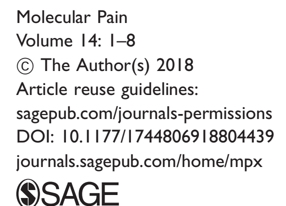

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Effect of single-nucleotide polymorphisms in *TRPV1* on burning pain and capsaicin sensitivity in Japanese adults

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Abstract

Transient receptor potential vanilloid 1 (TRPV1) is a nonselective cation channel that is expressed in the sensory neurons and responds to various noxious stimuli including heat and capsaicin. The molecular properties of TRPV1 have been clearly examined; however, there are obvious individual differences in human sensitivity to thermal stimuli and capsaicin. Here, we examined the possibility that different genome sequence of human *TRPV1* caused the different sensitivity to heat or capsaicin. The sensitivities to burning pain and capsaicin of Japanese adult subjects were compared with their *TRPV1* genome sequence, and we detected 6 single-nucleotide polymorphisms and 11 single-nucleotide polymorphisms related to burning pain and capsaicin sensitivity, respectively. In particular, homozygous I585V, a single-nucleotide polymorphism with amino acid substitution, significantly related to higher capsaicin sensitivity.

Keywords

TRPV1, capsaicin, burning pain, single-nucleotide polymorphism

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Introduction

TRPV1, which belongs to the TRP family,[1] is a nonselective cation channel that is expressed in the sensory afferent neurons innervating the skin and oral cavity, and it functions to integrate external noxious stimuli. As a polymodal receptor, it responds to various external stimuli, including protons, thermal stimuli, and capsaicin, and transmits heat sensations in the skin and spicy tastes in the oral cavity.[2,3]

The molecular properties of TRPV1, acting a receptor for both heat and capsaicin, have been previously examined, and the heat and capsaicin thresholds were reported to be ~43°C[4] and ~0.6 μM,[3,5] respectively. However, there are clearly individual differences in sensitivity to thermal stimuli and capsaicin; people have personal preferences when it comes to the temperature of their bathing water and their food, and some people cannot eat spicy food containing capsaicin, while others like to eat such foods.

The mechanisms underlying such differences among individuals are thought to involve both qualitative and quantitative differences in the TRPV1 molecules. A TRPV1-knockout mouse is not lethal[6]; therefore, although TRPV1 has been evolutionarily conserved in mammals,[7] it is not essential for survival. Many single-nucleotide polymorphisms (SNPs), with or without associated amino acid substitutions, have been identified in the human *TRPV1* gene,[8,9] and some of them have been reported to be associated with differences in disease-related molecular properties.[10,11] However, most SNPs

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have little or no effect on human health.[9,12–14] Such findings suggest that the SNPs within the human *TRPV1* gene may be the underlying cause of the observed differences in sensitivity to burning pain and capsaicin in healthy adults. Moreover, a study of monozygotic and dizygotic twins showed that genetic factors contribute to the sensitivity and preferences for capsaicin.[15]

In this study, we examined sensitivities to burning pain and capsaicin in Japanese adults and compared the sequences of the *TRPV1* gene to look for correlations. Although significant individual differences in sensitivity to burning pain and capsaicin were observed, there were no clear correlations between the two sensitivities, even though both are detected by TRPV1. Furthermore, in the sequencing analysis, many SNPs were detected in the *TRPV1* gene, including novel ones, and some were associated with sensitivity to burning pain or capsaicin. For one SNP that led to an amino acid substitution, I585V, capsaicin sensitivity was higher for the homozygous Val-Val phenotype. However, other SNPs associated with the sensitivities were located in noncoding regions, which suggest that they affect TRPV1 function without altering the amino acid sequence.

Materials and methods

Study subjects

This study was approved by the research ethical review committee of Matsumoto Dental University (Permission number: 173 and 174). The study participants consisted of 26 healthy volunteers aged 20–35 years (15 males and 11 females). Written and verbal informed consent was obtained from all participants prior to the sampling and behavioral testing. The participants were asked their age, sex, height, and weight, and then the sensory tests and sampling for genome extraction were carried out.

Sensory testing

The participants underwent a burning pain sensitivity test and a capsaicin sensitivity test after they had rested for 5 min while listening to quiet music with headphones on inside a room set at 20°C–25°C.

