The role of angiotensin-converting enzyme gene insertion/deletion polymorphism for blood pressure regulation in areca nut chewers

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Areca tannin has been suggested as having a blood pressure regulatory effect through its ability to inhibit the pressor response to both angiotensin I and II. As genetic and environmental factors determine the susceptibility and development of diseases and no report has been published concerning the genetic interaction of metabolic effects in areca nut/betel quid (BQ) chewers, it is proposed that the cardiovascular effects of chronic BQ usage can be affected by the polymorphism of the *angiotensin converting enzyme* (ACE) gene. This is a cross-sectional community-based study. A total of 189 BQ chewing subjects and 256 non-chewing controls were studied. BQ chewing status was determined by using a structured questionnaire, and insertion/deletion (I/D) polymorphisms were determined by a polymerase chain reaction. BQ chewers with the DD genotype had significantly lower blood pressure, pulse pressure, and prevalence of hypertension compared with those of chewers with II or ID genotypes. Multiple stepwise regression analysis confirmed that the ACE I/D genotype was independently associated with systolic blood pressure (SBP). BQ chewers with the DD genotype accounted for a significant age, sex, and waist-to-hip ratio adjusted decrease in SBP (-5.9 mm Hg, $P = 0.021$). This finding suggests that BQ chewing may be related to blood pressure regulation, which supports the hypothesis that concomitant genetic susceptibility and environmental factors determine the level of blood pressure. (Translational Research 2007; 150:58–65)

Abbreviations: ACE = angiotensin converting enzyme; ACEI = ACE inhibitor; ANOVA = analysis of variance; BMI = body mass index; BQ = betel quid; DBP = diastolic blood pressure; GOT = glutamic oxaloacetic transferase; HDL = high-density lipoprotein; I/D = insertion/deletion; LDL = low-density lipoprotein; SBP = systolic blood pressure; WHR = waist-to-hip ratio

Areca nut/betel quid (BQ; *Areca catechu*) is said to be the fourth most commonly used psychoactive substance in the world, after caffeine, nicotine, and alcohol, and it is chewed regularly by at least 10% of the world's population.¹ It is estimated that 2

million persons in Taiwan have the habit of chewing BQ, which is especially common among aborigines. The prevalence may be up to 50% in women.^{2,3} Many people have acquired the habit because of its physiologic effects, in-

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cluding increased stamina and a general feeling of well-being.¹ Both benign and malignant diseases can be induced by BQ chewing, with deleterious effects on the oral soft tissues, which has been published extensively in the dental literature.⁴ Reports have shown that chronic BQ chewing is associated with diverse systemic effects, including hyperglycemia, insulin resistance, obesity, cardiorespiratory disease, and dependence syndrome.^{5–10} Recently, it was observed that chronic BQ use is associated with a higher risk of type 2 diabetes mellitus and metabolic syndrome, determined by a epidemiologic survey in Taiwan.^{11,12}

The major compounds of BQ are polyphenolic compounds, alkaloids, tannin, and fibers.¹³ Interestingly, areca tannin has been suggested to have properties as a hypotensive drug through its ability to inhibit the pressor response to both angiotensin I and II.¹⁴ Acute cardiovascular responses occur, such as increased heart rate and blood pressure in new BQ chewers, whereas no significant effects on blood pressure have been observed in chronic BQ chewers.¹⁵

The angiotensin converting enzyme (ACE) plays a key role in modulating vascular tone and electrolyte balance by hydrolyzing angiotensin I to angiotensin II, which is a potent vasopressor and aldosterone-stimulating peptide.¹⁶ The individual variation of plasma ACE activity is largely affected by polymorphism of the *ACE* gene.¹⁷ Specifically, *ACE* gene I/D polymorphism has been found to be associated, albeit somewhat controversially, with the development of hypertension and the response of ACE inhibitor (ACEI) treatment in patients with essential hypertension.^{18–20}

In a recent report by the authors, it was observed that *ACE* insertion/deletion (I/D) polymorphism is associated with the risk of oral mucosal lesions in BQ chewers,²¹ which indicates the relative contribution of genetic and environmental factors that determine the susceptibility and development of diseases. As no report has been published concerning the genetic interaction of metabolic effects in BQ chewers, we propose that the cardiovascular and metabolic effects of chronic BQ usage may be affected by the polymorphism of the *ACE* gene. To test this hypothesis, the role of the *ACE* gene I/D polymorphism has been investigated in the systemic effects of BQ chewing in an aboriginal community with a high prevalence of BQ chewing habit.

