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Quantitative Analysis of TRP Channel Genes in Mouse Organs

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The transient receptor potential (TRP) channel superfamily is a set of channel genes that mediate numerous physiological functions such as sensing irritants or detecting temperature changes. Despite their functions, expressional information on TRP channels in various organs is largely elusive. Therefore, we conducted a systematic quantitative comparison of each mRNA expression level of 22 mouse TRP channels in various organs. As a result, we found that average levels of TRP channel transcripts were very low reaching ~3% of the GAPDH transcript level. Among 22 TRP channels, TRPC1 and TRPM7 were most abundant in the majority of organs. In contrast, TRPV3, TRPV5, TRPV6, TRPC7, TRPM1, and TRPM5 elicited very low message profiles throughout the major organs. Consistent with their functions as molecular sensors for irritants and temperature changes, TRPV1, TRPM8 and TRPA1 showed exclusive expression in sensory ganglia. TRPC3 and TRPM3 were abundant in the sensory ganglia and brain. High levels of transcripts of TRPV2, TRPC6, TRPM4, and TRPM6 were observed in the lung. In addition, channel transcript levels were very low except TRPM7 in the liver. In summary, the expression profile of TRP channels in major tissues provides insight to their physiological functions and therefore application to new drug development.

Key words: TRPV, TRPC, TRPM, TRPA1, TRP channels

INTRODUCTION

Since firstly characterized as transient receptor potential of *Drosophila* mutant photoreceptor (Hardie and Minke, 1992), transient receptor potential (TRP) channel family has elicited diverse physiological functions ranging from thermosensation to spermatocyte maturation, drawing immense attention for its possible therapeutic application (Bang et al., 2010; Wu et al., 2010). Until now, about 30 different types of TRP homologues throughout the various species were cloned and classified into five major subfamilies based on their structural similarity (Wu et al., 2010). Structurally, analogous to voltage-gated K^+ channels, TRP channels typify with six-transmembrane domain and pore-forming loop between transmembrane domain 5 and 6. Most of the TRP channels are nonselective cation channels permeable to various cations including Ca^{2+}, thus participating in intracellular Ca^{2+} signaling pathways, which mediates various cellular processes: muscle contraction, transmitter release, cell proliferation, gene transcription and cell death (Cho et al., 2010; Guibert et al., 2011; Shapovalov et al., 2011; Jang et al., 2012).

Most notably, TRP channels act as molecular sensors for detecting irritants such as acid, temperature change, osmolarity and mechanical stimuli (Dadon and Minke, 2010; Wu et al., 2010). For instance, TRPV1 is highly expressed in the nociceptive sensory neurons and activated by capsaicin, arachidonic acid metabolites, and noxious heat (higher than 42°C) (Caterina et al., 1997; Shin et al., 2002; Lee et al., 2005). TRPV4 is originally found to be activated by hypotonic stimuli, suggesting that it may be a sensor for osmolarity and osmotic stretch. Later, TRPV4 is known to be activated by warm temperature (25-34°C) as well (Liedtke et al., 2000; Guler et al., 2002). TRPM8 is strongly expressed in sensory neurons of the dorsal-root ganglia (DRG) and trigeminal ganglia (TG) here is activated by tem-

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peratures below 26°C and a cooling agent, menthol (McKemy et al., 2002; Peier et al., 2002). TRPA1 is also activated by cold with a lower threshold as compared to TRPM8 (below 17°C) as well as a variety of chemicals including cinnamalaldehyde, allicin, acrolein, and mustard oil (Bandell et al., 2004; Bautista et al., 2006).

Although physiological roles of many of TRP channels are well characterized in sensory neurons, brain and some other tissues, but functions of TRP channels in other major organs are not identified. Therefore, it is necessary to determine overall expression levels of mRNAs of TRP channels in major tissues. Thus, we sought to determine the expression level of each TRP channel in major organs to predict its physiological functions.