Burning pain sensitivity test. The participants placed their hand on a prewarmed hot plate and were instructed to remove their hand once the heat became unbearable. This withdrawal latency was measured with a stopwatch. The longest latency was 25 s (0.0–25.0 s). The hot plate was set at 48°C, so that it would not overlap with the temperatures of other TRP family receptors.[2] The participants were first tested by using their left hand and then their right hand.

Capsaicin sensitivity test. A capsaicin working solution (3 mg/ml in 80% ethanol, 7% Tween 80, and 0.1 M phosphate-buffered saline) was diluted with H_2O, and eight test solutions of various concentrations (0, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, and 0.15 μg/ml) were prepared. In the test, the participants put 5 ml of the least-concentrated capsaicin solution into their mouth. The solution was spat out after 5 s, and after their mouth was rinsed out and their sensitivity was recorded, the participants would then move to the next least concentrated solution. The time interval from one sample to the next was fixed at 30 s. For each sample, the participants chose one of the following responses to the question of whether they detected a capsaicin taste: “No, there was not,” “Probably not,” “I feel as if there was,” and “Yes, there was.” The participants were not told the concentration of each solution. The sensitivity threshold was defined as the concentration at which the response changed from “Probably not” to “I feel as if there was.”

Genome sequencing analysis

Genomic DNA was extracted from buccal mucosal cells using the ISOHAIR kit (TOYOBO, Osaka, Japan) and purified using a Mag Extractor (TOYOBO). Then polymerase chain reaction (PCR) was performed by using 1–5 μg of the purified genomic DNA and KOD plus neo enzyme (TOYOBO) according to the manufacturer’s protocol. The amplified fragments were purified using the NucleoSpin Gel and PCR Clean-up (Takara Bio, Inc., Kusatsu, Japan) and were sent to Macrogen Japan Corporation (Tokyo, Japan) along with primers for sequencing. The primers were designed by using Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/) and are listed in Table 1. The obtained sequences were compared to the Human Genome chromosome 17 sequence (NCBI NC_000017.11, 3610706–3561707). Haploview 4.2 software was used to calculate the D’ and logarithm of odds (LOD) parameters and was used for the haplotype analysis.

Statistical analysis

The data were analyzed with EZR software.[16] Spearman’s rank correlation coefficient was used to examine the correlation between two variables. After the Shapiro–Wilk test, data for two groups were analyzed by the Mann–Whitney *U* test, and data for three groups were analyzed by the Kruskal–Wallis and the Steel–Dwass tests. The level of significance was set at $p < 0.05$. Hardy–Weinberg equilibrium was assessed by Fisher’s exact test to detect whether the SNPs were in genetic equilibrium.

Results

Sensory testing

Burning pain sensitivity test. For the burning pain sensitivity test, the subjects' withdrawal latencies from the 48°C hot plate were measured, and the average values for the left and right hands were used. Five subjects with a difference that was more than double between their two hands were excluded, due to the unreliability of the data. Therefore, the results for 21 participants (12 males and 9 females) were analyzed: the minimum withdrawal latency was 2.05 s, the maximum was 25 s, and the median was 5.05 s (Figure 1(a)). No correlation was observed between burning pain sensitivity and sex, age, or body mass index (data not shown).

Table 1. Sequencing primer.

	Name	Sequence
995F	1F	CACATTCCAGAAGCCCTCAT
1630R	1R	GGTATCCCCATGTTGGTCAG
1892F	2F	GAGCTTGGTTTGGGAGGTTG
13375F	3F	GGAGCCACAGGTTTGAGAGG
14639F	4F	CCATCACACAGCCAACACTC
17643F	5F	CAGGATGCTTGACAGATGTTG
18797R	5R	ACGAGGTCACATGAGGAAGG
19193F	6F	TGCTTTTCTGGACAGTCACC
20192F	7F	CAGGCACTTTCTGCCCAGTT
21547F	8F	GCAGGCAGGAGCTTTAGGTA
22765R	8R	AGGAGGGTCCTTTTGTCCAT
24533F	9F	AGTTTCACCCCATTCACTGC
27041F	10F	GCGGCACAGCATTGTAAGAT
29967F	11F	CCGGCTTCCACTGTGTATTT
32754F	12F	CAATTTCTGCTCCTTGGGGACGA
33728R	12R	TCAGTGTGTCCTCTGTCCACCC
36517F	13F	GGCTGTATTCACATTGCACA
37457R	13R	CTCTGAGGTGGACACCCTGT
38295F	14F	CGCCAGGCTACAGAAACAAAG
39535R	14R	AGAGTGAGGAACAGGGCTGA
44093F	15F	CCCCAAGTGAATCTCCTAACA
44183R	15R	GGGCCTAACATGTCCCAGTA
44726F	16F	CAGGCTGGTCTCGAACTCTT
44879R	16R	TTCCCAAAGGTGTTTTCCTG
45384R	17R	ATGCATGCACACACCCACAC

