Ethnicity-specific distribution of TRPM8 gene variants in Eurasian populations: signs of selection

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Abstract. The TRPM8 gene encodes the ion channel, which is a cold receptor in afferent neurons of the mammalian somatosensory system. We studied the frequency of haplotype distribution from six SNPs in the TRPM8 gene in Eurasian human populations, including Russians, Kazakhs and Chukchi. Four of the six SNPs are located in exon 7 (rs13004520, rs28901637, rs11562975, rs17868387), rs7593557 is in exon 11. These exons encode parts of the N-terminus, which is necessary for channel functioning in the plasma membrane of neurons. The rs11563071 is in exon 23 encoding part of the C-terminus. The primary difference in population distribution of haplotypes determines the SNP from exon 11 which leads to Ser419Asn substitution in protein. The most pronounced differences in the patterns of diversity and frequencies of haplotypes were observed between Chukchi and Russians. The frequency of major H1 haplotype encompassing the 419Ser variant differs in examined populations; 0.738 (Russians), 0.507 (Kazakhs) and 0.337 (Chukchi), p < 0.001. The TRPM8 gene variants encoding 419Asn and carrying the minor alleles of rs28901637 (P249P) and rs11562975 (L250L) in exon 7 are characteristic of Asian populations. The frequency of all 419Asn variants in Chukchi is comparable to that in Africans, however, the minor allele frequencies of rs28901637, rs11562975 in Africans is low. Apparently in the process of human colonization of Eurasia, minor alleles of these SNPs diverged depending on rs7593557 structure in exon 11. We analyzed sequences of five TRPM8 mRNA isoforms extracted by researchers from different tissues. Sequence analysis demonstrates that they are transcribed from major H1 variant of the TRPM8 gene but contain different translation start codons, which are generated by alternative splicing from pro-mRNA.

Key words: TRPM8 gene; haplotypes; Eurasian human populations; alternative start codons.

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Этно-специфическое распределение вариантов гена TRPM8 в евразийских популяциях: знаки отбора

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Аннотация. Ген TRPM8 кодирует ионный канал, который является холодовым рецептором в афферентных нейронах сомatosенсорной системы мlekопитающих. Мы изучили распределение частот гаплотипов из шести ОНП гена TRPM8 в евразийских популяциях человека, включая русских, казахов и чукчей. Четыре из шести ОНП расположены в экзоне 7 (rs13004520, rs28901637, rs11562975, rs17868387). ОНП rs7593557 находится в экзоне 11. Эти экзоны кодируют фрагменты N-терминального домена, необходимого для функционирования канала в плазматической мембране афферентных нейронов. ОНП rs11563071 расположен в экзоне 23, кодирующем фрагмент C-терминального домена канала. Особое значение в популяционном распределении гаплотипов определяет ОНП rs11563071 в экзоне 11, обусловливающий Ser419Asn замещение в белке. Конtrasные различия в многообразии и частотах гаплотипов наблюдали между популяциями чукчей и русских. Частоты основного гаплотипа H1, относящегося к 419Ser вариантом гена TRPM8, существенно различались в изученных популяциях: 0.738 у русских, 0.507 у казахов, 0.337 у чукчей (р < 0.001). Для азиатских популяций характерны варианты гена TRPM8, кодирующие 419Asn и содержащие миорные аллели ОНП rs28901637 (P249P) и rs11562975 (L250L) в экзоне 7. Суммарная частота таких гаплотипов у русских составляет 0.032, по сравнению с 0.142 у казахов и 0.358 у чукчей (р < 10^{-3} для обоих сравнений). Частота всех 419Asn вариантов у чукчей сопоставима с таковой у африканцев, однако частота миорных аллелей rs28901637 и rs11562975 у африканцев низкая. По-видимому, в процессе колонизации человеком Евразии миорные аллели этих ОНП дивергировали в зависимости от структуры rs7593557 в экзоне 11. Нами проанализированы
Introduction
The gene TRPM8 encodes a subunit of Ca\textsuperscript{2+}-permeable non-selective cation channel, belonging to the TRPM (transient receptor potential melastatin) subfamily of TRP domain-containing proteins (Tsavaler et al., 2001). TRP channels are formed by oligomerization of subunits sharing common structural features, including six putative transmembrane segments (S1–S6), a pore loop linking segments S5 and S6, and cytoplasmic N- and C-terminal (Ramsey et al., 2006). The majority of TRP proteins carry a conserved TRP box ('VWKFQR' in TRPM channels) in the C-terminal domain, adjacent to the S6 segment. Many of these proteins, including TRPM8, are involved in Ca\textsuperscript{2+} homeostasis in response to extracellular and intracellular physical and chemical factors. TRPM8 expression has been observed in somatic afferent neurons, myocytes, epithelial cells of the lung, bronchi, prostate, bladder and others (Sabnis et al., 2008; Babes et al., 2011). The modulation of TRPM8 protein activity is coupled with basic biochemical and physiological processes related to thermal sensitivity, proliferation, and apoptosis (Zhang, Barritt, 2006; Yee, 2015).