MATERIALS AND METHODS

Subjects. All data were derived from the Aboriginal Health Interview and Integrated Health Care Program for rural indigenous people over 30 years old, which was conducted by the Bureau of National Health Insurance of Taiwan from January 2000 to December 2003 as recently reported.^{21,22} Using population registries, this study selected

a stratified random sample of 1466 subjects, representative for age, gender, and aboriginal community. The overall participation rate was 59% according to the 2002 Official Household Registry. Subjects with hypertension were defined as having a systolic blood pressure (SBP) ≥ 140 mm Hg, diastolic blood pressure (DBP) ≥ 90 mm Hg, or both, or as receiving antihypertensive medication indicated for treatment of hypertension. For the purposes of the current study, 84 participants were excluded with known major systemic diseases (eg, coronary heart disease, hypertrophic cardiomyopathy, liver cirrhosis, renal insufficiency, and malignancies). Subjects of 813 areca nut chewers who smoked or consumed alcohol were excluded to avoid interfering effects on the metabolic measurements. Also, 124 participants were excluded for other reasons, including 58 persons with an age <40 years old, 34 persons with residence outside aboriginal communities, and 32 persons with missing information. This study was approved by the Human Research Ethics Committee of the participating hospital, and informed consent was obtained from each participant.

All study subjects lived in the same region at the time of the study and were of Pai-Wan ethnicity without known ancestry from other ethnicities. Each subject was personally interviewed with regard to demographic information, occupation, physical activity, BQ chewing, smoking history, alcohol drinking habits, as well as personal and family history of various diseases using a structured questionnaire.²¹ The physical activity of the study subjects was evaluated by 2 types of exercise status: nonexercisers and moderate exercisers. The main varieties of chews used by the aborigines studied were those prepared by combining unripe areca fruit with a piece of inflorescence (flower head) of the *Piper betle* vine and red lime paste or by wrapping unripe areca fruit in leaves of the *Piper betle* vine with white lime paste.³ It should be noted that no tobacco was added to the chews used in Taiwan. Subjects who had chewed 1 BQ or more per day for at least 1 year were defined as those having chewed. Among them, current chewers were those who practiced this habit within the previous 1 year, and ex-chewers were those who had stopped for less than 1 year before the interview. In this study, current chewers and ex-chewers were analyzed as a group and compared with those who had never been chewers and those who had chewed before who had stopped the habit for at least 1 year. For all of those who had chewed before, the duration of BQ use and the amount of BQ chewed each day were also recorded. The cumulative exposure to BQ chewing was derived by multiplying the daily consumption of BQ chewing (in quid/day) by the duration of BQ chewing (in years).

Venous blood samples were drawn for biochemical analyses after an overnight fasting period of at least 8 h. The plasma biochemical parameters were measured after overnight fasting as previously described.²³ Data on the biological factors associated with the risk of hypertension were collected, including plasma triglycerides, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, uric acid, creatinine, glucose, and glutamic oxaloacetic transferase (GOT). Body height, weight, and waist and hip circumferences were measured, and the body mass index (BMI) and waist-to-hip ratio (WHR) were

calculated. Blood pressure was measured in the morning (readings were taken twice, at least 2 min apart), on the right upper arm in the line of the heart, in a sitting position after a minimum of 5 min of rest. BP was read by a mercury column sphygmomanometer. The average value of these measurements was used for analysis.

ACE I/D polymorphism. Genomic DNA was prepared from peripheral blood using standard techniques. For the *ACE* I/D polymorphism, the primer pairs used and the annealing temperatures were as follows: forward 5'-CTGGAGACCACTCCCATCCTTTCT-3' and reverse 5'-GATGTGGC-CATCACATTCGTCAGAT-3', which amplify the intron 16 region where the I/D fragment is located.²⁴ To avoid ID/DD mistyping of the heterozygote as DD-homozygote,²⁵ all DD-genotype samples were confirmed using a pair of primers that produced an amplified product only in the presence of the insertion and was used to verify the polymorphism: forward, 5'-TGGGACCACAGCGCCCGCCACTAC-3' and reverse, 5'-TCGCCAGCCCTCCCATGCCCATAA-3'. All PCR products were visualized after electrophoresis on a 2% agarose gel and ethidium bromide staining. To assure that the genotyping was of sufficient quality, we performed random duplicates in more than 10% of the samples and we carried controls from carriers and noncarriers in each genotyping assay. The assays were performed by a technician who was blind to the phenotype. No genotype errors were detected in the random duplicates.