Capsaicin sensitivity test. For the capsaicin sensitivity test, the capsaicin sensitivity of all 26 study subjects (15 males and 11 females) was assessed. The minimum sensitivity was 0.05 µg/ml, the maximum was 0.15 µg/ml, and the median was 0.08 µg/ml (Figure 1(b)). No correlation was observed between capsaicin sensitivity and sex, age, and body mass index (data not shown).

There was also no correlation between burning pain sensitivity and capsaicin sensitivity ($r = 0.241$, Figure 1(c)).

TRPV1 sequencing analysis

Approximately 19 kbp of the ~45 kbp *TRPV1* region on human chromosome 17 was analyzed, with a focus on the exons and surrounding areas. Compared to the GenBank human chromosome 17 sequence (NC_000017.11, 3610706–3561707), the sequence of the 26 subjects contained 89 SNPs. Of these, 40 SNPs with a minor allele frequency > 0.05 were examined (Table 2).

Of the 12 SNPs in the exon region, two were in the 5′-untranslated region (UTR), seven were in the coding region, and three were in the 3′-UTR. The seven SNPs in the coding region were rs56095209 (P60P), rs222749

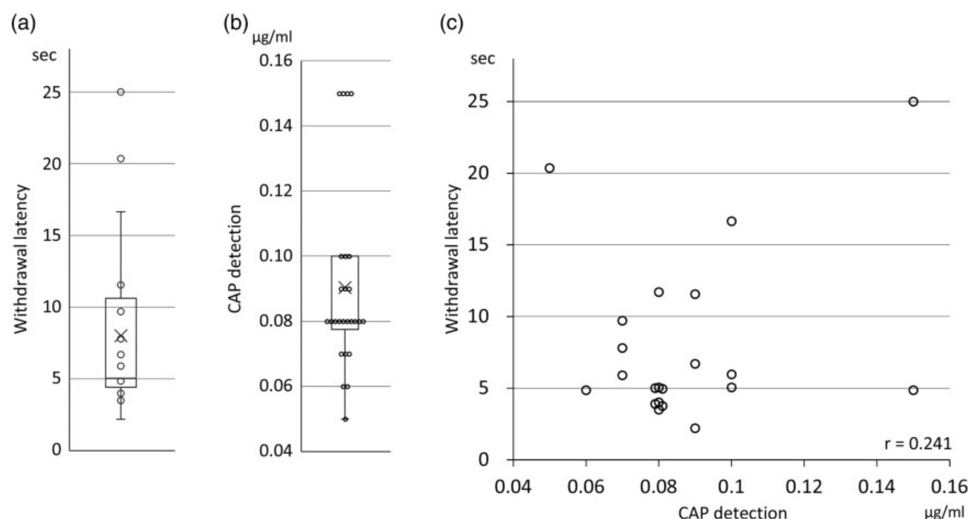

Figure 1. Sensitivities for burning pain or capsaicin. (a) Sensitivity for burning pain of the participants hand was measured as withdrawal latency from the 48°C hot plate. (b) Sensitivity for capsaicin in oral cavity was tested by capsaicin solutions. (c) Comparison of two sensitivities. r: coefficient of correlation; CAP: capsaicin.

Table 2. SNPs detected in TRPV1 gene.

