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Original Article

Combined effect of acid-sensing ion channel 3 and transient receptor potential vanilloid 1 gene polymorphisms on blood pressure variations in Taiwanese

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Abstract

Objectives: Both acid-sensing ion channel acid-sensing ion channel 3 (ASIC3) and transient receptor potential vanilloid 1 (TRPV1) have been proposed to be involved in the pathophysiology of hypertension. Common colocalization of ASIC3 and TRPV1 channels in the same sensory neuron has been reported. We aimed to study the combined *ASIC3* and *TRPV1* gene polymorphisms in the risk of hypertension. **Materials and Methods:** To test the statistical association between genetic polymorphisms of the *ASIC3* and *TRPV1* genes and blood pressure (BP) variations in Taiwanese, 551 unrelated individuals (286 men and 265 women) having routine health examinations were recruited. The participants had no history of cardiovascular disease or use of medication for hypertension. **Results:** Six *ASIC3* and four *TRPV1* gene polymorphisms were genotyped, and only the *ASIC3* rs2288646 polymorphism was associated with variations in BP in the participants. In subgroup analysis, we found participants carrying the combined *ASIC3* rs2288646 *AA* or *AG* and *TRPV1* rs8065080 *CC* genotypes (combined genotypes) had significantly higher systolic, mean and diastolic BP compared with the other subgroups (P = 0.009, 0.003, and 0.006, respectively, after Bonferroni correction). Interaction analysis also revealed significant gene-gene interaction in the systolic, mean, and diastolic BP in the *ASIC3* and *TRPV1* genotypes (interaction P = 0.006, 0.002, and 0.002, respectively). A trend of increasing frequencies of the combined genotype was observed in normotensive, prehypertensive, and hypertensive subgroups (P for trend = 0.001), as well as in those with higher systolic and diastolic BPs (P for trend = 9.13×10^{-4} and P for trend = 5.5×10^{-5}, respectively). **Conclusion:** Our data show a combined effect of *ASIC3* and *TRPV1* gene polymorphisms in BP variations in Taiwanese. These results suggest that the interaction between *ASIC3* and *TRPV1* is involved in BP regulation.

Keywords: *Acid-sensing ion channel 3, Blood pressure variations, Combined genotypes, Interaction effect, Transient receptor potential vanilloid 1*

Introduction

Acid-sensing ion channels (ASICs) are ligand-gated cation channels activated by extracellular protons [1] and belong to a large epithelial Na^+ channels (EnaC)/degenerin (DEG)/ASIC family, which include hypertension-related ENaCs [2-4]. One member of the ASICs, Acid-sensing ion channel 3 (ASIC3), is the most sensitive acid sensor ($pH_{0.5}$ activation: ~6.7) predominantly expressed in the peripheral snsory neurons [5]. It can be activated by low extracellular pH to evoke both transient and sustained inward currents, which can be further enhanced by lactate [1,6]. ASIC3 has been associated with myocardial ischemic pain, muscle pressor reflex, and possible imbalanced autonomic regulation [7-10]. The *ASIC3* gene, located on chromosome 7q35-36.1, has three transcript variants encoding distinct isoforms generated by alternative splicing [11,12]. Previous studies have shown a genetic variant in the *ASIC3* gene was associated with interindividual variation in blood pressure (BP) levels and insulin resistance in Taiwanese [13,14].

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Transient receptor potential (TRP) channels belong to the subfamily of cation channels formed by a tetramer of six transmembrane domains subunits which enclose a pore near the C-terminal end [15]. Unlike voltage-gated ion (Ca^{++} and K^{+}) channels, TRP subunits do not possess a voltage-sensing moiety, making their activity insensitive to change in membrane potential. TRP channels, therefore, function as voltage-independent, nonselective cation channels which are permeable to Na^{+}, K^{+}, Cs^{+}, Li^{+}, Ca^{++}, and Mg^{++} [16]. The TRP vanilloid 1 (TRPV1) channel, a member of the TRP sub-family, is identified by expression cloning using the "hot" pepper-derived vanilloid compound capsaicin as a ligand. For this reason, TRPV1 is also referred to as the vanilloid receptor (VR1) or the capsaicin receptor. VR1 is mainly expressed in a subpopulation of primary afferent neurons that project to the cardiovascular and renal tissues [17-19]. These capsaicin-sensitive primary afferent neurons are not only involved in the perception of somatic and visceral pain but also have a "sensory-effector" function. The most studied of the sensory neuropeptides are calcitonin gene-related peptide (CGRP) and substance P, both of which are potent vasodilators and natriuretic/diuretic factors. Genetic variants of the *TRPV1* gene have been previously associated with various phenotypes and disease states, including a somatosensory function in patients with neuropathic pain, cortical excitability, migraine, salt taste perception and cough, as well as a lower risk of childhood asthma [20-26].