Three types of TRPM8 polypeptides potentially able to form Ca\textsuperscript{2+} channels by tetramerization have been described. The full-length variant of 1104 amino acids (aa) identified in sensory neurons contain the long N-terminal sequence (693 aa), six transmembrane segments and C-terminal domain with TRP box (Latorre et al., 2011). This channel is able to respond to changes in ambient temperature (threshold of ~22–34 °C). Another TRPM8 isoform of 1054 aa, with a rearranged N-terminal domain, was identified in prostate tumor cells (Lis et al., 2005). In addition, a truncated variant (304 aa), lacking the entire N-terminal sequence and the first two transmembrane segments (S1 and S2), but retaining parts of the voltage-dependent sensory module (S3 and S4), the pore-forming components (S5 and S6 and the loop between them), and the C-terminal domain, was identified in human epithelial cells of the bronchi and lung (Sabnis et al., 2008). Full-length TRPM8 is located to the plasma membrane, while the truncated variant is located in the endoplasmic reticulum membrane and is associated with the release of Ca\textsuperscript{2+} ions from intracellular stores (Bidaux et al., 2007).

Studies clarifying the contribution of the N-terminal domain to the TRPM8 channel function have shown that the first 40 amino acids of full-length TRPM8 modulate sensitivity of the protein to cold and menthol, and the region between residues 40 and 60 is involved in trafficking of the protein to the plasma membrane (Phelps, Gaudet, 2007; Bidaux et al., 2012; Pertusa et al., 2014). It is also an essential element in ensuring the proper folding and assembly of TRPM8.

In this study, we evaluated the distribution of the alleles at five SNPs located in TRPM8 gene exons 7 and 11 in geographically dispersed Eurasian human populations in order to clear up the potential importance of the N-domain parts encoded by these modificable exons. The composition and localization of the SNPs assayed in the TRPM8 gene are unique. Four of six SNPs are densely clustered in exon 7; three in successive codons (encoding P249P, L250L, and Y251C), and the fourth in the codon but one upstream (encoding R247T). The SNP (S419N) in exon 11 and two SNPs (R247T and Y251C) from exon 7 can potentially influence on function of the TRPM8 channel by altering the protein structure and accessibility of these sites to post-translational modification. The two SNPs (P249P and L250L) in exon 7 and the SNP (encoding V1058S in the C-terminal domain) in exon 23 cannot directly influence on protein structure. However, recurrent emergence of the minor alleles of these SNPs in different TRPM8 gene variants suggests that they may have influence on gene expression regulation.

Materials and methods
Human populations that have resided in geographically dispersed territories in Eurasia were examined, including Russians (N = 170, Novosibirsk), Kazakhs (N = 119, Kosh-Agach district, the Altai Republic) and Tundra Chukchi (N = 80, Kanchalan settlement, Chukotka Autonomous district). Ethnicity of individuals was determined by special questioning with elucidation of a nationality of the ancestors (at least in three generations). Blood samples were collected from unrelated representatives of the ethnic group. All subjects gave their informed consent for participation in the experiment.

The work was approved by the Bioethical Committee of the Institute of Cytology and Genetics of Siberian Branch of the Russian Academy of Sciences.

The general characteristics of the assayed SNPs are listed in Table 1. The information about variants and reference sequences (NM_024080.4 and BC143819.1) were extracted from dbSNP (https://www.ncbi.nlm.nih.gov/snp/) and GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Genomic DNA from blood samples was isolated by phenol-chloroform extraction. DNA was genotyped by polymerase chain reaction. The details of the assay (primers and PCR parameters) developed for genotyping were described previously (Potapova et al., 2014). Primers for rs17868387 fragment

| Table 1. TRPM8 single nucleotide polymorphisms included in this study |
|-------------------|-----|----------------|----------------|
| SNP               | Exon| Nucleotide substitution | Amino acid position in protein |
| rs13004520        | 7   | G>C | R247T |
| rs28901637        | 7   | A>T | P249P |
| rs11562975        | 7   | G>C | L250L |
| rs17868387        | 7   | A>G | Y251C |
| rs7593557         | 11  | G>A | S419N |
| rs11563071        | 23  | C>G | V1058S |