	SNP ID	Region		Allele [major/minor]	Major homo	Hetero	Minor homo	MAF
2193	rs460716	**exon1d2**	5' UTR	T[C/T]C	16	10	0	0.192[a]
13461		intron1d2		G[C/G]A	21	5	0	0.096
13491	rs2277675	intron1d2		C[A/G]G	16	8	2	0.231
13894	rs74441350	intron1c		G[T/A]G	20	4	2	0.154
13983	rs161383	intron1c		G[C/G]C	9	9	8	0.481
14727		intron1c		C[C/G]A	15	11	0	0.212[a]
14907	rs79821076	**exon1b**	5' UTR	T[C/T]G	21	5	0	0.096
17775	rs161385	intron1b		G[C/G]A	13	6	7	0.385[a,d]
17821	rs3837858	intron1b		G[-/TGGGTG]G	23	2	1	0.077
18536	rs56095209	**exon2**	P60P	C[G/A]G	24	1	1	0.058
18627	rs222749	**exon2**	P91S	C[C/T]C	17	7	2	0.212
19274	rs117112057	intron2		C[G/A]T	23	3	0	0.058[b]
19640	rs222748	**exon4**	H317H	A[C/T]G	19	7	0	0.135[c]
19802	rs3216758	intron4		G[G/-]C	10	11	5	0.404
20801	rs222747	**exon6**	M315I	T[G/C]C	10	14	2	0.346
21003	rs12936340	intron6		G[C/T]G	17	6	3	0.231[b]
21682	rs7220415	intron6		G[G/T]C	17	6	3	0.231[b]
21881	rs520671	intron7		T[G/T]T	17	8	1	0.192[c]
24650	rs74389246	intron8		C[T/G]C	18	7	1	0.173
24752	rs3744686	intron8		G[G/A]G	17	6	3	0.231[b]
24757	rs161394	intron8		C[T/C]T	10	11	5	0.404
27299	rs224534	**exon10**	T469I	A[T/C]T	16	5	5	0.288[d]
30524	rs161365	intron11		C[G/A]T	17	7	2	0.212
30580	rs57716901	intron11		G[A/G]G	8	12	6	0.462[a,b]
30599	rs61387317	intron11		C[T/G]G	8	12	6	0.462[a,b]
33226	rs2277679	intron12		A[C/G]C	18	6	2	0.192
33554	rs8065080	**exon13**	I585V	C[G/A]T	12	12	2	0.308[b]
33605	rs8078936	intron13		G[A/G]G	9	11	6	0.442[b]
36828	rs375458057	**exon14**	P619P	C[T/G]G	24	1	1	0.058
37092	rs113287435	intron14		G[G/A]G	24	1	1	0.058
37167	rs71153373	intron14		T[G/GG]G	24	1	1	0.058
37280	rs57405156	intron14		C[T/C]G	9	11	6	0.442[b]
38436	rs877611	intron14		C[T/G]A	18	7	1	0.173
38983	rs224547	intron15		G[T/C]T	18	8	0	0.154
39243	rs1018658001	intron16		G[G/A]G	23	3	0	0.058
39341	rs3826503	intron16		A[T/C]G	9	12	5	0.423[b]
43524	rs4790523	intron16		G[T/G]T	8	12	6	0.462[a,b]
44148	rs4790522	**exon17**	3' UTR	T[G/T]T	18	8	0	0.154
44235	rs4790521	**exon17**	3' UTR	C[A/G]C	15	8	3	0.269
45193	rs150895493	**exon17**	3' UTR	C[TTC/-]T	24	1	1	0.058

Note: $p < 0.05$. MAF: minor allele frequency; SNP: single-nucleotide polymorphism; dbSNP: database of single-nucleotide polymorphism.
[a]SNPs related to burning pain sensitivity; †: SNPs related to capsaicin sensitivity
[b]SNPs related to capsaicin sensitivity
[c]SNPs not in Hardy-Weinberg equilibrium
[d]MAFs significantly different from reported in dbSNP
bold font: SNPs cited in exons

(P91S), rs222748 (H317H), rs222747 (M315I), rs224534 (T469I), rs8065080 (I585V), and rs375458057 (P619P). The remaining 28 SNPs were located in the introns, and two of these (13461 and 14727) were not in the NCBI database of single-nucleotide polymorphism (dbSNP). The allele frequencies of two others, 19640 (rs222748) and 21881 (rs520671), were significantly different from those reported in dbSNP, and 17775 (rs161385) and 27299 (rs224534) were not in Hardy–Weinberg equilibrium (Table 2). Haplotype analysis identified two haplotype blocks (block 1: 21003–24752 and block 2: 30580–39341) for the 14 SNPs that are related to burning pain and/or capsaicin sensitivity (Figure 2).