Both ASIC3 and TRPV1 have been proposed to be involved in the pathophysiology of hypertension [13,17,27-29]. Our previous study revealed a significant association of *ASIC3* polymorphism with a risk of hypertension [13]. Common colocalization of ASIC3 and TRPV1 channels in the same sensory neuron has been reported [30]. The aim of this study was thus to elucidate the role and interaction of *ASIC3* and *TRPV1* genes in the risk of hypertension in Taiwanese.

Materials and methods

Subjects

The study participants were recruited during routine cardiovascular health examinations, and only those with no known history of major systemic and cardiovascular diseases and no history of medication for hypertension were enrolled. A total of 551 participants were included in the analysis (286 men with a mean age of 44.1 ± 10.4 years; 265 women with a mean ± standard deviation [SD] age of 45.9 ± 10.2 years). Baseline characteristics and biometrical features of the study population are summarized in Table 1. After a 5-min rest period in the supine position, their BP was measured with a random-zero sphygmomanometer by trained physicians or nurses. Two BP measurements were made at 5-min intervals with the participants in the seated position, and the mean of the two values was used as a measure of BP. Mean BP was calculated as the diastolic BP plus one-third of the pulse pressure. Hypertension was defined as a systolic BP of at least 140 mmHg, a diastolic BP of at least 90 mmHg, or both. In the absence of the use of antihypertensive medication, individuals with a systolic BP of 120–139 mmHg or a diastolic BP of 80–89 mmHg were considered to have prehypertension. This study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee of the institution (IRB number: 99-IRB-036-XD). Informed written consent was obtained from all patients before their enrollment in this study.

Genomic DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leukocytes according to a standard method with proteinase K digestion of the nuclei. Phenol and chloroform extraction was followed by isopropanol precipitation of DNA. From the published sequence of the ASIC3 and TRPV1 genes, oligonucleotide primers were generated to amplify fragments of genomic DNA containing genetic polymorphisms reported on the websites of GenePipe (http://genepipe.ngc.sinica.edu.tw/visualsnp) and GeneCards. Genotyping for the *ASIC3* gene polymorphisms was performed as we previously reported [13] and *TRPV1* gene polymorphisms were genotyped by TaqMan assays or polymerase chain reaction and restriction enzyme digestion. The data are shown in Table 2.

Statistical analysis

The Chi-square test or Chi-square test for trend was used to examine statistical differences in the distribution of categorical

Table 1: Baseline characteristics of the study participants according to blood pressure status

	Total	Normotension	Prehypertension	Hypertension	*P*
Number of participants	551	305	192	54	
Age (years)	45.0±10.1	42.8±9.4	46.8±10.0	51.2±10.1	<0.001
Sex (male/female)	286/265	142/163	118/74	26/28	0.068
Systolic BP (mmHg)	112.9±16.2	102.2±8.9	121.5±8.5	142.7±12.6	<0.001
Diastolic BP (mmHg)	74.9±10.1	68.3±6.1	81.0±5.3	91.0±10.0	<0.001
Mean BP (mmHg)	87.6±11.3	79.6±6.4	94.5±4.2	108.3±6.9	<0.001
Total cholesterol (mmol/L)	5.14±0.95	4.99±0.89	5.23±1.01	5.33±0.96	0.001
HDL cholesterol (mmol/L)	1.45±0.36	1.46±0.39	1.39±0.35	1.39±0.29	0.033
LDL cholesterol (mmol/L)	3.01±0.86	2.90±0.82	3.10±0.90	3.14±0.89	0.006
Triglycerides (mmol/L)	1.56±1.23	1.43±0.94	1.66±1.21	1.95±2.28	0.001
BMI (kg/m^2)	24.2±3.5	23.4±3.2	24.9±3.2	26.3±4.4	<0.001
Diabetes mellitus (%)	2.2	2.6	6.3	1.9	0.393
Smokers (%)	25.0	21.3	17.2	18.5	0.351