MATERIALS AND METHODS

Mouse tissue extraction and total RNA purification

Nine major tissues (brain, heart, lung, kidney, liver, testis and skeletal muscle, TG, and DRG) were isolated from seven-week-old ICR male mice. Small fragment of each organ was immersed into RNA*later*® solution (Ambion) in order to prevent possible RNA degradation. Total RNA from each organ (~30 mg of each tissue) was purified with easy-BLUE™ (iNtRON Biotech) solution, as recommended by the manufacturer. To eliminate possible genomic DNA contamination, total RNA sample was treated with Turbo-DNaseI (DNA-free™ kit, Ambion).

First-strand cDNA synthesis

To design specific primers for TRP channels, sequences of TRP channel genes were aligned (ClustalW, http://www-ebi.ac.uk/clustalw). Specific primers for each TRP channel were designed for reverse transcription (RT)-PCR using Primer Bank software (Wang and Seed, 2003). cDNAs were synthesized using SuperScript™ III (Invitrogen). Total RNA (2 μg) was used as a template, and random hexamers were added as primer. After subsequent synthesis reaction, final products were diluted twice by adding the same volume of distilled water. cDNAs (1 μL) were used in the subsequent PCR.

Quantitative Real time-polymerase chain reaction (RTQ-PCR)

Selected sets of cDNAs and target primers were used for real time quantitative PCR. The iQTM™ SYBR Green Supermix PCR Reagents were used for real-time amplification detection. Briefly, selected sets of 1 μL of cDNA and 5 pmol forward and reverse primers were mixed with 12.5 μL of 2X SYBR Green Supermix (BIO-RAD) containing 100 mM KCl, 40 mM Tris-HCl at pH 8.4, 0.4 mM dNTP, 50 U/mL iTaq DNA polymerase, 6 mM $MgCl_2$, SYBR Green I, 20 nM fluorescein, and stabilizers. The PCR amplification conditions were as follows: 3 min of initial denaturation at 95°C, followed by 40 cycles of each 30 sec at 95°C, 30 sec at 60°C and 30 sec at 72°C, and then 60 sec at 95°C, 60 sec at 55°C finally, 10 sec at 55°C. The information about primers of each TRP family is listed in Table I, Table II, and Table III.

Table I. RTQ-PCR primers and conditions

Target gene	Location	Primer sequence	T_m (°C)	*E*
GAPDH	493-512	AACTTTGGCATTGTGGAAGG	55	1.76
	715-696	ACACATTGGGGGTAGGAACA	57	
TRPV1	330-349	AAGGCTCTATGATCGCAGGA	57	1.71
	498-479	CAGATTGAGCATGGCTTTGA	55	
TRPV2	66-87	TGCTGAGGTGAACAAAGGAAAG	58	1.72
	212-192	TCAAACCGATTTGGGTCCTGT	57	
TRPV3	163-181	ACGGTCACCAAGACCTCTC	59	n/a
	307-287	GACTGTTGGGATTGGATGGGG	61	
TRPV4	39-102	ATGGCAGATCCTGGTGATGG	59	1.76
	214-193	GGAACTTCATACGCAGGTTTGG	60	
TRPV5	39-103	ATGGGGGCTAAAACTCCTTGG	59	1.71
	209-190	CCTCTTTGCCGGAAGTCACA	59	
TRPV6	26-48	GGGGTTAATACTCTGCCTATGG	59	1.77
	217-196	GCACCTCACATCCTTCAAACTT	58	

PCR primers used for the RTQ-PCR analysis of target TRPV and GAPDH are listed with their location in coding nucleotide sequences. Validated q-PCR conditions including annealing temperature (T_m) and amplification efficiency (*E*) calculated from standard curves are listed.