Statistical analysis. Descriptive data were examined for all variables. For continuous variables, results were presented as the mean $\pm$ SD, geometric mean (1-SD interval), or median (interquartile range) when appropriate. Statistical differences in variables were compared using unpaired Student *t*-tests and 1-way analysis of variance (ANOVA) for normally distributed variables, followed by Turkey's pair-wise comparison. Categorical variables were recorded as frequency counts, and intergroup comparisons were analyzed by the χ^2 -test. General linear modeling function analysis was used to control for potential confounders other than age (eg, sex, BMI, WHR, total cholesterol and HDL-cholesterol levels, use of antihypertensive medication, diabetes, and physical activity status).

Associations between systolic blood pressure and other parameters by BQ chewing were first analyzed by simple linear regression and then by multiple stepwise regression analysis. Age, gender, BMI, WHR, fasting glucose, total-cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, uric acid, creatinine, GOT, and *ACE* gene I/D genotypes were included in the analysis. The interactions between BQ chewing and *ACE* genotypes were assessed using a cross-product term between genotypes and BQ chewing status. Statistical significance was accepted if $P < 0.05$. All statistical analysis was performed using SAS statistical software, version 8.2 (SAS Institute Inc., Cary, NC).

RESULTS

Data were collected from a total of 1466 rural indigenous people, of which 1021 persons for reasons listed in the subjects and methods section were excluded. A total of 189 BQ chewing subjects without alcohol

drinking and smoking habits were selected as case subjects, and 256 without any BQ chewing, drinking, and smoking habits were used as controls. None of the study subjects had coffee consumption habits.

Table I shows the clinical characteristics of the study subjects grouped by their BQ usage status. BQ-chewing subjects had female predominance, higher BMI, WHR, and serum triglyceride levels and lower creatinine and uric acid levels than nonchewers. The systolic and diastolic BPs, fasting plasma glucose, serum total cholesterol, HDL- and LDL-cholesterol, GOT levels, and the prevalence of hypertension, diabetes, physical activity status, and antihypertensive drug use were not statistically different between the study groups. The mean duration of BQ chewing in the case group was 34 years.

Table II presents the general characteristics of the 2 study groups stratified by *ACE* I/D genotypes with ANOVA, followed by Turkey's pair-wise comparison and the χ^2 -test. In all study subjects, the genotype distributions of this polymorphism were in Hardy-Weinberg equilibrium. Within the non-BQ chewing group, no significant differences in general characteristics were observed among the genotypes. The prevalence of hypertension, SBP, and pulse pressure in BQ chewers with DD genotypes was significantly lower compared with those with ID and II genotypes even after adjustment for age, sex, BMI, WHR, total cholesterol, HDL-cholesterol, use of antihypertensive medication, diabetes, and physical activity status. The SBP and prevalence of hypertension of BQ chewers with the II genotype (150 ± 22 mm Hg and 68%, respectively) were different from those of nonchewers (134 ± 23 mm Hg and 52.6%, respectively) with the II genotype, whereas the difference was not statistically significant after adjusting by general linear models for age, sex, BMI, WHR, total cholesterol, HDL-cholesterol, use of antihypertensive drugs, diabetes, and physical activity status.

Simple linear regression analysis has revealed that age, BMI, fasting glucose, triglyceride, creatinine, and the *ACE* I/D genotype are associated with SBP in BQ chewers. Age, WHR, uric acid, and creatinine are associated with SBP in nonchewers. Multiple stepwise regression analysis confirmed that age, fasting glucose, and the *ACE* I/D genotype are independent risk factors for SBP in BQ chewers, whereas age and BMI are factors for SBP in nonchewers (Table III). The interaction between the *ACE* I/D genotype and BQ chewing in relation to SBP (P value for interaction = 0.006) is statistically significant.

Figure 1 shows the prevalence of hypertension stratified by *ACE* genotypes in BQ chewers and nonchewers. Within the chewers group, the hypertension rate

