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

Leay-Kiaw Er*, Ming-Sheng Teng*, Semon Wu**, Lung-An Hsu¹, I-Shiang Tzeng*, Ching-Feng Cheng¹, Hsin-I Chang*, Hsin-Hua Chou⁴, Yu-Lin Ko³**

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 (pH₉.₅ activation: ~6.7) predominantly expressed in the peripheral sensory 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].

© 2018 Tzu Chi Medical Journal | Published by Wolters Kluwer - Medknow
Transient receptor potential (TRP) channels belong to the subfamily of cation channels formed by a tetramer of six transmembrane domain subunits which enclose a pore near the C-terminal end [15]. Unlike voltage-gated ion (Ca\(^{2+}\) 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\(^{2+}\), and Mg\(^{2+}\) [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 capsicain as a ligand. For this reason, TRPV1 is also referred to as the vanillloid receptor (VR1) or the capsicain receptor. VR1 is mainly expressed in a subpopulation of primary afferent neurons that project to the cardiovascular and renal tissues [17-19]. These capsicain-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 sphygmonanometer 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 protease 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.nge.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 |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 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/m²) | 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        |
| rs22747    | F: 5'-GACAACAGCCGCACACGAGT-3' | 226 bp/BstNI  | C/G    | Exon 6   | Missense   |
|           | R: 5'-TGAGTCAGCTCCTCTCCCATGC-3' |            |        |          |            |
| rs224534   | F: 5'-GGTGATAATGGAAACTCACA-3' | 180 bp/Tsp509I | A/G   | Exon 9   | High       |
|           | R: 5'-ATGAAACGATGATGAATTGG-3' |            |        |          | Missense   |
| rs8065080  | F: 5'-TCAGAAGGCTCAGCCAAGCA-3' | 250 bp/Hpy99I | C/T   | Exon 12  | High       |
|           | R: 5'-GGCGTTGTGCTTGTGCTTC-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^(-4) 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<sup>+</sup> | 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) | 0.915 | 0.544 |
| CC | 112.1±15.9 (296) | 0.199 | 0.667 | 74.9±10.2 (296) | 75.0±10.0 (296) |
| TT + TC | 113.9±16.5 (247) | 0.199 | 0.667 | 74.9±10.0 (247) | 75.0±10.0 (247) |
| CC | 112.1±15.9 (296) | 0.199 | 0.667 | 74.9±10.2 (296) | 75.0±10.0 (296) |
| rs222747 | GG | 112.3±14.6 (198) | 0.619 | 0.965 | 74.7±9.9 (198) | 74.8±9.9 (198) |
| GC | 112.9±17.5 (233) | 0.782 | 0.733 |
| CC | 114.2±15.9 (105) | 0.733 | 0.733 |
| GG + GC | 112.6±16.2 (431) | 0.365 | 0.910 | 74.7±10.2 (431) | 75.5±9.1 (105) |
| CC | 114.2±15.9 (105) | 0.365 | 0.910 | 74.7±10.2 (105) | 75.5±9.1 (105) |
| rs224534 | GG | 114.5±19.0 (19) | 0.137 | 0.174 | 77.4±11.6 (19) | 74.8±9.9 (337) |
| GA | 111.0±15.6 (178) | 0.782 | 0.733 |
| AA | 113.9±16.3 (345) | 0.687 | 0.719 | 77.4±11.6 (345) | 74.8±9.9 (345) |
| GG | 114.5±19.0 (19) | 0.687 | 0.719 | 77.4±11.6 (19) | 74.8±9.9 (19) |
| GA + AA | 112.9±16.1 (523) | 0.269 | 0.111 |
| rs8065080 | TT | 110.7±15.5 (93) | 0.302 | 0.152 | 74.8±9.2 (93) | 74.8±9.2 (93) |
| TC | 113.2±16.7 (264) | 0.863 | 0.434 |
| CC | 113.8±15.8 (182) | 0.863 | 0.434 |
| TT + TC | 112.6±16.4 (357) | 0.397 | 0.190 | 74.8±9.6 (357) | 75.3±10.8 (182) |
| CC | 113.8±15.8 (182) | 0.397 | 0.190 | 74.8±9.6 (182) | 75.3±10.8 (182) |