Continuous variables are presented as mean±SD. Triglyceride values were logarithmically transformed before statistical testing to meet the assumption of normal distributions; however, the untransformed data are shown. BP: Blood pressure, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, SD: Standard deviation, BMI: Body mass index

Table 2: Primer sequences and restriction enzymes used in transient receptor potential vanilloid 1 polymorphisms

SNP number	Primer sequence	PCR size and RE	Allele	Location	Risk types
rs222749	TaqMan SNP genotyping assays		C/T	Exon 2	Low
					Missense
rs222747	F: 5'-GACAACACGGCCGACAACACGAAGT-3'	226 bp/BstNI	C/G	Exon 6	High
	R: 5'-TGAGTCAGGCAGTCCTCTCCCATGC-3'				Missense
rs224534	F: 5'-GGTGATAATGGAAACTCACA-3'	180 bp/Tsp509I	A/G	Exon 9	High
	R: 5'-ATGAACGAATGAGTAAATGG-3'				Missense
rs8065080	F: 5'-TCAGAAGGCTCAGCCAAGCA-3'	250 bp/Hpy99I	C/T	Exon 12	High
	R: 5'-GCGGTTTGCTTTGCTGGTTC-3'				Missense

SNP: Single-nucleotide polymorphism, PCR: Polymerase chain reaction, RE: Restriction enzymes

data. The clinical characteristics of the continuous variables were expressed as means ± SDs and were tested by a two-sample *t*-test or analysis of variance. A generalized linear model was used to analyze systolic, diastolic, and mean BP with respect to predictors of the investigated genotypes and confounders. Multiple logistic regression analysis was used to evaluate the independent effect of the investigated genotypes on BP levels and the risk of hypertension. Triglyceride levels were logarithmically transformed before statistical analysis to adhere to a normality assumption. To address the accumulated errors from multiple testing in the genetic association analysis, the Bonferroni correction was used to determine the corrected cutoff by multiplying each *P* value with the total numbers of tests (*n*). The $P < 0.05$ using a two-sided test was considered statistically significant. The analysis of deviation from the Hardy–Weinberg equilibrium and estimation of linkage disequilibrium between polymorphisms were performed using Golden Helix SVS Win32 7.3.1 software (Golden Helix, Inc., Bozeman, MT, USA). In this study, power analysis for a logistic regression was conducted using free R software (version 3.1.0) (R Foundation for Statistical Computing, Vienna, Austria 2008) and the package powerMediation.

Results

Baseline characteristics in the study population

A summary of basic data, clinical and lipid profiles, and genotypic characteristics of the study participants stratified by BP status is provided in Table 1. No statistically significant differences in the sex ratio or frequency of diabetes mellitus and current smoking were observed between participants with normotension, prehypertension, or hypertension. On the other hand, the analysis showed that several other variables were significantly different statistically between participants with different hypertensive statuses. Compared with those with lower values, participants with higher BP values were older ($P < 0.001$) and had a higher body mass index (BMI) ($P < 0.001$), and higher total cholesterol ($P = 0.001$), low-density lipoprotein cholesterol ($P = 0.006$) and triglyceride levels ($P = 0.001$), and lower high-density lipoprotein cholesterol levels ($P = 0.033$).