Table II. RTQ-PCR primers and conditions

Target gene	Location	Primer sequence	T_m (°C)	E
TRPC1	926-945	AGCCTCTTGACAAACGAGGA	57	1.84
	1096-1077	TCTTACAGGTGGGCTTACGG	59	
TRPC2	55-76	CTCAAGGGTATGTTGAAGCAGT	58	1.79
	240-220	AGCCGTCTTCCTGTTTGGTTC	59	
TRPC3	262-280	GCCTTCATGTTCGGTGCTC	59	1.8
	416-398	GCGTTCTGGCCCATGTAGT	59	
TRPC4	1270-1290	GGCGGACTCCAGGATTACATC	61	1.74
	1404-1384	CCATGATTCCCGTGGGTTCAG	61	
TRPC5	71-89	GGGCTGAGACTGAGCTGTC	61	1.76
	317-297	TTGCGGATGGCGTAGAGTAAT	57	
TRPC6	82-103	AGCCAGGACTATTTGCTGATGG	60	1.76
	233-213	AACCTTCTTCCCTTCTCACGA	57	
TRPC7	573-591	GCGGCCCCATGACTACTTC	61	1.8
	703-684	TGGATAGGGACAGGTAGGCG	61	
TRPA1	49-68	GTCCAGGGCGTTGTCTATCG	61	1.79
	211-191	CGTGATGCAGAGGACAGAGAT	59	

PCR primers used for the RTQ-PCR analysis of target TRPC and TRPA1 are listed with their location in coding nucleotide sequences. Validated q-PCR conditions including annealing temperature (T_m) and amplification efficiency (*E*) calculated from standard curves are listed.

Table III. RTQ-PCR primers and conditions

Target gene	Location	Primer sequence	Tm (°C)	E
TRPM1	1021-1040	TACTGCGACGAAGGAGGAGT	59	n/a
	1237-1218	TTCCTTTGAGCAAGGCAGTT	55	
TRPM2	476-494	AGCACTGGGGCCTAGATGT	59	1.75
	679-659	TCAGACTGAAGTCCCGTACCG	61	
TRPM3	1043-1063	AGGACATGGAGCTAACAGCAA	57	1.68
	1182-1163	CACGATGGGTGCGTTGTAGA	59	
TRPM4	65-83	TGGCTGTAAGGGACCACCA	59	1.77
	195-176	GTACCTTGCGGGGAATGAGC	61	
TRPM5	78-98	AGAGATCAACTTCGGAGGGTC	59	n/a
	250-230	GCCACGACTTCATAGCCAAAG	59	
TRPM6	107-128	TCTGCCACAATTTAGTCAGGTG	58	1.72
	265-245	TGGTGCCGAAGGTATCTGTAG	59	
TRPM7	93-115	AGGATGTCAGATTTGTCAGCAAC	58	1.73
	220-199	CCTGGTTAAAGTGTTCACCCAA	58	
TRPM8	279-301	ACATACCAAGGAGTTTCCAACAG	58	1.69
	406-387	GCTGGGTCAGCAGTTCGTAG	61	

PCR primers used for the RTQ-PCR analysis of target TRPM are listed with their location in coding nucleotide sequences. Validated q-PCR conditions including annealing temperature (T_m) and amplification efficiency (*E*) calculated from standard curves are listed.

Data analysis

The standard-curve method was used to measure the quantity of each template. Each TRP template was diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} ng/μL to create a standard series. Using a standard curve generated with each PCR products, the threshold cycle value obtained from each cDNA sample was further processed to calculate the initial quantity of cDNA template per well. To normalize the inter-sample variation in quality that was inherently associated with RNA preparation

from each mouse tissues, GAPDH amount per each sample was quantified to obtain the normalized each TRP's copy number. Finally, the TRPs quantity expressed in normalized ratio was illustrated as bar graph.

RESULTS

The levels of mRNAs of TRP channels in mouse tissues

We firstly performed conventional RT-PCR to determine mRNA transcript levels of TRP channels in the major nine organs such as brain, heart, lung, kidney, liver, testis, skeletal muscle, TG, and DRG. Sets of primers specific for each TRP ion channel were designed in reference to Primer Bank (Wang and Seed, 2003).

