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

Nozomu Okamoto¹, Masayo Okumura², Osamu Tadokoro², Norio Sogawa³, Mihoko Tomida⁴, and Eiji Kondo¹,²

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

Date Received: 19 June 2018; revised: 20 August 2018; accepted: 6 September 2018

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°C⁴ 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 lethal⁶; 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...
have little or no effect on human health.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsubscript{2}O, 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). Haplov4.2 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.\textsuperscript{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).

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).

Table 1. Sequencing primer.

| Name | Sequence                      |
|------|-------------------------------|
| 995F | 1F CACATTCCAGAAGCCCTCAT       |
| 1630R| 1R GTATCCCCCAAGTGGTCAG        |
| 1892F| 2F CAGCTTTGGTTGAGGGTTG        |
| 13375F| 3F GGAGCCACAGTGGAGGAAGG      |
| 14639F| 4F CCATCACAGACACACACCT       |
| 17643F| 5F CAGGATGCTTGCAGATGTTG      |
| 18797R| 5R ACGAGTTCATAGAGGAAGG       |
| 19193F| 6F TGGCTTTCTGGACATGCACC      |
| 20192F| 7F CAGGACCCTTCTGCCAGTT       |
| 21547F| 8F GCAGGACCTTCTTGTAGGA       |
| 22765R| 8R AGGAGGTCCTTGTCCCAT        |
| 24533F| 9F ATTTTACCCCCATTTGC         |
| 27041F| 10F GCCGGCAGACATTTGAAGAT     |
| 29967F| 11F CCGGGCTTCCAGTGTGATTT    |
| 32754F| 12F CAATTCTTCTCTTGGGAGCA     |
| 33728R| 12R TCAAGTGTCTCTTGCTTCCCA    |
| 36517F| 13F GGCTTACACCTTGCACA       |
| 37457R| 13R CTCCTAGTTGCACCCCTTG     |
| 38295F| 14F GCAGGCTACAGAAACAAAG     |
| 39535R| 14R AGAGTAGGAAACAGGGCTGA    |
| 44093R| 15F CCGGGTAAACTCCTAAACA    |
| 44183R| 15R GGGCTTAACTTGCCAGTA      |
| 44726F| 16F CAGGCTGTCTCGAACTCTT     |
| 44879R| 16R TTCCCAAAGGTGTCTTCTCG    |
| 45384R| 17R ATGCATGCACACACCCAC      |

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.
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).

Table 2. SNPs detected in TRPV1 gene.

| SNP ID     | Region       | Allele [major/minor] | Major homo | Hetero | Minor homo | MAF     |
|------------|--------------|----------------------|------------|--------|------------|---------|
| rs460716   | exon1d2      | T[C/T]C              | 16         | 10     | 0          | 0.192   |
| rs222747   | intron1d2    | G[C/G]A              | 21         | 5      | 0          | 0.096   |
| rs222765   | intron1d2    | C[A/G]G              | 16         | 8      | 2          | 0.231   |
| rs7441350  | intron1c     | G[T/A]G              | 20         | 4      | 2          | 0.154   |
| rs161383   | intron1c     | G[C/G]C              | 9          | 9      | 8          | 0.481   |
| rs12936340 | intron6      | C[C/G]A              | 15         | 11     | 0          | 0.212   |
| rs79821076 | 5’ UTR       | T[C/T]G              | 21         | 5      | 0          | 0.096   |
| rs161385   | intron1b     | G[C/G]A              | 13         | 6      | 7          | 0.385   |
| rs3837858  | intron1b     | G[-/TGGGTG]G         | 23         | 2      | 1          | 0.077   |
| rs56095209 | exon2        | C[G/A]G              | 24         | 1      | 1          | 0.058   |
| rs222749   | exon2        | C[C/T]C              | 17         | 7      | 2          | 0.212   |
| rs11712057 | intron2      | C[G/A]T              | 23         | 3      | 0          | 0.058   |
| rs222748   | exon1b       | T[C/T]G              | 21         | 5      | 0          | 0.096   |
| rs161385   | intron1c     | G[C/G]A              | 13         | 6      | 7          | 0.385   |
| rs3837858  | intron1c     | G[-/TGGGTG]G         | 23         | 2      | 1          | 0.077   |
| rs56095209 | exon2        | C[G/A]G              | 24         | 1      | 1          | 0.058   |
| rs222749   | exon2        | C[C/T]C              | 17         | 7      | 2          | 0.212   |
| rs11712057 | intron2      | C[G/A]T              | 23         | 3      | 0          | 0.058   |
| rs222748   | exon1b       | T[C/T]G              | 21         | 5      | 0          | 0.096   |
| rs161385   | intron1c     | G[C/G]A              | 13         | 6      | 7          | 0.385   |
| rs3837858  | intron1c     | G[-/TGGGTG]G         | 23         | 2      | 1          | 0.077   |
| rs56095209 | exon2        | C[G/A]G              | 24         | 1      | 1          | 0.058   |
| rs222749   | exon2        | C[C/T]C              | 17         | 7      | 2          | 0.212   |
| rs11712057 | intron2      | C[G/A]T              | 23         | 3      | 0          | 0.058   |
| rs222748   | exon1b       | T[C/T]G              | 21         | 5      | 0          | 0.096   |
| rs161385   | intron1c     | G[C/G]A              | 13         | 6      | 7          | 0.385   |
| rs3837858  | intron1c     | G[-/TGGGTG]G         | 23         | 2      | 1          | 0.077   |
| rs56095209 | exon2        | C[G/A]G              | 24         | 1      | 1          | 0.058   |
| rs222749   | exon2        | C[C/T]C              | 17         | 7      | 2          | 0.212   |
| rs11712057 | intron2      | C[G/A]T              | 23         | 3      | 0          | 0.058   |
| rs222748   | exon1b       | T[C/T]G              | 21         | 5      | 0          | 0.096   |