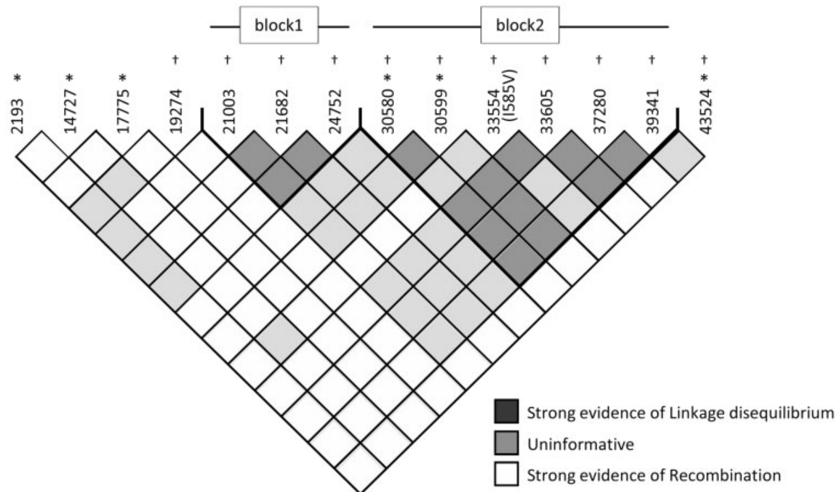

Figure 2. Haplotype analysis. The *TRPV1* SNPs related to burning pain or capsaicin sensitivity were analyzed, and two haplotype blocks (block 1 and block 2) were detected. *: SNPs related to burning pain sensitivity; †: SNPs related to capsaicin sensitivity.

TRPV1 *SNPs and burning pain sensitivity*

For each SNP, the study subjects were divided into three groups according to genotype (major allele homozygous, heterozygous, or minor allele homozygous), and the groups were assessed for any differences in burning pain sensitivity. A significant difference was detected for six SNPs, 2193 (rs460716), 14727, 17775 (rs161385), 30580 (rs57716901), 30599 (rs61387317), and 43524 (rs4790523).

For 2193 (rs460716) and 14727, there were only two genotypes, and for both SNPs, the heterozygote (2193CT and 14727CG) had higher burning pain sensitivity. In addition, for 17775 (rs161385), the heterozygote (CG) had higher burning pain sensitivity than the other two genotypes (CC + GG). Two other SNPs, 30580 (rs57716901) and 30599 (rs61387317), had a strong linkage (D' = 1, LOD = 11.97, block2 in Figure 2), and the double heterozygote (30580AG/30599TG) showed significantly higher burning pain sensitivity than the other types (AA/TT + GG/GG). For 43524 (rs4790523), T carriers (TT + GT) had significantly higher burning pain sensitivity than the GG genotypes (Figure 3).

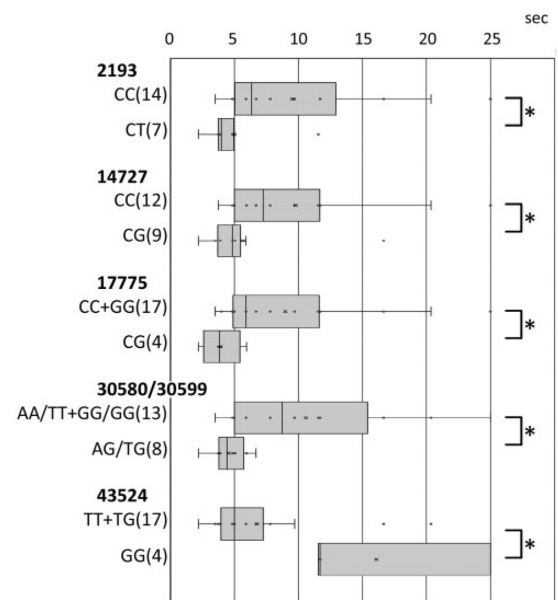

Figure 3. *TRPV1* SNPs related to burning pain sensitivity. A significant difference was detected for six SNPs, and 30580/30599 had a strong linkage. For each SNP, the withdrawal latency from 48°C hot plate of each genotype group was indicated. (n): subject number in each group. *: significant difference detected by the Mann–Whitney *U* test, $p < 0.05$.

TRPV1 *SNPs and capsaicin sensitivity*

For each SNP, the subjects were divided into three groups according to genotype (major allele homozygous, heterozygous, or minor allele homozygous), and the groups were examined for any differences in capsaicin sensitivity. A significant difference was detected for 11 SNPs, 19274 (rs117112057), 21003 (rs12936340), 21682 (rs7220415), 24752 (rs3744686), 30580 (rs57716901), 30599 (rs61387317), 33554 (rs8065080), 33605 (rs8078936), 37280 (rs57405156), 39341 (rs3826503), and 43524 (rs4790523).