Note. Amino acid positions are given to the full-length protein from neurons.
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Table 2. TRPM8 haplotypes in Russian, Kazakh, and Chukchi populations

| Haplotype designation | Haplotype structure | Haplotype frequencies in Russians (N = 340) | Haplotype frequencies in Kazakhs (N = 238) | Haplotype frequencies in Chukchi (N = 160) |
|-----------------------|---------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|
| H1                    | GAGAGC              | 0.738 ± 0.024                           | 0.508 ± 0.032                           | 0.337 ± 0.037                           |
| H2                    | GTGAGC              | 0.006 ± 0.004                           | 0.038 ± 0.012                           | 0.025 ± 0.012                           |
| H3                    | GACAGC              | 0.085 ± 0.015                           | 0.097 ± 0.019                           | 0.056 ± 0.018                           |
| H4                    | GAGGCC              | 0                                        | 0.004 ± 0.004                           | 0                                        |
| H5                    | GAGAGG              | 0.062 ± 0.013                           | 0.021 ± 0.009                           | 0.006 ± 0.006                           |
| H6                    | GTCAAG              | 0                                        | 0.004 ± 0.004                           | 0.012 ± 0.009                           |
| H7                    | GTCAAG              | 0                                        | 0.008 ± 0.006                           | 0                                        |
| H8                    | GACAGG              | 0.018 ± 0.007                           | 0.050 ± 0.014                           | 0.031 ± 0.014                           |
| H9                    | GTCAAG              | 0                                        | 0.013 ± 0.007                           | 0                                        |
| H10                   | GAGACC              | 0.018 ± 0.007                           | 0.064 ± 0.016                           | 0.119 ± 0.026                           |
| H11                   | GTGAC               | 0.011 ± 0.006                           | 0.050 ± 0.014                           | 0.113 ± 0.025                           |
| H12                   | GAGACC              | 0.003 ± 0.003                           | 0.021 ± 0.009                           | 0.063 ± 0.019                           |
| H13                   | GTCAAC              | 0.006 ± 0.004                           | 0.008 ± 0.006                           | 0.062 ± 0.019                           |
| H14                   | GACAGC              | 0.003 ± 0.003                           | 0.004 ± 0.004                           | 0.025 ± 0.012                           |
| H15                   | GTGAAG              | 0                                        | 0.030 ± 0.011                           | 0.050 ± 0.017                           |
| H16                   | TCAAG               | 0                                        | 0.017 ± 0.008                           | 0.044 ± 0.016                           |
| H17                   | GAGAGA              | 0                                        | 0.004 ± 0.004                           | 0.025 ± 0.012                           |
| H18                   | GAGACG              | 0                                        | 0.004 ± 0.004                           | 0                                        |
| H19                   | CAGAGC              | 0.026 ± 0.009                           | 0.043 ± 0.013                           | 0.019 ± 0.011                           |
| H20                   | CAGAGC              | 0.006 ± 0.004                           | 0.008 ± 0.006                           | 0                                        |
| H21                   | CGTAGG              | 0.003 ± 0.003                           | 0.004 ± 0.004                           | 0                                        |
| H22                   | CAGAGG              | 0.015 ± 0.006                           | 0                                        | 0.012 ± 0.009                           |

Notes: A: number of chromosomes.
Comparison among the populations revealed considerable differences in the frequencies of the H1 haplotype, with 0.738 in Russians versus 0.508 and 0.337 in Kazakhs and Chukchi, respectively (Table 3). The 419Ser H2–H5 singleton variants, except H3, have its own specific distribution in the examined populations. The H2 haplotype is present at very low frequency in the Russian population. The frequency of the haplotype H5 is higher in Russians in comparison with Asian populations, while haplotype H4 is absent in the Russian and Chukchi populations and present at a very low frequency in Kazakhs (0.004). The total frequency of the H2–H5 singleton variants is lower in Chukchi in comparison with Russian and Kazakh populations. The total frequency of haplotypes containing the rs28901637 and rs11562975 SNPs (7 exon) in the examined populations was detected. The exception is the Japanese population, in which the MAF at this SNP comprises 0.034, compared with 0.183–0.194 in Africans and Chukchi, and 0.100–0.141 in Russians and others Europeans. Consequently, it may be supposed that both of the Ser and Asn TRPM8 gene variants were present in the ancestral population of anatomically modern humans emerging from Africa.