Note: p < 0.05. MAF: minor allele frequency; SNP: single-nucleotide polymorphism; dbSNP: database of single-nucleotide polymorphism.

*SNPs related to burning pain sensitivity; †: SNPs related to capsaicin sensitivity

**SNPs related to capsaicin sensitivity

^SNPs not in Hardy-Weinberg equilibrium

bMAFs significantly different from reported in dbSNP

bold font: SNPs cited in exons

(P91S), rs222748 (H317H), rs222747 (M315I), rs224534 (T469I), rs8065080 (I585V), and rs375458057 (P619P).
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).

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 2), and subjects with the TT/TG/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. 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, 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, TRPA1, and TRPM3, 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. In addition, Anoctamin1 (ANO1), a calcium-activated chloride channel, was described as a heat sensitive channel expressed in nociceptive neurons. 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. 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, transmembrane domain, and C-terminal domain.

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 M315I9 were previously suggested to be gain-of-function phenotypes, and I585V was to be comparable to WT9 or loss-of-function phenotypes9,11,25 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 SNPs was examined by comparing the EC50 or maximum functional property of the TRPV1 molecule with each 

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 SNPs was examined by comparing the EC50 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 mRNA29,30 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.

### Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by a grant from the Matsumoto Dental University.

### References

1. Montell C. The TRP superfamily of cation channels. *Sci STKE* 2005; 2005: re3.
2. Numazaki M and Tominaga M. Nociception and TRP channels. *Curr Drug Targets CNS Neurol Disord* 2004; 3: 479–485.
3. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD and Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997; 389: 816–824.
4. Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI and Julius D. The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 1998; 21: 531–543.
5. Wood JN, Winter J, James IF, Rang HP, Yeats J and Bevan S. Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture. *J Neurosci* 1988; 8: 3208–3220.
6. Caterina MJ, Leflter A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Kolzenburg M, Basbaum AI and Julius D. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 2000; 288: 306–313.
7. Saito S and Tominaga M. Evolutionary tuning of TRPA1 and TRPV1 thermal and chemical sensitivity in vertebrates. *Temperature (Austin)* 2017; 4: 141–152.
8. Allen AL, McGearry JE and Hayes JE. Polymorphisms in TRPV1 and TAS2R3 associate with sensations from sampled ethanol. *Alcohol Clin Exp Res* 2014; 38: 2550–2560.
9. Wang S, Joseph J, DIatchenko L, Ro JY and Chung MK. Agonist-dependence of functional properties for common
nonsynonymous variants of human transient receptor potential vanilloid 1. *Pain* 2016; 157: 1515–1524.

10. Tahara T, Shibata T, Nakamura M, Yamashita H, Yoshioha D, Hirata I and Arisawa T. Homozygous TRPV1 315C influences the susceptibility to functional dyspepsia. *J Clin Gastroenterol* 2010; 44: e1–e7.

11. Cantero-Recasens G, Gonzalez JR, Fandos C, Duran-Tauleria E, Smit LA, Kauffmann F, Antó JM and Valverde MA. Loss of function of transient receptor potential vanilloid 1 (TRPV1) genetic variant is associated with lower risk of active childhood asthma. *J Biol Chem* 2010; 285: 27532–27535.

12. Forstennofter M, Forster M, May D, Hofschulte F, Cascorbi I, Wasner G, Gierthmüller J and Baron R. Short Report: TRPV1-polymorphism 1911 A>G alters capsaicin-induced sensory changes in healthy subjects. *PLoS One* 2017; 12: e0183322.

13. van Esch AA, Lamberts MP, Te Morsche RH, van Oijen MG, Jansen JB and Drenth JP. Polymorphisms in gene encoding TRPV1-receptor involved in pain perception are unrelated to chronic pancreatitis. *BMC Gastroenterol* 2009; 9: 97.