Associations between acid-sensing ion channel 3 and transient receptor potential vanilloid 1 gene polymorphisms and metabolic parameters including blood pressure

Six *ASIC3* and four *TRPV1* gene polymorphisms were genotyped, and only the *ASIC3* rs2288646 polymorphism was associated with variations in BP among participants, as previously reported [13]. In that study, significantly higher BP levels were observed in participants carrying the rs2288646-*A* allele (*AA* + *AG* genotypes) than in noncarriers (*GG* genotype) after adjustment for age, sex, BMI, and smoking status. There were no significant associations between the *TRPV1* polymorphisms and BP levels [Table 3]. However, in subgroup analysis, participants with both the *ASIC3* genotype rs2288646 *AA* + *AG* and *TRPV1* genotype rs8065080 *CC* were found to have significantly higher systolic, diastolic, and mean BP (128.9 ± 16.2, 85.1 ± 13.0, and 99.7 ± 13.5 mmHg, respectively) compared with the other 2 subgroups (rs2288646 *AA* + *AG* with rs8065080 *TT* + *TC*, and rs2288646 *GG* with rs8065080 *CC*, respectively) [Table 4]. Further, we performed interaction analysis between *ASIC3* and *TRPV1* gene variants for BP values. The results show that the *ASIC3* genotypes rs2288646 *AA* + *AG* were associated with BP levels only in participants with the *TRPV1* genotype rs8065080 *CC* subgroup ($P = 0.008$, $P = 0.004$ and $P = 0.012$ for systolic, mean, and diastolic BP, respectively, after adjusting for age, sex, BMI, and smoking with Bonferroni correction and the interaction ($P = 0.006$, 0.002, and 0.002, respectively). Further logistic regression analysis with combined genotypes also indicated that participants with both the *ASIC3* genotype rs2288646 *AA* + *AG* and *TRPV1* genotype rs8065080 *CC* (genotype B) had higher systolic and diastolic BP, and a higher risk of hypertension compared with those with a combination of other genotype subgroups (genotype A) ($P = 0.001$, 5.5×10^{-5} and 0.001, respectively) [Table 5]. The results of power analysis for logistic regression with the independent effect of the investigated genotypes showed at least 0.8 for each binary outcome (i. e., higher systolic and diastolic BP and higher risk of hypertension).

In the analysis of genotypes A and B, there were no significant differences in other baseline characteristics including age, total cholesterol levels, low-density lipoprotein and high-density lipoprotein cholesterol levels, triglyceride levels, and BMI, except for the fasting plasma glucose level which was higher with borderline significance in genotype B. There were also no significant differences in various electrocardiographic parameters, including heart rate and corrected QT interval [Table 6].

Associations between acid-sensing ion channel 3 and transient receptor potential vanilloid 1 gene polymorphisms and blood pressure status

Analysis showed significantly higher frequencies of both the *ASIC3* genotype rs2288646 *AA* + *AG* and *TRPV1* genotype rs8065080 *CC* (genotype B), in individuals with hypertension than in those without hypertension (9.8% vs.

Table 3: Association between transient receptor potential vanilloid 1 genotypes and systolic and diastolic blood pressure levels

TRPV1	Genotypes	Systolic BP levels, means±SD (*n*)	*P*	*P**	Diastolic BP levels, means±SD (*n*)	*P*	*P**
rs222749[#]	TT	114.5±15.4 (34)	0.429	0.672	74.4±8.8 (34)	0.957	0.535
	TC	113.8±16.7 (213)			74.9±10.2 (213)		
	CC	112.1±15.9 (296)			75.0±10.0 (296)		
	TT + TC	113.9±16.5 (247)	0.199	0.667	74.9±10.0 (247)	0.915	0.544
	CC	112.1±15.9 (296)			75.0±10.0 (296)		
rs222747	GG	112.3±14.6 (198)	0.619	0.965	74.7±9.9 (198)	0.782	0.733
	GC	112.9±17.5 (233)			74.7±10.5 (233)		
	CC	114.2±15.9 (105)			75.5±9.1 (105)		
	GG + GC	112.6±16.2 (431)	0.365	0.910	74.7±10.2 (431)	0.483	0.679
	CC	114.2±15.9 (105)			75.5±9.1 (105)		
rs224534	GG	114.5±19.0 (19)	0.137	0.174	77.4±11.6 (19)	0.342	0.796
	GA	111.0±15.6 (178)			74.3±9.2 (178)		
	AA	113.9±16.3 (345)			75.1±10.3 (345)		
	GG	114.5±19.0 (19)	0.678	0.719	77.4±11.6 (19)	0.269	0.111
	GA + AA	112.9±16.1 (523)			74.8±10.0 (523)		
rs8065080	TT	110.7±15.5 (93)	0.302	0.152	74.8±9.2 (93)	0.863	0.434
	TC	113.2±16.7 (264)			74.8±9.8 (264)		
	CC	113.8±15.8 (182)			75.3±10.8 (182)		
	TT + TC	112.6±16.4 (357)	0.397	0.190	74.8±9.6 (357)	0.587	0.431
	CC	113.8±15.8 (182)			75.3±10.8 (182)		