For 19274 (rs117112057), there were only two genotypes, and the heterozygous type (19274GA) had significantly higher capsaicin sensitivity. Three SNPs, 21003 (rs12936340), 21682 (rs7220415), and 24752 (rs3744686), had strong linkages (D' = 1, LOD = 10.39, block1 in

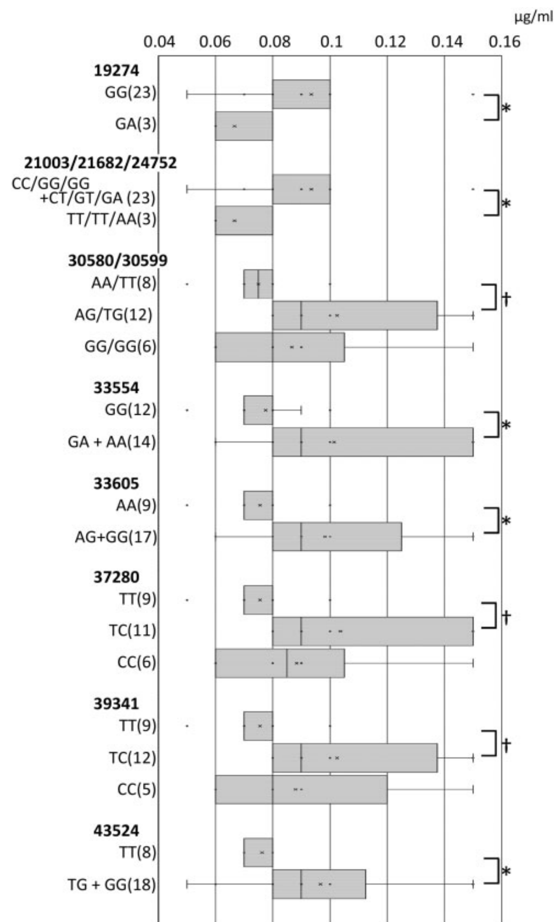

Figure 4. *TRPV1* SNPs related to capsaicin sensitivity. A significant difference was detected for 11 SNPs and 21003/21682/24752 and 30580/30599 had a strong linkage. For each SNP, the capsaicin sensitivity threshold of each genotype group was indicated. (n): subject number in each group. *: significant difference detected by the Mann–Whitney *U* test, $p < 0.05$. †: significant difference detected by both of the Kruskal–Wallis and the Steel–Dwass tests, $p < 0.05$.

Figure 2), and subjects with the TT/TT/AA genotype for 21003/21682/24752 had significantly higher capsaicin sensitivity than the other types (GT/GT/GA + GG/GG/GG). For two other SNPs, 30580 (rs57716901) and 30599 (rs61387317), the capsaicin sensitivity of the 30580AA/30599TT subjects was significantly higher than that of AG/TG genotypes subjects. The 33554 (rs8065080) SNP was accompanied by an amino acid substitution (I585V), and the capsaicin sensitivity of the GG genotype (amino acid phenotype Val-Val) was higher. The capsaicin sensitivities of subjects with 33605AA and 43524TT had significantly higher capsaicin sensitivity than the other genotypes. The 37280TT and 39341TT were significantly higher than those of their heterozygous genotypes, 37280TC and 39341TC, respectively (Figure 4).

Discussion

TRPV1 is a polymodal receptor that is receptive to both thermal stimulation at 43°C and capsaicin.[2,3] In the present study, no correlation was found between burning pain sensitivity and capsaicin sensitivity. This result is consistent with the fact that the thermosensitive site on TRPV1 is distinct from the capsaicin-sensitive site,[17–20] and it also indicates that none of the detected SNPs affected the gating property of the TRPV1 channel. However, whether these two sensitivities are independent is still controversial, in part because of the methodological differences in testing, since burning pain sensitivity was measured as a pain threshold (upper limit), whereas capsaicin sensitivity was measured as a detection threshold (lower limit).

