCGGA-TAMRA. Each 25 μ L of polymerase chain reaction (PCR) contained 30 ng of genomic DNA, 900 nmol/L of primers, 250 nmol/L of probe, and 12.5 μ L of master-mix (ABI). Amplification was done under the following conditions: 50°C, 2 min then at 95°C, 10 min, followed by 40 cycles of 94°C, 15 sec, and 62°C, 1 min in a Perkin-Elmer 9600 thermocycler (Perkin-Elmer, Applied Biosystems, Foster City, CA). After PCR, fluorescence was read by an ABI 7700 machine.

Statistical Analysis Only subjects with measured BP or medicated hypertensives were included in the analysis, which was based on siblings and no parents. For all subjects, we calculated age- and sex-standardized SBP and DBP measurements by subtracting the population mean BP and dividing by the standard deviation for that age and sex group. Normative data were obtained for the Chinese population of Taiwan (W. Harn pers. comm.; obtained from the Nutrition and Health Survey in Taiwan 1993-1996). We grouped the subjects into those who were taking at least one antihypertensive medication (and thus have distorted BP

readings) and those on no antihypertensive medication.

To evaluate the relationship between α -adducin genotype and the BP data for untreated subjects and the hypertension status of the treated subjects (but not their BPs) simultaneously, we used a likelihood analysis, as follows. Because we used age- and sex-standardized BP measurements, we assume that BP has a normal distribution with mean 0 and variance 1 in the population. For the tested α -adducin polymorphism, we assume three parameters: 1) the allele frequency "p" for the high value allele, 2) the difference, or displacement "t" in mean values for the two homozygotes, and 3) the degree of dominance "d" of the high value allele; a value of d = 1 means the high risk allele is dominant; a value of 0 means it is recessive; and a value of 0.5 means an additive model. The variance contributed by the tested locus can then be calculated as $V_A + V_D$, where $V_A = 2pqt^2(p(1-d) + qd)^2$ and $V_D = (pqt(1-2d))^2$. This is the total genetic variance due to the tested locus, and cannot exceed one. The residual variance within genotype is given by $V_R = 1 - V_A - V_D$. Individuals with treated hypertension are assumed to have a BP value above some threshold T determined by the upper K percentile of the population BP distribution, where K is the prevalence of that definition of hypertension. In our case, the prevalence of stage II hypertension as we have defined it is about 20%, so we assumed that K is 20%, and defined the threshold accordingly. In the likelihood analysis, the threshold is defined iteratively at each step as a function of the genotype means and frequencies.

We calculated a likelihood of the genotype and BP data (or hypertension status) for each sibship, summing all the six possible mating-type combinations in the parents, therefore not assuming the genotypes to be independent among sibs. However, we did assume the BP (or hypertension status) to be independent in the sibships; the actual population correlation for BP among sibs is probably in the range of 10 to 15%; therefore, assumption is reasonable as a first approximation. Although our families were ascertained as a function of their BP or hypertension status, forcing the mean and variance of BP to be 0 and 1, respectively, and not estimating these parameters, leads to unbiased estimates of the parameters p, t, and d. These parameters were estimated by maximum likelihood, and likelihood ratio tests were performed using the computer program MAXLIK.¹⁰ The test of positive gene effect is obtained by comparing twice the log likelihood difference at t = 0 versus at t > 0 (estimated) with a χ^2 distribution with one degree of freedom. Similarly, hypotheses regarding dominance can be tested in terms of the parameter d.

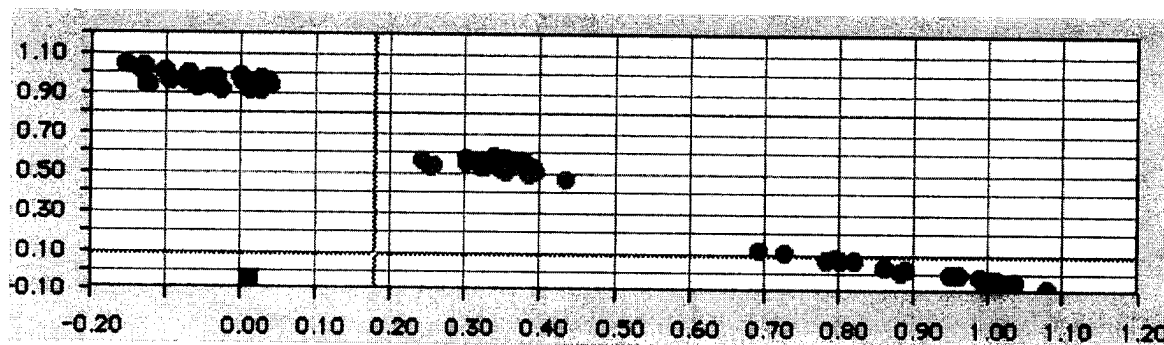


FIGURE 1. Representative genotyping data for the G460W polymorphism in α -adducin. PCR was performed and fluorescence values read as described (see Methods). Red dots represent individuals homozygous for the G allele; blue dots represent individuals homozygous for the W allele, and green dots are G/W heterozygotes. Black squares are control reactions that had no DNA.