The genotype call rates were 543/551 (98.5%) for rs222749, 536/551 (97.2%) 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 |
|---------------------------|---------------------------|----------------|---------------|
| Systolic BP, means±SD (n) | AA + AG | GG | 0.626 | 0.006 |
| TT + TC | 112.7±15.9 (20) | 112.6±15.6 (337) | 0.002 (0.008) | 0.002 |
| CC | 128.9±16.2 (11) | 112.9±15.3 (171) | 0.002 (0.012) | 0.002 |
| P* | 0.040 | 0.432 | 0.841 | 0.618 |
| Diastolic BP, means±SD (n) | TT + TC | 75.5±10.1 (20) | 74.8±9.6 (337) | 0.001 (0.004) | 0.001 |
| CC | 85.1±13.0 (11) | 76.7±10.3 (171) | 0.001 (0.004) | 0.001 |
| P* | 0.034 | 0.841 | 0.618 | 0.618 |
| Mean BP, means±SD (n) | TT + TC | 87.9±11.6 (20) | 87.4±11.0 (337) | 0.001 (0.004) | 0.001 |
| CC | 99.7±13.5 (11) | 87.4±11.2 (171) | 0.001 (0.004) | 0.001 |
| P* | 0.031 | 0.841 | 0.618 | 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, P*: Comparison of TRPV1 genotypes on systolic, diastolic or mean BP values in different subgroups of ASIC3 genotypes

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 × 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/m²)                            | 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) |
|--------------------------|-----------------------------|-------------|
| Systolic BP, mmHg (n)    |                             |             |
| ≥140 (35)                | 4 (11.4)                    | 9.13×10⁻⁴    |
| <140 (504)               | 7 (1.4)                     |             |
| Diastolic BP, mmHg (n)   |                             |             |
| ≥90 (34)                 | 5 (14.7)                    | 5.5×10⁻⁴     |
| <90 (505)                | 6 (1.2)                     |             |
| Hypertension, (n)        |                             | 0.001       |
| Yes (51)                 | 5 (9.8)                     |             |
| No (488)                 | 6 (1.2)                     |             |

For Bonferroni correction (α=3). Genotype B: Combined ASIC3 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. 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

**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 (ASIC3−/−) 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 ASIC3−/− 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.
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 rutacearpine 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 HPO_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.

Financial support and sponsorship

This study was supported by grants from the Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, and assistance in expert statistical analysis from researcher Dr. Dao-Peng Chen of Kim Forest Enterprise Co., Ltd.

Conflicts of interest

There are no conflicts of interest.
REFERENCES

1. Waldmann R, Champigny G, Bassilana F, Heurteaux C, Lazdunski M. A proton-gated cation channel involved in acid-sensing. Nature 1997;386:173-7.

2. Hansson JH, Nelson-Williams C, Suzuki H, Schild L, Shimkets R, Lu Y, et al. Hypertension caused by a truncated epithelial sodium channel gamma subunit: Genetic heterogeneity of Liddle syndrome. Nat Genet 1995;11:76-82.

3. Babinski K, Lê KT, Séguéla P. Molecular cloning and regional distribution of a human proton receptor subunit with biphasic functional properties. J Neurochem 1999;72:27411-4.

4. Immke DC, McCleskey EW. Lactate enhances the acid-sensing Na+ channel on ischemia-sensing neurons. Nat Neurosci 2001;4:869-70.

5. Yagi J, Wenk HN, Naves LA, McCleskey EW. Sustained currents through ASIC3 ion channels at the modest pH changes that occur during myocardial ischemia. Circ Res 2006;99:501-9.

6. Gao Z, Henig O, Kehoe V, Sinoway LI, Li J. Vanilloid type 1 receptor and the acid-sensing ion channel mediate acid phosphate activation of muscle afferent nerves in rats. J Appl Physiol (1985) 2006;100:421-6.