[#]The genotype call rates were 543/551 (98.5%) for rs222749, 536/551 (97.3%) for rs222747, 542/551 (98.4%) for rs224534, and 539/551 (97.8%) for rs8065080, respectively. *n*: Number of participants, *P*: Unadjusted *P*, **P*: *P* adjusted for age, sex, BMI, and current smoker, BP: Blood pressure, SD: Standard deviation, BMI: Body mass index, TRPV1: Transient receptor potential vanilloid 1

Table 4: Interactive effects of the transient receptor potential vanilloid 1 genotypes on the association between acid-sensing ion channel 3 genotypes and blood pressure levels

	TRPV1 genotypes rs8065080	*ASIC3* genotypes rs2288646		*P* (adjusted *P*)	Interaction *P*
		AA + AG	GG		
Systolic BP, means±SD (*n*)	TT + TC	112.7±15.9 (20)	112.6±16.5 (337)	0.626	
	CC	128.9±16.2 (11)	112.9±15.3 (171)	0.002 (0.008)	0.006
*P**		0.040	0.432		
Diastolic BP, means±SD (*n*)	TT + TC	75.5±10.1 (20)	74.8±9.6 (337)	0.695	
	CC	85.1±13.0 (11)	76.7±10.3 (171)	0.003 (0.012)	0.002
*P**		0.034	0.841		
Mean BP, means±SD (*n*)	TT + TC	87.9±11.6 (20)	87.4±11.0 (337)	0.634	
	CC	99.7±13.5 (11)	87.4±11.2 (171)	0.001 (0.004)	0.002
*P**		0.031	0.618		

For Bonferroni correction (*n*=3). *n*: Number of participants, *P*: *P* adjusted for age; sex; BMI and current smoker, BMI: Body mass index, BP: Blood pressure, SD: Standard deviation, TRPV1: Transient receptor potential vanilloid 1, ASIC3: Acid-sensing ion channel 3, *P**: Comparison of TRPV1 genotypes on systolic, diastolic or mean BP values in different subgroups of ASIC3 genotypes

Table 5: Logistic regression analysis between combined acid-sensing ion channel 3 and transient receptor potential vanilloid 1 genotypes and blood pressure levels and the risk of hypertension

	Systolic BP		Diastolic BP		Hypertension	
	β estimate	*P*	β estimate	*P*	β estimate	*P*
Age (per years)	0.111	<0.001*	0.041	0.047*	0.082	<0.001*
Sex (male vs. female)	−0.591	0.167	0.184	0.674	−0.401	0.257
BMI	0.164	0.001*	0.272	<0.001*	0.201	<0.001*
Current smoker	0.108	0.855	0.284	0.585	0.194	0.674
Genotype (B vs. A)	2.312	0.001*	2.753	<0.001*	2.145	0.001*

**P* value was significant at 0.05. The models were adjusted for age, sex, BMI, and smoking status. Genotype A: Participants without combined *ASIC3* rs2288646 *AA* or *AG* genotypes + *TRPV1-CC* genotypes, Genotype B: Combined *ASIC3* rs2288646 *AA* or *AG* genotypes + *TRPV1-CC* genotypes, BMI: Body mass index, BP: Blood pressure, TRPV1: Transient receptor potential vanilloid 1, ASIC3: Acid-sensing ion channel 3