Recently, three TRP family proteins, TRPV1,[3,4] TRPA1,[21] and TRPM3,[22] were reported to have a redundant role in heat sensitivity. That is, triple knockout (Trpv1−/−Trpm3−/−Trpa1−/−) mice lacked the acute withdrawal response to noxious heat, while heat responsiveness was observed when at least one of these TRP channels was functional.[23] In addition, Anoctamin1 (ANO1), a calcium-activated chloride channel, was described as a heat sensitive channel expressed in nociceptive neurons.[24] Because ANO1 is a chloride channel, the opening of ANO1 can lead to the hyperpolarization or depolarization of neurons in an intracellular chloride concentration-dependent manner. In the nociceptive neurons, the heat-dependent ANO1 opening induced depolarization and activation due to their high concentration of intracellular chloride.[24] These heat sensitive proteins can be activated at ~45°C, and they were predicted to be involved in our study using a 48°C hot plate. This redundancy and complexity of the heat sensing system produced by many sensor proteins compared to capsaicin sensing may cause a low correlation between burning pain sensitivity and capsaicin sensitivity.

The SNPs associated with burning pain sensitivity were located throughout the *TRPV1* gene. However, a genetic linkage was only observed for 30580 (rs57716901) and 30599 (rs61387317), which suggests that these SNPs independently influence the burning pain sensitivity of TRPV1. This may be related to the complexity of the temperature threshold determining domain of the TRPV1 molecule, which, in various reports, were identified in the N-terminus,[18] transmembrane domain,[19] and C-terminal domain.[20]

Like the SNPs associated with burning pain sensitivity, the SNPs associated with capsaicin sensitivity are widely scattered. However, unlike burning pain sensitivity, linkages were observed for each SNP, so they were largely divided into two haplotype blocks. For this reason, it was impossible to specify which SNP in the haplotype block affected capsaicin sensitivity.

Furthermore, in this study, the analysis was focused on the whole exons and the introns in the vicinity of the splice sites; however, there are many unanalyzed regions in the long introns, where there may be many SNPs. There may also be many unanalyzed SNPs in the haplotype block1 and block2 (Figure 2), which may also affect capsaicin sensitivity.

For the SNPs associated with an amino acid substitution, I585V was the only one that was related to significantly high capsaicin sensitivity (the VV type). Nonsynonymous SNPs detected in TRPV1, Q85R,[9,14] P91S,[14] and M315I[9] were previously suggested to be gain-of-function phenotypes, and I585V was to be comparable to WT[14] or loss-of-function phenotypes[9,11,25] of capsaicin by electrophysiological or calcium-imaging experiments, and our result conflicted with these previous studies. However, there are some possible explanations for these differences. First, in many reports, the functional property of the TRPV1 molecule with each SNP was examined by comparing the EC_{50} or maximum amount of Ca2+ entry into the cells.[11,14,25] Our results measured the detectable threshold (lower limit), which may not reflect the property of TRPV1. Second, the cultured cells did not mimic the in vivo environment. TRPV1 functions will be affected by many other factors, such as functional-associated proteins PKC,[26] PIP2,[27] and PIRT,[28] which are related to TRPV1 phosphorylation. Because TRPV1 cDNA with SNPs were transfected into nonneuronal cell lines to investigate its function in previous studies,[11,14,21,25] their protein expression profile may differ from neurons. Another factor is genome sequences within noncoding regions. It has been reported that the sequences in 5′- and 3′-UTR can affect protein functions by changing localization, stability, and translational efficiency of the mRNA[29,30] and that intron sequences are able to alter protein expression by regulating RNA splicings.[31] Thus, 2193 (rs460716) located in the 5′-UTR may have a similar effect; however, these noncoding regions cannot be used and examined in cDNA transfection experiments. In addition, nonmolecular factors, such as influences of the autonomic nerve, and cognition or preference produced by brain function, may produce differences between the results of the cultured cell and in vivo experiments. Actually, capsaicin application to human skin can detect high pain sensitivity of I585V,[12] rather than loss-of-function. To identify the roles of these factors, which could not be reproduced by in vitro experiments, further investigation will be needed in the future.

Many of the SNPs linked to burning pain and capsaicin sensitivity were located in the intron, ~50–350 bp away from the splice sites. In some reports, protein expression was affected by intron sequence changes, mainly via splicing control following transcription.[32] However, these changes were located close to the splice site; it is unclear by what mechanisms sequence changes, like SNPs, far from splice sites could affect splicing.

In this study, a number of SNPs associated with burning pain sensitivity and capsaicin sensitivity were detected. Although the findings suggest that the intron sequence may affect protein function, further research is to ascertain the detailed mechanisms involved.

Author Contributions

NO and MO designed and performed experiments, analyzed data, and prepared the manuscript. NS and MT contributed to experiment designs and statistical analyses. OT and EK supervised the experiments and finalized the manuscript. All the authors have read and approved the paper.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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