Table I. Clinical and biochemical characteristics of the study subjects

Variable	All subjects (N = 445)	BQ chewers (N = 189)	Non-BQ chewers (N = 256)	P value
Age (years)	61.5 ± 13.0	62.4 ± 12.9	60.8 ± 13.0	0.186
Sex, male, (%)	25.2	15.3	32.4	<0.0001
BMI (kg/m ²)	27.1 ± 4.9	27.7 ± 5.4	26.6 ± 4.4	0.031
WHR	0.87 ± 0.08	0.88 ± 0.09	0.86 ± 0.07	0.041
Hypertension (%)	51.9	52.7	51.3	0.776
Diabetes mellitus (%)	14.0	14.4	13.7	0.818
Antihypertensive drug use (%)	25.2	25.4	25.0	0.923
Systolic BP (mmHg)	136 ± 22	138 ± 22	135 ± 22	0.186
Diastolic BP (mmHg)	81 ± 12	81 ± 12	80 ± 12	0.396
Fasting glucose (mmol/L)	6.1 ± 2.7	6.1 ± 2.5	6.1 ± 2.8	0.785
T-cholesterol (mmol/L)	5.08 ± 0.89	5.07 ± 0.89	5.08 ± 0.89	0.915
Triglyceride (mmol/L)*	1.26 (0.75–1.47)	1.38 (0.80–1.52)	1.17 (0.72–1.38)	0.007
HDL-cholesterol (mmol/L)	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	0.947
LDL-cholesterol (mmol/L)	3.20 ± 0.79	3.14 ± 0.77	3.24 ± 0.80	0.213
SGOT (U/L)	27.8 ± 10.9	27.6 ± 9.5	27.9 ± 11.9	0.804
Creatinine (μmol/L)	82.2 ± 34.5	78.7 ± 23.0	84.9 ± 40.7	0.044
Uric acid (mmol/L)	0.38 ± 0.09	0.37 ± 0.08	0.40 ± 0.10	0.008
Moderate exercise (%)	72.8	71.3	73.8	0.627
Betel chewing duration (years)†	—	34.0 (20.0–48.0)	—	
Cumulative amount of quid consumption (quid-years)†	—	95,760 (43,680–194,544)	—	

Abbreviation: SGOT, serum glutamic oxaloacetic transferase.

Note: Data are expressed as means ± SD.

*Geometric mean (1-SD interval).

†Median (interquartile range). Comparisons between BQ chewers and non-BQ chewers are performed by unpaired Student *t*-test or χ^2 -test.

decreased significantly with the number of D alleles present (*P* value for trend < 0.05). However, in non-BQ chewers, there was no statistical difference in the prevalence of hypertension among subjects with different *ACE* I/D genotypes.

In multiple linear regression analysis using the II genotype as a reference group, the presence of the DD genotype is associated with both SBP (−5.9 mm Hg, *P* = 0.021) and pulse pressure (−5.8 mm Hg, *P* = 0.004) in BQ-chewers after adjustment for age, sex, and WHR.

DISCUSSION

These results demonstrate that BQ chewers with the *ACE* DD genotype have significantly decreased SBP and a decreased risk of hypertension compared with chewers who carry the I allele. The current study confirmed the recent observation in aborigines that genetic-environmental factors contribute to the susceptibility and development of diseases in BQ consumption.²¹ This report seems to be the first to demonstrate that *ACE* I/D genotypes may affect blood pressure homeostasis in BQ chewers.

ACE inhibitor-like activity of the areca nut extract was observed by Inokuchi et al,¹⁴ and this activity was comparable with that of captopril. The vasopressor response to norepinephrine and vasodepressor responses to bradykinin and acetylcholine were not ap-

preciably changed by intravenous treatment with areca extract, although it did produce a dose-related inhibition of the pressor responses to angiotensin I and II. These findings suggest that the areca extract has favorable properties as a hypotensive drug through its ability to inhibit the pressor responses to both angiotensin I and II.¹⁴ *ACE* is a key component in the rennin-angiotensin system, converting angiotensin I to angiotensin II. It also inactivates the vasodilator bradykinin. Both peptides play central roles in blood pressure regulation and are believed to be important in the pathogenesis of cardiovascular diseases.²⁶ *ACE* levels in plasma and tissue are under genetic control. An I/D polymorphism exists in the *ACE* gene characterized by the presence or absence of a 287 bp *alu* repeat. Subjects with the DD genotype have higher plasma *ACE* activity compared with those with ID and II genotypes.^{17,27} In Caucasians, 44% of the variance in circulating *ACE* activity was accounted for by genetic polymorphism,²⁷ and similar results have been found in the Chinese population.²⁸ Subjects with the DD genotype have markedly higher plasma *ACE* levels than those with genotype II. The *ACE* gene polymorphism has also been shown to contribute to the differences in response to ACEI treatment.²⁹ Among hypertensive patients treated with ACE inhibitors, patients with the D allele of the *ACE* gene are associated with significantly greater blood pressure reductions.^{29–32} The chewers