1.2%, $P = 0.003$, with Bonferroni correction). This was also found in individuals with a high systolic BP (≥ 140 mmHg) compared with those with a low systolic BP (11.4% vs. 1.4%, $P = 0.003$, with Bonferroni correction), as well as in those with a high diastolic BP (≥ 90 mmHg) compared with those with a low diastolic BP (14.7% vs. 1.2%, $P = 1.7 \times 10^{-4}$, with Bonferroni correction) [Table 7]. A significantly higher frequency of combined *ASIC3* genotype

rs2288646 *AA* + *AG* and *TRPV1* genotype rs8065080 *CC* (genotype B) was also noted in the hypertension category, with frequencies of 0.7% for the normotensive, 2.1% for the prehypertensive, and 9.6% for the hypertensive subgroups (*P* for trend = 0.001); [Figure 1]. When BP levels were divided into categories (<120, 120–139, and ≥140 for the systolic BP and <80, 80–89, and ≥90 for the diastolic BP), there was also a significant trend toward a higher prevalence of the combined genotypes in the highest systolic and diastolic categories; 0.6% versus 3.3% versus 11.4% (*P* for trend = 5.93×10^{-5}) and 1.1% versus 1.3% versus 14.7% (*P* for trend = 3.75×10^{-4}), respectively [Figure 1].

Table 6: Baseline data of combined transient receptor potential vanilloid 1 (rs8065080) and acid-sensing ion channel 3 (rs2288646) genotypes

Baseline data factors	Genotype A	Genotype B	*P*	*P**
Number of participants	528	11		
Age (years)	44.9±10.1	49.0±5.4	0.184	0.145
Cholesterol (mg/dL)	198.1±36.3	221.2±47.9	0.048	0.090
HDL cholesterol (mg/dL)	55.4±14.5	55.9±7.5	0.911	0.697
LDL cholesterol (mg/dL)	116.0±33.1	134.4±37.5	0.083	0.143
Triglycerides (mg/dL)	137.9±110.1	129.4±57.2	0.809	0.586
Fasting plasma glucose (mg/dL)	95.1±21.2	109.6±49.7	0.031	0.050
BMI (kg/m^2)	24.2±3.5	25.0±2.7	0.447	0.590
Heart rate (per minute)	65.1±8.5	63.1±8.4	0.441	0.491
QTc (ms)	399.7±22.5	398.2±17.3	0.828	0.850
QTcd (ms)	23.8±20.7	29.1±13.8	0.402	0.476
TPE (ms)	75.8±15.0	77.6±23.1	0.695	0.884

**P*: *P* adjusted for age; sex; BMI and current smoker. *P*: Unadjusted *P*. Genotype A: Participants without combined *ASIC3* rs2288646 *AA* or *AG* genotypes + *TRPV1-CC* genotypes, Genotype B: Combined *ASIC3* rs2288646 *AA* or *AG* genotypes + *TRPV1-CC* genotypes, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, QTc: Corrected QT interval, QTcd: QTc dispersion, TPE: Peak and end of the T-wave interval, BMI: Body mass index, TRPV1: Transient receptor potential vanilloid 1, ASIC3: Acid-sensing ion channel 3

Table 7: Genotype frequencies of the combined transient receptor potential vanilloid 1 (rs8065080) and acid-sensing ion channel 3 (rs2288646) polymorphisms by blood pressure status in the study population

Hypertensive status	Genotype B frequency, *n* (%)	*P* (adjusted *P*)
Systolic BP, mmHg (*n*)		
≥140 (35)	4 (11.4)	9.13×10^{-4}
<140 (504)	7 (1.4)	
Diastolic BP, mmHg (*n*)		5.5×10^{-5}
≥90 (34)	5 (14.7)	
<90 (505)	6 (1.2)	
Hypertension, (*n*)		0.001
Yes (51)	5 (9.8)	
No (488)	6 (1.2)	

For Bonferroni correction (*n*=3). Genotype B: Combined *ASIC3* rs2288646 *AA* or *AG* genotypes + *TRPV1-CC* genotypes, *P*: *P* adjusted for age, sex, BMI and current smoker, *n*: Number of participants, BP: Blood pressure, BMI: Body mass index, TRPV1: Transient receptor potential vanilloid 1, ASIC3: Acid-sensing ion channel 3

Discussion

This study investigated the combined *ASIC3* and *TRPV1* gene polymorphisms in BP levels and the risk of hypertension. Although there was no evidence of an association between *TRPV1* polymorphisms and BP levels, our data showed a combined effect of *ASIC3* and *TRPV1* gene polymorphisms on BP variations in Taiwanese. These results suggest that the interaction between *ASIC3* and *TRPV1* genes, a combined effect, is involved in BP regulation.