As shown in Fig. 1, transcript levels of 22 TRP channels were variable to different organs. Even though the relative amount of mRNA of each TRP channel can be determined in some extent, it is difficult to estimate exact copies of channel genes in tissues with PCR products. Therefore, we then conducted the real-time quantitative (RTQ) PCR analysis to obtain precise amount of mRNA transcripts of TRP channels.

In order to validate the binding efficiency and specificity of the primers, we carried out RTQ-PCR using 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} ng/μL of each TRP template to create standard curves for each TRP channel. The average binding efficiency of each primer obtained from standard curves was 1.75 ± 0.01 (n = 20), indicating good binding of the primers to corresponding TRP genes (Pfaffl, 2001). However, the levels of TRPV3, TRPM1, and TRPM5 transcripts were too low to calculate their binding efficiency. Furthermore, the specificity of PCR amplification of each TRP channel gene was also confirmed by checking single peak at the melting curve as well as discrete single band from agarose gel electrophoresis of final PCR products (data not shown) (Ririe et al., 1997).

Relative abundances of mRNAs of TRP channels

As an internal control, the threshold cycle (C_t) value of a house-keeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was measured for each organ. Thus, the relative abundance of each TRP channel was obtained by comparing its C_t value with that of GAPDH gene. This normalization makes it possible to compare overall abundance of each TRP channel mRNA levels in different organs. The experiments were repeated five times in the same condition.

Fig. 2 shows the relative abundance of all TRP channels in 9 organs. The average C_t values of all TRP channels in 9 organs were 29.9 ± 0.3 cycles (n = 153).