Table II. Variables according to *ACE* genotypes by betel quid chewing status

Variable	BQ chewers (n = 189)					Non-BQ chewers (n = 256)			
	II	ID	DD	P	P ^a	II	ID	DD	P
Age (yr)	63.9 ± 13.4	62.1 ± 11.8	62.5 ± 14.2	0.802		59.3 ± 14.8	63.0 ± 14.0	62.2 ± 12.7	0.856
Sex, male, (%)	21.4	15.1	13.0	0.580		27.7	34.4	31.7	0.695
Cumulative amount of quid consumption (quid-years)	141,500 ± 165,500	157,700 ± 263,200	186,100 ± 234,900	0.694		—	—	—	
BMI (kg/m ²)	27.7 ± 4.5	27.7 ± 6.0	27.7 ± 5.0	0.998		26.4 ± 4.2	26.4 ± 4.5	27.2 ± 4.3	0.445
WHR	0.89 ± 0.08	0.90 ± 0.11	0.86 ± 0.05	0.036		0.88 ± 0.09	0.85 ± 0.08	0.87 ± 0.06	0.120
Hypertension (%)	68.0	59.3	37.3	0.009	0.035	52.6	50.4	52.0	0.962
Systolic BP (mmHg)	150 ± 22	136 ± 23	134 ± 18	0.007	0.044	134 ± 23	134 ± 22	137 ± 22	0.639
Diastolic BP (mmHg)	81 ± 16	82 ± 12	81 ± 12	0.909		81 ± 12	79 ± 13	82 ± 11	0.318
Pulse pressure (mmHg)	69.1 ± 19.1	55.7 ± 17.6	52.7 ± 13.5	0.0003	0.028	52.7 ± 16.8	55.3 ± 17.9	55.3 ± 17.4	0.712
Fasting glucose (mmol/L)	6.1 ± 3.0	6.0 ± 2.6	6.1 ± 2.3	0.962		6.4 ± 3.9	6.3 ± 2.9	5.8 ± 1.7	0.335
T-cholesterol (mmol/L)	5.04 ± 0.91	5.14 ± 0.92	5.01 ± 0.85	0.627		5.29 ± 0.83	4.97 ± 0.86	5.21 ± 1.11	0.066
Triglyceride (mmol/L)	1.48 ± 1.28	1.35 ± 0.84	1.39 ± 1.02	0.832		1.30 ± 0.82	1.13 ± 0.63	1.20 ± 0.79	0.404
HDL-cholesterol (mmol/L)	1.1 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	0.458		1.2 ± 0.2	1.2 ± 0.3	1.1 ± 0.2	0.635
LDL-cholesterol (mmol/L)	3.15 ± 0.76	3.17 ± 0.84	3.11 ± 0.70	0.877		3.39 ± 0.83	3.13 ± 0.75	3.32 ± 0.84	0.104
SGOT (U/L)	25.3 ± 7.2	29.2 ± 11.3	26.4 ± 7.0	0.065		25.9 ± 8.0	27.4 ± 13.0	29.7 ± 11.8	0.169
Creatinine (μmol/L)	81.3 ± 23.0	78.7 ± 27.4	76.9 ± 14.1	0.689		81.3 ± 22.1	89.3 ± 53.9	81.3 ± 19.4	0.309
Uric acid (mmol/L)	0.37 ± 0.08	0.38 ± 0.08	0.37 ± 0.07	0.568		0.38 ± 0.09	0.40 ± 0.11	0.39 ± 0.08	0.756
Moderate exercise (%)	75.0	70.3	71.1	0.921		87.5	72.1	68.5	0.134
Antihypertensive drug use (%)	25.0	31.2	16.1	0.107		23.4	25.0	25.9	0.951

Abbreviation: SGOT, serum glutamic oxaloacetic transferase.

Notes: Data are expressed as mean ± SD. Comparisons between *ACE* genotype are performed by 1-way ANOVA or χ^2 -test.

P^a: Data are adjusted by general linear models for age, sex, BMI, WHR, total cholesterol, HDL-cholesterol, use of antihypertensive drug, diabetes, and physical activity status.