RESULTS

High-Throughput Genotyping of the G460W Variant

We adapted the 5' nuclease detection assay or TaqMan to distinguish the G and W alleles of the α -adducin gene, and using this method, we typed more than 1000 individuals for this polymorphism. Typical results are presented in Figure 1. The method is reliable—>500 samples were typed in duplicate with no discordant calls—and rapid—with five 96-well PCR machines, a thousand individuals can be genotyped in 1 day. The key advantage of this method of genotyping, compared to the gel-based assays used in previous studies, is that genotypes can be called without any post-PCR processing.

Association Tests We calculated average standardized BP within each genotype class, as well as allele frequencies in treated and untreated subjects. Subjects

were separated by ethnicity (Chinese and Japanese) and medication status (treated and untreated). These data are presented in Table 1.

The frequency of the W allele was slightly increased in the treated subjects in the Chinese population (0.458 v 0.423) but not the Japanese population (0.549 v 0.558). Examination of genotypes reveals a relative increase in the W/W homozygote in both populations in the treated group (0.225 v 0.177, Chinese; 0.284 v 0.229, Japanese), possibly suggestive of a recessive effect of this locus. Examination of genotype-specific mean adjusted BP among the untreated subjects did not reveal a consistent pattern, however. SBP were slightly higher in the W/W group in Chinese, but not Japanese (who showed the opposite). DBP showed little difference across genotypes.

We analyzed the BP data for untreated subjects and the hypertension status of treated subjects simulta-

TABLE 1. ADDUCIN GENOTYPE FREQUENCIES AND BLOOD PRESSURES BY ETHNICITY

Ethnicity	Med	Genotype					
		G/G		G/W		W/W	
		N(%)	BP(SD)	N(%)	BP(SD)	N(%)	BP(SD)
Chinese	0	116 (0.331)	-0.28 (1.47) -0.72 (1.14)	172 (0.491)	-0.41 (1.46) -0.74 (1.22)	62 (0.177)	-0.14 (1.46) -0.71 (1.16)
	>0	132 (0.310)	0.62 (1.36) 0.00 (1.04)	198 (0.465)	0.52 (1.24) 0.00 (1.08)	96 (0.225)	0.33 (1.27) -0.24 (0.93)
	0	8 (0.114)	0.32 (1.55) -0.19 (1.17)	46 (0.657)	-0.19 (1.30) -0.46 (1.15)	16 (0.229)	-0.51 (1.03) -0.55 (0.91)
Japanese	>0	40 (0.186)	0.18 (0.91) -0.46 (0.82)	114 (0.530)	0.36 (0.93) -0.30 (0.82)	61 (0.284)	0.11 (0.91) -0.40 (0.90)
	0	124 (0.295)	-0.24 (1.47) -0.69 (1.14)	218 (0.519)	-0.36 (1.43) -0.68 (1.21)	78 (0.186)	-0.21 (1.38) -0.68 (1.11)
	>0	172 (0.268)	-0.52 (1.28) -0.11 (1.00)	312 (0.487)	0.46 (1.14) -0.11 (0.99)	157 (0.245)	0.24 (1.13) -0.30 (0.91)

Med = 0 means subjects not taking antihypertensive medications; Med >0 means subjects taking at least 1 antihypertensive medication. Within each group, the first line corresponds to systolic blood pressure, the second line to diastolic blood pressure.

TABLE 2. LIKELIHOOD ANALYSIS OF ADDUCIN AND BLOOD PRESSURE VARIATION

Ethnicity	Parameter	t = 0	Model		
			d = 0.0	d = 0.5	d = 1.0
Chinese	p	0.434 (0.017)	0.409 (0.020)	0.413 (0.022)	0.432 (0.020)
			0.422 (0.019)	0.422 (0.019)	0.434 (0.017)
	t	—	0.186 (0.076)	0.145 (0.088)	0.011 (0.062)
			0.128 (0.071)	0.113 (0.081)	0.030 (0.070)
2lnL + C	0.00	6.84	2.68	0.04	
		3.30	1.94	0.22	
Japanese	p	0.539 (0.028)	0.580 (0.049)	0.677 (0.056)	0.524 (0.046)
			0.550 (0.045)	0.517 (0.101)	0.524 (0.043)
	t	—	-0.166 (0.160)	-0.685 (0.295)	0.074 (0.175)
			-0.047 (0.157)	0.107 (0.464)	0.081 (0.167)
2lnL + C	0.00	1.02	2.56	0.16	
		0.08	0.04	0.22	