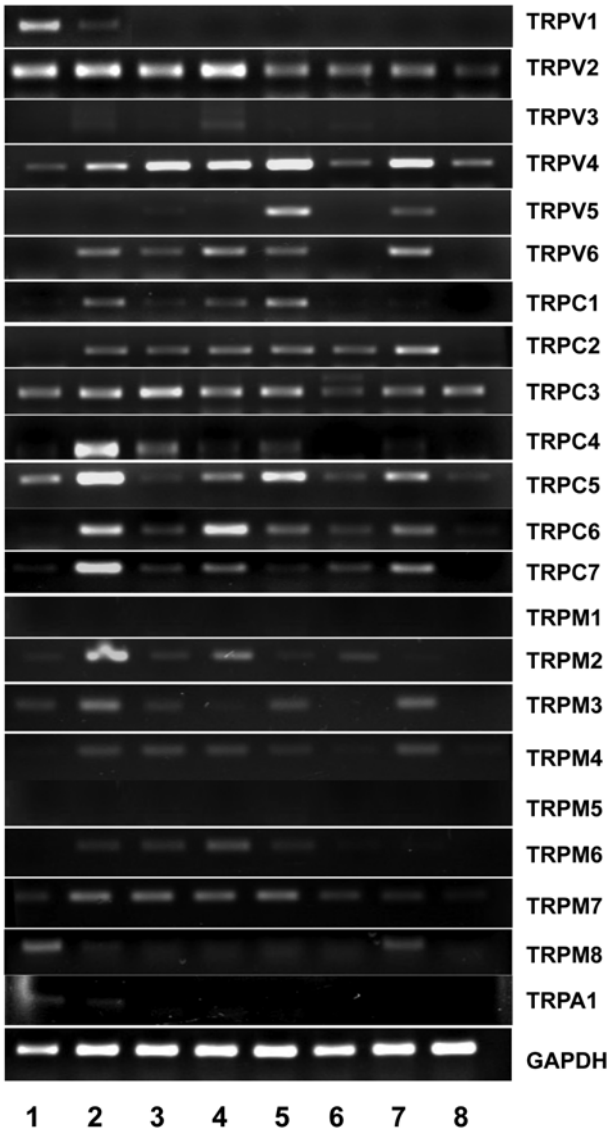

Fig. 1. Expression level of TRP channel mRNAs in eight major tissues using standard RT-PCR. PCR was done with each specific primer pairs in 35 cycles. The PCR products were run on the 1.3% agarose gel. No detectable band means no or very low level of that transcript. 35 cycle PCR products visualized under UV illuminator (1; DRG, 2; brain, 3; heart, 4; lung, 5; kidney, 6; liver, 7; testis, 8; skeletal muscle).

The average C_t values of GAPDH in the 9 organs were 18.2 ± 0.7 cycles (n = 9). The average relative C_t value of all TRP channel genes (C_t of TRP genes/C_t of GAPDH) were 1.67 ± 0.02 (n = 153). Thus, overall mRNA levels of TRP channels were by far much lower than that of GAPDH. The average relative copy number of all TRP channel genes corresponding to this C_t difference (number of TRP genes/number of GAPDH) is 0.032 ± 0.006 (n = 153). Among 22 TRP channels, the most abundant one was TRPC1 (Fig. 2A). Moreover, testis and lung contained riches amount of TRP transcripts

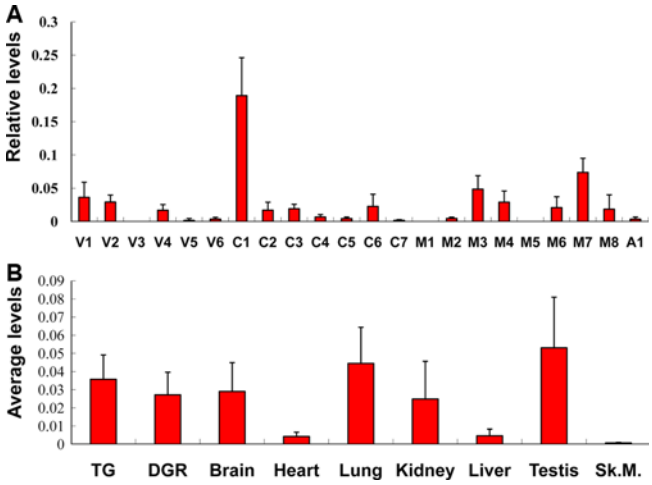

Fig. 2. Relative message abundance of TRP family. The relative abundance of each TRP channel was obtained by comparing its C_t value with that of GAPDH gene. Average levels of each TRP channels in nine mouse tissues (**A**). Total levels of TRP channels in mouse tissues (**B**).

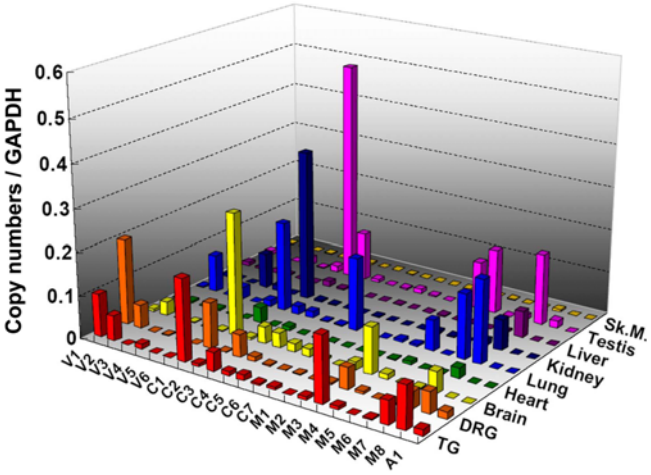

Fig. 3. Relative expression levels of 22 TRP channels in nine organs in the mouse obtained by real-time quantitative PCR (average data from five different experiments). Threshold cycle (C_t) of each TRP channel in each organ that is considered to represent its mRNA level was normalized to that of GAPDH in the organ for relative abundance of the channel message. Blank indicates that PCR products of the specific gene were not detectable in regular RT-PCR and thus the C_t values by RTQ-PCR were not obtained.

whereas skeletal muscle contained the smallest amount of mRNA transcripts (Fig. 2B).