7. Ko YL, Hsu LA, Wu S, Teng MS, Chang HH, Chen CC, et al. Association between single nucleotide polymorphisms of the transient receptor potential vanilloid 1 (TRPV1) gene and patients with irritable bowel syndrome in Korean populations. Acta Gastroenterol Belg 2012;75:222-7.

8. Zhu H, Tucker HM, Grear KE, Simpson JF, Manning AK, Cupples LA, et al. A common polymorphism decreases low-density lipoprotein receptor exon 12 splicing efficiency and associates with increased cholesterol. Hum Mol Genet 2007;16:1765-72.

9. Smith SA, Mitchell JH, Garry MG. The mammalian exercise pressor reflex in health and disease. Exp Physiol 2006;91:89-102.

10. Watanabe H, Murakami M, Ohba T, Takahashi Y, Ito H. TRP channel and cardiovascular function and disease. Circ Res 2006;99:119-31.

11. Wang DH. The vanilloid receptor and hypertension. Acta Pharmacol Sin 2005;26:286-94.

12. Inoue R, Jensen LJ, Shi J, Morita H, Nishida M, Honda A, et al. Transient receptor potential channels in cardiovascular function and disease. Circ Res 2006;99:119-31.

13. Binder A, May D, Baron R, Maier C, Tölle TR, Treede RD, et al. Transient receptor potential channel polymorphisms are associated with the somatosensory function in neuropathic pain patients. PLoS One 2011;6:e17387.

14. Ko YL, Hsu LA, Wu S, Teng MS, Chang HH, Chen CC, et al. Transient receptor potential gene expression in dorsal root ganglion neurons. Brain Res Mol Brain Res 2005;136:125-33.

15. Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, et al. Liddle’s syndrome: Heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. Cell 1994;79:407-14.

16. Smith SA, Mitchell JH, Garry MG. The mammalian exercise pressor reflex in health and disease. Curr Opin Nephrol Hypertens 2013;22:170-6.

17. Liu J, Gao DH. Transient receptor potential vanilloid in blood pressure regulation. Curr Opin Nephrol Hypertens 2010;19:125-33.

18. Liu J, Gao Z, Li J. Femoral artery occlusion increases expression of ASIC3 in dorsal root ganglion neurons. Brain Res Mol Brain Res 2005;136:125-33.

19. Xu X, Li Q, Shrestha K, Cornet-Boyaka E, Chen L, Smith PR, et al. Interregulation of proton-gated Na (t) channel 3 and cystic fibrosis transmembrane conductance regulator. J Biol Chem 2006;281:36960-8.

20. Smit LA, Kogevinas M, Antó JM, Bouzigon E, González JR, Le Moul N, et al. Transient receptor potential genes, smoking, occupational exposures and cough in adults. Respir Res 2012;13:26.

21. Carreño O, Corominas R, Fernández-Morales J, Caminé M, Sobrido MJ, Fernández-Fernández JM, et al. SNP variants within the vanilloid TRPV1 and TRPV3 receptor genes are associated with migraine in the Spanish population. Am J Med Genet B Neuropsychiatr Genet 2012;159B:94-103.

22. Chen Y, Yang KC, Kuo TB, Chang HH, Chen CC, et al. Genetic influence on variability in human acute experimental pain sensitivity associated with gender, ethnicity and psychological temperament. Pain 2004;109:488-96.
41. Valdes AM, De Wilde G, Doherty SA, Lories RJ, Vaughn FL, Laslett LL, et al. The ile585Val TRPV1 variant is involved in risk of painful knee osteoarthritis. Ann Rheum Dis 2011;70:1556-61.

42. Dias AG, Rousseau D, Duizer L, Cockburn M, Chiu W, Nielsen D, et al. Genetic variation in putative salt taste receptors and salt taste perception in humans. Chem Senses 2013;38:137-45.

43. Li J, Maile MD, Sinoway AN, Sinoway LI. Muscle pressor reflex: Potential role of vanilloid type 1 receptor and acid-sensing ion channel. J Appl Physiol (1985) 2004;97:1709-14.

44. Gao Z, Li JD, Sinoway LI, Li J. Effect of muscle interstitial pH on P2X and TRPV1 receptor-mediated pressor response. J Appl Physiol (1985) 2007;102:2288-93.