### Discussion

According to the current paradigm, anatomically modern humans have spread out of Africa over 60,000–80,000 years ago, following different routes to colonize Europe and Asia (Stoneking, Delfin, 2010; Stewart, Stringer, 2012). Respectively, to assist in interpretation of our results, we considered additional data describing the MAFs distribution of the six SNPs within the TRPM8 gene included in this study in African, European, Chinese, and Japanese populations, extracted from the dbSNP (Table 4).

This analysis produced the following unambiguous inferences: (1) haplotypes comprising the rs7593557 A allele (exon 11) are widely distributed among Africans and Eastern Asians (Chinese, Japanese, and Chukchi), in comparison with Russians and Europeans. (2) There is absent the rs11562975 minor C allele (exon 7) in the African population and it’s present at frequencies of 0.170–0.300 in Asian haplotypes. A similar situation is observed for the rs28901637 T allele (exon 7), which occurs at a relatively low frequency in African and European populations and at higher frequencies (0.142–0.306) in Asians. (3) No pronounced continental or ethnic specificity in the distribution of the haplotypes containing the rs11563071 minor G allele (exon 23) in the examined populations was detected. The exception is the Japanese population, in which the MAF at this SNP comprises 0.034, compared with 0.183–0.194 in Africans and Chukchi, and 0.100–0.141 in Russians and others Europeans. Consequently, it may be supposed that both of the Ser and Asn TRPM8 gene variants were present in the ancestral population of anatomically modern humans emerging from Africa.

### Table 3. Frequencies of TRPM8 haplotype groups in Russian, Kazakh and Chukchi populations and significance of the inter-population differences

| Haplotype group | Frequency | P<sub>r</sub> | p<sub>c</sub> | Russians<sup>1</sup> | Kazakhs<sup>2</sup> | Chukchi<sup>3</sup> | 1–2 | 1–3 | 2–3 |
|-----------------|-----------|-------------|-------------|---------------------|------------------|-------------------|-----|-----|-----|
| H1              | 0.738     |             |             | 0.508               | 0.337            |                   | 0.000 | 0.000 | 0.001 |
| H2–H5           | 0.153     | 0.160       | 0.087       | n                   | 0.044            | 0.036             |
| H6–H9           | 0.018     | 0.075       | 0.043       | n                   | 0.001            | n                 |
| H10–H17         | 0.041     | 0.198       | 0.501       | 0.000               | 0.000            | 0.000             |
| H18–H22         | 0.050     | 0.059       | 0.031       | n                   | n                | n                 |
| H11–H16, H20, H21 | 0.032 | 0.142       | 0.357       | 0.000               | 0.000            | 0.000             |

**Note:** n – differences are unreliable.
Table 4. Minor allele frequencies of TRPM8 gene SNPs in African and Eurasian populations

| Population | rs13004520 | rs28901637 | rs11562975 | rs17868387 | rs7593557 | rs11563071 |
|------------|------------|------------|------------|------------|------------|------------|
|            | C (R247T)  | T (P249P)  | C (L250L)  | G (Y251C)  | A (S419N)  | G (V1058V) |
| Russians   | 0.050 ± 0.008 | 0.026 ± 0.006 | 0.121 ± 0.012 | 0.050 ± 0.008 | 0.091 ± 0.011 | 0.100 ± 0.011 |
| Europeans  | 0.058 ± 0.015 | 0.008 ± 0.006 | 0.092 ± 0.019 | 0.058 ± 0.015 | 0.067 ± 0.016 | 0.141 ± 0.022 |
| Kazakhs    | 0.055 ± 0.010 | 0.172 ± 0.017 | 0.223 ± 0.019 | 0.063 ± 0.011 | 0.256 ± 0.020 | 0.151 ± 0.016 |
| Chukchi    | 0.031 ± 0.010 | 0.306 ± 0.026 | 0.294 ± 0.025 | 0.031 ± 0.010 | 0.531 ± 0.028 | 0.194 ± 0.022 |
| Chinese    | 0.100 ± 0.022 | 0.142 ± 0.022 | 0.300 ± 0.034 | 0.093 ± 0.022 | 0.456 ± 0.034 | 0.122 ± 0.024 |
| Japanese   | 0.090 ± 0.021 | 0.142 ± 0.022 | 0.170 ± 0.028 | 0.088 ± 0.015 | 0.412 ± 0.027 | 0.034 ± 0.014 |
| Africans   | 0.008 ± 0.006 | 0.050 ± 0.014 | 0           | 0.009 ± 0.006 | 0.650 ± 0.031 | 0.183 ± 0.025 |

Notes: a The MAFs in Europeans (CEU), Chinese (CHB), Japanese (YPT), and Africans (YRI) are extracted from dbSNP. b MAF – minor allele frequency.