Overall profile of TRP channels in major organs

In order to see the relative expression of all TRP channels in different organs, we plotted the expression levels of 22 TRP channel genes relative to the transcript level of GAPDH at each organ. As shown in Fig. 3, mRNA transcripts of mouse TRPV1 showed significantly greater amount in DRG compared to other organs examined ($p < 0.001$). Transcripts of TRPV1 in the brain, lung, liver and testis were at the level of about 3% of TRPV1 in DRG. Relatively high levels of TRPV2 were observed in the DRG, brain, and lung with the highest levels in the lung ($p < 0.001$). TRPV2 in the brain was approximately detected 5-fold lower than the one in the lung. TRPV4 mRNA was predominantly detected in the kidney and lung compared to other organs. However, TRPV4 transcripts in the lung were 3-fold less than those observed in the kidney. The TRPV4 mRNA in the heart and liver was only about 4% of that found in the kidney. In case of TRPV5 and TRPV6, transcripts levels were low throughout nine organs in comparison to genes of other TRPV subtypes. However, TRPV5 and TRPV6 were found at relative high expression levels in the kidney and in the lung, respectively (Fig. 3).

TRPC channels appeared to be expressed widely throughout organs such as DRG, brain, lung, kidney, and testis. Among these, TRPC1 was the most abundant in all organs. The highest level of TRPC1 was detected in testis, about 2-fold higher than brain, lung, and kidney. TRPC2 mRNA was observed the greatest amounts in the testis. TRPC2 transcripts were also found in the lung reaching about 20% of that observed in the testis. TRPC3 mRNA was mainly detected in the DRG and brain. TRPC4 mRNA was found at considerably lower expression levels in all tissues except in the brain, TG and DRG. TRPC5 also showed relatively low expression levels throughout organs except in the brain. TRPC6 mRNA was predominantly detected in the lung. TRPC7 expression was limited to DRG, brain, and testis.

Relatively small quantity of TRPM2 transcript was expressed in the DRG, brain, lung, and liver with highest amount in the brain. Large amount of TRPM3 mRNA was found mainly in the TG, DRG, brain and testis. TRPM4 mRNA was primarily detected in the lung and testis. As stated earlier, TRPM5 transcripts were rarely found throughout these organs. TRPM6 transcripts were detected in the lung, kidney and testis. TRPM7 is the only subtype among TRPM family which showed broad distribution throughout various organs. TRPM7 was highly expressed in the lung and testis followed by DRG, brain, heart, kidney, liver, and skeletal muscle (20%, 26%, 13%, 37%, 31%, and 3% of the levels observed in the lung). Finally, TRPM8 transcripts were exclusively detected in the DRG, brain and testis. Its mRNA levels in the brain and testis were about 1.5% and 35% of that detected in the DRG (Fig. 3).

Consistent with its role in somatosensation, TRPA1 expression was visibly limited to TG and DRG and

considerably low in other organs (Fig. 3).