Acid-sensing ion channel 3 and hypertension

The *ASIC3* gene is in the same ENaC/DEG/ASIC family of the *ENaC* gene, whose mutations result in Liddle's syndrome, one form of monogenic hypertension [2,31,32]. *ASIC3* is known to be involved in the exercise pressor reflex, which has been noted to result in systemic vasoconstriction and marked increases in BP that accompany static exercise [33-35]. The presence of ASICs in sensory neurons might serve as a distributed pH sensor and drive the excitatory receptor potential to subsequently release vasoactive substances from the peripheral nerve endings, which might form a closed feedback loop for local vascular control, thereby affecting BP [36]. Tan *et al*. [9] provided evidence that ASICs, including ASIC3, may contribute to chemotransduction of low pH by carotid body chemoreceptors that may elicit both hyperventilation and sympathetic activation. A recent investigation in our group suggested that the null mutation of *ASIC3* in mice ($ASIC^{-/-}$ mice) results in imbalanced autonomic regulation with decreased sympathetic function and was associated with a lower BP, slower heart rate, and higher incidence of sinus arrhythmia than in $ASIC^{+/+}$ mice [37]. We have shown that a genetic variant in the *ASIC3* gene was associated with interindividual variation in BP levels in Taiwanese [13]. Chemoreceptor hypersensitivity, sympathetic excitation, and overexpression of ASICs before the onset of hypertension was also reported in spontaneous hypertension rats [28]. These results point to the importance of the ASIC3 in hypertension both in animals and in humans.

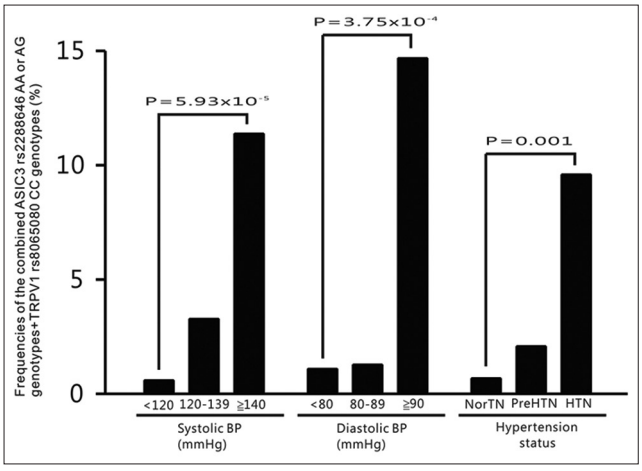

Figure 1: Frequencies of the combined acid-sensing ion channel 3 rs2288646 *AA* or *AG* and transient receptor potential vanilloid 1 rs8065080 *CC* genotypes in Taiwanese participants according to systolic blood pressure, diastolic blood pressure, and blood pressure status. The frequency of combined genotypes increased with increases in systolic blood pressure, diastolic blood pressure, and blood pressure status. HTN: Hypertension, NorTN: Normotension, PreHTN: Prehypertension