Table III. Linear regression analysis of variables associated with systolic blood pressure by betel quid chewing status

Variable	Betel quid chewers (n = 189)					Non-betel quid chewers (n = 256)				
	Simple		Multiple			Simple		Multiple		
	Estimate	SE	P	Estimate	SE	P	Estimate	SE	P	95% CI
Age	0.64	0.12	<0.0001	0.64	0.12	<0.0001	0.71	0.11	<0.0001	0.56~0.98
Sex	-0.34	4.56	0.940	-0.34	4.56	0.940	3.99	3.10	0.199	
BMI	-0.69	0.32	0.031	-0.69	0.32	0.031	0.56	0.34	0.103	
WHR	29.97	20.54	0.147	29.97	20.54	0.147	89.78	21.82	<0.0001	
Fasting glucose	0.08	0.04	0.039	0.08	0.04	0.039	-0.01	0.03	0.712	
T-cholesterol	0.06	0.05	0.238	0.06	0.05	0.238	0.06	0.04	0.142	
Triglyceride	0.04	0.02	0.039	0.04	0.02	0.039	0.05	0.03	0.067	
HDL-cholesterol	-0.12	0.18	0.494	-0.12	0.18	0.494	-0.08	0.16	0.630	
LDL-cholesterol	0.03	0.06	0.667	0.03	0.06	0.667	0.05	0.05	0.327	
Uric acid	-1.43	1.28	0.268	-1.43	1.28	0.268	1.81	0.86	0.035	
Creatinine	12.79	6.27	0.043	12.79	6.27	0.043	13.02	5.46	0.018	
SGOT	0.18	0.18	0.317	0.18	0.18	0.317	-0.02	0.12	0.894	
ACE genotype Reference group: II genotype	-7.07	2.43	0.004	-5.73	2.23	0.011	1.98	2.19	0.366	

Abbreviations: SE, standard error; SGOT, serum glutamic oxaloacetic transaminase.
Note: In multiple stepwise regression analysis, all covariates were used for analysis.

Fig 1. Prevalence of hypertension in study subjects stratified by ACE genotypes and BQ chewing status. *BQ chewers with ACE DD genotype had a significantly lower hypertension rate than that of II genotype ($P < 0.05$) after adjusting by general linear models for age, sex, BMI, waist-to-hip ratio, total cholesterol, HDL-cholesterol, use of antihypertensive drugs, diabetes status, and physical activity status. † $P_{\text{trend}} < 0.05$. No statistical difference was found in the prevalence of hypertension among nonchewers with different ACE I/D genotypes.

with the DD genotype who presented with lower SBP and prevalence of hypertension observed in the current study may indicate that BQ chewers with this genotype have greater hypotensive responses to the tannin substance contained in the areca nut than subjects with II or ID genotypes.

Areca nut extract has also been reported to induce higher basal secretion of catecholamines from adrenal chromaffin cells.³³ However, data indicate that BQ chewing can affect cardiovascular function and that tolerance to the pressor response occurs in habitual users.¹³ The observation here reveals no significant difference in blood pressure measurements and prevalence of hypertension between controls and BQ users, which indicates that complex gene-environment factors exist in blood pressure homeostasis.

Johnston et al³⁴ using rat brain slices demonstrated that BQ constituents arecholine and guvacoline hydrolyzed products arecaidine and guvacine, which act as substrate-competitive inhibitors of GABA uptake. Inhibition of GABA activity can promote an increase in appetite and is believed to be contributor to the pathogenesis of obesity in BQ chewers.⁵ The mechanism of BQ chewing associated with hyper-triglyceridemia is still unknown. Another issue that needs to be addressed is that the effect of the D allele, and BQ chewing seems to be restricted to SBP only. In accordance with our findings, Schut et al³⁵ have also observed the smoking-dependent effects of the ACE I/D polymorphism on SBP and systolic hypertension and not on DBP. Findings regarding the ACE I/D polymorphism and SBP or

isolated systolic hypertension have not, however, been very conclusive as of yet.^{36,37}

Several limitations to this study exist. As in all studies that rely on participant reports, recall bias and social desirability bias may have produced a spurious association. Furthermore, the sample size of this study remained small because of the strict selection criteria designed to exclude BQ chewers with smoking or alcohol usage. The definition of a nonchewer (ie, those who never consumed BQ in their lives) most likely included some people who were infrequent chewers. The inclusion of infrequent chewers in the nonchewer category causes the ORs for hypertension to be underestimated. Only the aborigines of Taiwan were studied, so caution is advised in generalizing these conditions to other populations.

In summary, the current data indicate that chronic BQ chewing may have diverse systemic effects, which suggests that a direct effect exists on blood pressure associated with BQ chewing and carrying of the D allele. It is hypothesized that synergistic effects on the regulation of blood pressure may explain the effects of the D allele and BQ chewing on SBP. Additional prospective and large-scale studies are required to confirm the blood pressure effect of BQ consumption.

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