DISCUSSION

Of functions known so far, TRPV1, TRPV2, TRPV3, TRPV4, TRPM3, TRPM8, and TRPA1 are thermoTRP channels that are activated by temperature change and therefore are known to mediate temperature sensation (Vay et al., 2012). TRPV1 is the best known channel for its ability of sensing heat over 43°C. TRPV2 is another heat sensor that responds to heat over 52°C (Caterina et al., 1999). However, mice deficient of *TRPV2* failed to show phenotype to heat sensing (Park et al., 2011). TRPV3 and TRPV4 are activated by warm temperature change (Guler et al., 2002; Smith et al., 2002; Xu et al., 2002). Recently, TRPM3 is also known to be activated by heat and thus becomes another heat sensor (Vriens et al., 2011). TRPM8 is activated by cool temperature whereas TRPA1 is activated by cold stimuli despite some controversies (Peier et al., 2002; Kwan et al., 2006). Because of their ability for sensing temperature change, these thermoTRPs are expected to be expressed in DRG neurons. Indeed, most of thermoTRPs were expressed highly in DRG except TRPV3. Sensory ganglia also contained high levels of TRPM7, the functions of which are not well understood except its role as a kinase (Runnels et al., 2001). The brain contained relative high levels of TRPV2, TRPC1, TRPC3-5, TRPM3-4, and TRPM7. Most of channels found in the DRG were also detected in the brain, except TRPV1, TRPM8 and TRPA1. The lung is another organ that expresses high levels of many TRP channel subtypes. Besides TRPC1 and TRPM7, high levels of transcripts of TRPV2, TRPC6, TRPM4, and TRPM6 were observed in the lung. High level of TRPC6 in the lung coincides with a report that TRPC6 expression is increased in the lung and smooth muscle cells of pulmonary artery origin in patients with idiopathic pulmonary hypertension (Yu et al., 2004). Kidney has high levels of TRPC1, TRPV4, TRPV5 and TRPM7 transcripts. Among these, high level of TRPV4 transcripts would suggest its role as a osmosensor because TRPV4 is known to be activated by changes in osmolarity (Liedtke and Friedman, 2003). The liver showed a limited expression of TRP channels because only TRPM7 transcript is found in the liver (Fig. 3). TRPC1, TRPC2, TRPM3, TRPM4, and TRPM7 are expressed highly in the testis. More importantly, TRPC2 transcripts were found only in the testis with no significant expression in other tissues. This is largely consistent with its role in pheromone signaling (Lucas et al., 2003).

TRPC1 once suggested to be a mechano-sensitive channel (Maroto et al., 2005) was expressed highly across various tissues except liver and skeletal muscle. It remains to be seen why TRPC1 is ubiquitous in many organs. TRPC7 was cloned firstly from mouse brain and activated by diacyl glycerol and Ca^{2+} (Okada et al., 1999). TRPC7 is abundant in the heart, lung, and eye and is known to be related to heart failure (Okada et al., 1999). In this study TRPC7 showed moderate expression in DRG and brain. However, we failed to detect the expression in the heart. Consistent with our data, a weak expression of TRPC7 in heart was also reported (Kunert-Keil et al., 2006). Variations among mouse strains would be one reason. TRPM7 is also widely expressed throughout the major organs. TRPM7 is a Ca^{2+} permeable channel with unique function as a kinase (Runnels et al., 2001). Its wide distribution profile may hint its potential roles in Ca^{2+} signaling, control of cell-cycle, and cell motility (Runnels et al., 2001).

Several previous studies made a comparison of TRP channel expression in various tissues. Two reports showed the expression patterns of TRPC or TRPM channels in various tissues in human using RTQ-PCR (Riccio et al., 2002; Fonfria et al., 2006). Staaf and colleagues reported changes of the distribution and expression of TRPM channels throughout embryonic development (Staaf et al., 2010). Sulk and colleagues looked into the expression patterns of TRP channels in chronic inflammatory skin disease (Sulk et al., 2012). In addition, Kunert-Keil and colleagues reported a rather complete analysis of tissue distributions of TRP channels in the three different mouse strains (Kunert-Keil et al., 2006). In the present study, we used tissues isolated from ICR mouse strain. Moreover, we stressed additional information on expressional profiles of TRP channels in trigeminal and dorsal-root ganglia.

Summarizing, we have characterized and compared the mRNA distributions of TRP channels in major organs. Specific expression patterns of TRP channels coincide with their known physiological functions. In addition, the expression pattern of each TRP channel would provide insights on its possible physiological or pathological role in the organ.

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