Transient receptor potential vanilloid 1 and hypertension

Hypertension is associated with profound alterations in Ca^{2+} homeostasis and smooth muscle cell proliferation. TRP channels are nonselective cation channels that are involved in hypertensive disease states. The association between TRPV1, a specific receptor for capsaicin, and hypertension has been summarized in several review articles [17,27,29]. Recent findings also suggest that TRPV1 may be activated by exogenous vanilloid or endovanilloid compounds, and its function modulated by vasoactive mediators. TRPV1 also interacts with various physiological and pathophysiological systems involved in salt and water homeostasis and cardiovascular homeostasis with sympathoinhibition, natriuresis/diuresis, and vasodilatation [29]. TRPV1 has been proposed to be involved in Dahl-sensitive hypertension, as determined in acute and short-term experiments: TRPV1 expression and function were observed to be impaired in Dahl salt-sensitive rats, rendering these animals sensitive to salt load in terms of BP regulation [17]. High salt intake in Dahl salt-sensitive rats results in activation and upregulation of TRPV1 expression, thereby acting to prevent salt-induced high BP. Adding support to these findings, Deng and Li [38] demonstrated that activation of TRPV1 in hypertensive rats by rutaecarpine led to an increase in CGRP release and a subsequent decrease in BP. Hao *et al*. [39] further showed that TRPV1 activation prevents high-salt diet-induced nocturnal hypertension in Dahl-sensitive hypertension. These results indicate the importance of TRPV1 in BP regulation.

Possible mechanisms of combined acid-sensing ion channel 3 and transient receptor potential vanilloid 1 in hypertension

The current study showed a combined effect between *ASIC3* and *TRPV1* polymorphisms in the risk of hypertension. Both *ASIC3* rs2288646 and *TRPV1* rs8065080 polymorphisms are located in the 3' coding region. It has been suggested that the *G* to *A* mutation in the rs2288646 polymorphism abolishes an exon splicing silencer motif and creates an exon splicing enhancer (ESE) motif [21]. The *de novo* creation of an ESE through mutation could either trigger splicing when unnecessary, resulting in a drastically altered protein, or it could enhance splicing above the current level that has been optimized by natural selection for that particular mRNA [25]. In contrast, the rs8065080 polymorphism is a missense mutation that could diminish an ESE motif and further abolish the protein domain. Previous studies reported associations of TRPV1 genotypes with pain sensation and salt taste perception, in which those with the genotype rs8065080 *CC* experienced lower sensitivity to pain and salt stimulation than carriers of the T allele [40-42]. These results provide evidence that this polymorphism modifies *TRPV1* function in humans where the *CC* genotype is associated with lower pain sensitivity and a decreased pain response. Both *ASIC3 and TRPV1* have been proposed to be involved in the pathophysiology of hypertension, muscle pressor reflex, and myocardial ischemia. Common colocalization of the ASIC3 and TRPV1 channels in the same sensory neuron has been reported [30]. It has been proposed that both ASIC3 and TRPV1 are likely to play a coordinated and interactive role in the processing of muscle afferent responses to $H^-PO^-_4$, which results in the exercise pressor reflex [8,43]. In analysis of the effects of muscle interstitial pH on the receptor-mediated pressor response, Gao *et al*. [44] revealed that ASICs were stimulated by mild or moderate muscle acidification, whereas the TRPV1 response increased in severe muscle acidosis. In isoproterenol-induced myocardial ischemia in mice, Cheng *et al*. [10] also showed that ASIC3 is activated first and works as an alarm in early myocardial ischemia, whereas TRPV1 is triggered later during tissue acidosis and works as damage control to limit inflammation/remodeling and to restore the left ventricular function. The above results suggest that the coordinated and sequential response between ASIC3 and TRPV1 channels to various stimuli may play an important role in various pathophysiological states, including animal and human hypertension.

Limitations of the study

The main limitation of the study was the modest sample size and the relatively lower number of participants with the combined genotype. However, the high significance of multiple results, even with the Bonferroni correction being stringently applied for multiple tests, indicates that it is very unlikely due to chance. Further, the study population was relatively young compared with usual hypertension patients. Independent association studies with older populations and with a larger sample size and functional data are needed to confirm these results before any definitive conclusions can be drawn.

Conclusion

Our data revealed an interaction of *ASIC3* and *TRPV1* gene polymorphisms in the risk of hypertension. These results also suggest that a combined analysis of *ASIC3* and *TRPV1* polymorphisms is more powerful in hypertension prediction in Taiwanese, and also supports evidence that both ASIC3 and TRPV1 are involved in the pathogenesis of human hypertension. The possible involvement of ASIC3 and TRPV1 makes them potential targets of therapy for hypertension. Further study may help to unveil novel pharmacological strategies for treating hypertension.

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Conflicts of interest

There are no conflicts of interest.

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