14. Xu H, Tian W, Fu Y, Oyama TT, Anderson S and Cohen DM. Functional effects of nonsynonymous polymorphisms in the human TRPV1 gene encoding TRPV1-receptor involved in pain perception are unrelated to chronic pancreatitis. *BMC Gastroenterol* 2009; 9: 97.

15. Tornwall O, Silventoinen K, Kaprio J and Tuorila H. Why do some like it hot? Genetic and environmental contributions to the pleasantness of oral pungency. *Physiol Behav* 2012; 107: 381–389.

16. Kanda Y. Investigation of the freely available easy-to-use software ‘EZR’ for medical statistics. *Bone Marrow Transplant* 2013; 48: 452–458.

17. Gavva NR, Klionsky L, Qu Y, Shi L, Edenson S, Zhang TJ, Viswanadhan VN, Toth A, Pearce LV, Vanderah TW, Porreca F, Blumberg PM, Lile J, Sun Y, Wild K, Louis JC and Treanor JJ. Molecular determinants of vanilloid sensitivity in TRPV1. *J Biol Chem* 2004; 279: 20283–20295.

18. Yao J, Liu B and Qin F. Modular thermal sensors in temperature-gated transient receptor potential (TRP) channels. *Proc Natl Acad Sci U S A* 2011; 108: 11109–11114.

19. Garcia-Sanz N, Valente P, Gomis A, Fernandez-Carvajal A, Fernandez-Ballester G, Viana F, Belmonte C and Ferrer-Montiel A. A role of the transient receptor potential domain of vanilloid receptor 1 in channel gating. *J Neurosci* 2007; 27: 11641–11650.

20. Vlachovič V, Teisinger J, Susánková K, Lyfenko A, Ettrich R and Vyklický L. Functional role of C-terminal cytoplasmic tail of rat vanilloid receptor 1. *J Neurosci* 2003; 23: 1340–1350.

21. Hoffmann T, Kistner K, Miermeister F, Winkelmann R, Wittmann J, Fischer MJ, Weidner C and Reeh PW. TRPA1 and TRPV1 are differentially involved in heat nociception of mice. *Eur J Pain* 2013; 17: 1472–1482.

22. Vrians J, Owsianik G, Hofmann T, Philipp SE, Stab J, Chen X, Benoit M, Xue F, Janssens A, Kerselaers S, Oberwinkler J, Vennekens R, Gudermann T, Nilius B and Voets T. TRPM3 is a nociceptor channel involved in the detection of noxious heat. *Neuron* 2011; 70: 482–494.

23. Vandewauw I, De Clercq K, Muller M, Held K, Pinto S, Van Ranst N, Segal A, Voet T, Vennekens R, Zimmermann K, Vriens J and Voets T. A TRP channel trio mediates acute noxious heat sensing. *Nature* 2018; 555: 662–666.

24. Cho H, Yang YD, Lee J, Lee B, Kim T, Jang Y, Back SK, Na HS, Harfe BD, Wang F, Raouf R, Wood JN and Oh U. The calcium-activated chloride channel anoctamin 1 acts as a heat sensor in nociceptive neurons. *Nat Neurosci* 2012; 15: 1015–1021.

25. Sadofsky LR, Cantero-Recasens G, Wright C, Valverde MA and Morice AH. TRPV1 polymorphisms influence capsaicin cough sensitivity in men. *J Thorac Dis* 2017; 9: 839–840.

26. Premkumar LS and Ahern GP. Induction of vanilloid receptor channel activity by protein kinase C. *Nature* 2000; 408: 985–990.

27. Prescott ED and Julius D. A modular PIP2 binding site as a determinant of capsaicin receptor sensitivity. *Science* 2003; 300: 1284–1288.

28. Kim AY, Tang Z, Liu Q, Patel KN, Maag D, Geng Y and Dong X. Pirt, a phosphoinositide-binding protein, functions as a regulatory subunit of TRPV1. *Cell* 2008; 133: 475–485.

29. Mignone F, Gissi C, Liuni S and Pesole G. Untranslated regions of mRNAs. *Genome Biol* 2002; 3: REVIEWS0004.

30. Pichon X, Wilson LA, Stoneley M, Bastide A, King HA, Somers J and Willis AE. RNA binding protein/RNA element interactions and the control of translation. *Curr Protein Pept Sci* 2012; 13: 294–304.

31. Wang Z and Burge CB. Splicing regulation: from a parts list of regulatory elements to an integrated splicing code. *RNA* 2008; 14: 802–813.

32. Lee Y and Rio DC. Mechanisms and regulation of alternative pre-mRNA splicing. *Annu Rev Biochem* 2015; 84: 291–323.