Within each parameter, the first line corresponds to systolic blood pressure, the second line to diastolic blood pressure.

neously by assuming that individuals undergoing treatment for hypertension had BP values above a certain threshold (see Methods). The results of the likelihood analysis are given in Table 2. There was a significant result for a recessive model for SBP in the Chinese population (χ^2 6.84, $df = 2$, $P < .05$), but only suggestive evidence for DBP (χ^2 3.30). These results are consistent with those in Table 1, where the WW genotype is increased in the treated subjects, and SBP is somewhat higher in individuals with the WW genotype among the untreated subjects. In contrast, in the Japanese population, there was no evidence for a positive association, and in fact the estimated value of t for a recessive model was negative.

The frequency of the W allele differs in the Chinese and Japanese populations (0.43 in Chinese, 0.54 in Japanese). Therefore, we could not perform a simple analysis combining the two samples. Instead, we analyzed the combined data allowing for different estimates of the allele frequency in the two populations, but assuming the value of t and d to be the same. Thus, there was a total of four parameters in this analysis (the two population-specific allele frequencies p_1 and p_2 , t , and d). For the recessive model for SBP, t was estimated to be 0.12, and the χ^2 test gave a value of 2.72. For the recessive model for DBP, t was estimated at 0.10, and the χ^2 value was 0.92. Thus, for the combined Chinese and Japanese samples, the evidence for association with α -adducin was not significant.

DISCUSSION

In our likelihood analysis, we ignored residual familial correlation in BP or hypertension status among sibs. Because this residual correlation is modest (10 to 15%), it is unlikely that this assumption has had much influence on our results. In fact, George and Elston¹¹

have shown that ignoring a large residual family correlation in testing for a candidate gene association in family data can lead to a modest inflation of the likelihood ratio test for significance of the gene effect. Thus, our negative results cannot be due to our exclusion of residual correlation.

Our results deviate from those of Cusi et al² and Iwai et al.³ There are several possible explanations for the differing conclusions. First, our study population (Chinese and Japanese) differs ethnically from the study of Cusi et al² (Italian and French whites). Although we tested exactly the same polymorphism in α -adducin, it may be that expression of this variant requires either a genetic or environmental background that is present in the white population but absent in the Chinese and Japanese populations we studied. On the other hand, Iwai et al³ studied a Japanese population and also found a positive association of the W allele with hypertension. They found an allele frequency (0.53) in their controls that was nearly identical to ours (0.54), although we did not find an increased frequency of the W allele in our Japanese hypertensive subjects. Both studies reveal that the W allele is much more frequent in Asian populations (Chinese and Japanese), than in European whites (allele frequency around 0.20). Our results are consistent with those from two other studies using Scottish and Japanese populations.⁵⁻⁷

Second, the studies differed in sample design. The prior two studies focused on unrelated subjects, as hypertensive cases and controls. We focused on sibships ascertained through at least one hypertensive proband and at least one additional hypertensive or hypotensive sib. Sampling from related individuals has the effect of reducing power (to some extent, but offers greater robustness to stratification artifact), whereas sampling from the tails of the BP distribution

has the opposite effect of enhancing power. In any event, it is unlikely that our negative result was due to low power, because we had a large sample size (>1000 subjects genotyped), and the high frequency of the W allele led to a reasonably balanced frequency of the three genotypes.

In the original study of Cusi et al² the effect of the α -adducin W allele on hypertension, based on a case-control comparison, was assumed to be dominant. The European populations studied had a relatively low frequency of the W allele, therefore, their sample had relatively few WW homozygotes. Because the W allele has a higher frequency in the Chinese and Japanese populations, we could more readily compare a dominant and recessive model, as we had a fair number of WW homozygotes. In our analyses, a dominant model was clearly not supported. There was suggestive evidence for a recessive model in the Chinese for SBP. However, there was no such evidence in the Japanese population, and the evidence for DBP in the Chinese was much weaker. An analysis combining both populations did not provide significant evidence for an association of the W allele with increased BP.

Finally, the difference in results may be due to the original finding simply being a type 1 error. Further studies of white and non-white groups, such as ours, may help resolve the degree to which α -adducin plays a role in BP regulation, and in which populations.

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