Table 4 shows that 419Ser gene variants have spread in Eurasia compared with African population. The H1 haplotype is the most frequent among study populations. In Russians the majority haplotypes is H1 (~3/4). The total frequency of 419Ser haplotypes, together with H1, comprises 0.909. The European population also has high frequency 419Ser gene variants (0.933). It is likely that their ancestral population experienced a bottleneck after the Asian-European split. The lower haplotype diversity among Europeans is not only the result of purifying selection from the presumed African Asn haplotype variants, but is also due to the limited distribution of haplotypes containing the minor alleles of the rs28901637 and rs11562975 SNPs in exon 7.

The majority of Asian Asn variants contain minor alleles of the rs28901637 and rs11562975 SNPs. Specific patterns of these haplotypes are observed among Asian populations. The Asn haplotypes (H10–H17) are more frequent in Chukchi (0.501) compared with Russian (0.041) population (see Table 3). Apparently, the relatively large population differentiation, with an Fst value of 0.1420 (p < 10^-5) between the Russians (Europeans) and Chukchi (East Asians), emerged under the influence of the two factors: (1) the effect of purifying selection, with persistent fixation of the Ser H1 haplotype, among Russian and Kazakh ancestors in the West of Central Asia and (2) the displacement of the Asn gene variants outside of Africa by the novel Asn derivatives containing the minor alleles of the rs28901637 and rs11562975 SNPs (exon 7) among the ancestors of East Asians.

Interestingly, in all examined populations, haplotypes belonging to the H18–H22 subgroup occurred with similar frequencies (see Table 3). These gene variants include the minor alleles of three SNPs (positions 1, 4, and 5), which lead to amino acid replacements in protein. The strong LD minor alleles of these SNPs. These SNPs (exon 7) and rs7593557 A allele (exon 11), detectable in all three populations, confirms the nonrandom character of their coevolution. Individuals carrying these haplotypes may be resistant to a specific non-coding RNA (ncRNA) region, comprising a potential 3′-UTR, and potential coding sequence with the alternative AUG codon in a segment of exon 3. In consequence the TRPM8-b mRNA isoform may translates the polypeptide with truncated N-terminal domain without the peptide sequences encoded by exons 2 and 3.

The truncated ERTRPM8 mRNA isoform discovered in the bronchial epithelial cells (Sabnis et al., 2008) most likely encodes the minimum structural component required for a protein function (see the Figure, c). Exons 18 and 19 located at the 5′-end of this mRNA, encode the S3 and S4 transmembrane segments, which are necessary to open the pore and activate the channel (Latorre et al., 2011; Kühn et al., 2013). It is clear, the potential-dependent sensory module of this protein (transmembrane segments S1–S4) is represented the only two...
transmembrane segments, S3 and S4, while the remaining S1 and S2, encoded by exons 16 and 17, are absent.

The absence of these exons is not unique, the another mRNA isoform (BC143819.1) with this feature has been identified (see the Figure, d, e). This mRNA after exon 8 contains the 257 nt insert containing a putative alternative start codon (AUG) and then through exons 9–25, without exons 16 and 17, up to UGA codon. Thus, the BC143819.1 mRNA isoform contain non-protein-coding spliced exons from the opposite parts of the translated transcript and have different AUG start codons: one in exon 2 and the other in the 257 nt insert.

NcRNA – non-coding mRNA; nt – nucleotide.

**Structural features of TRPM8 mRNA isoforms.**

a – the reference sequence NM_024080.1 comprises 26 exons and encodes the full-length protein (1104 aa) from sensory neurons translated from the AUG codon in exon 2. The location of the transmembrane segments (S1–S6) and the studied SNPs in the mRNA are shown; b – the TRPM8-b mRNA isoform with a rearranged 5′-terminal region contain the 15 nt fragment of intron 2 (2i), exon 3, and the 46 nt sequence from intron 3 end (3i). The AUG codon is located in the 3i insert. The rearranged RNA region without AUG codon is looked as a potential 5′-UTR. The TRPM8-b mRNA region, encoded by exons 4–25, corresponds to the neuronal mRNA structure; c – the truncated TRPM8 mRNA isoform includes the second half of the exon 18, containing an AUG codon and the remaining part, up to exon 25, identical with neuronal mRNA isoform; d – this mRNA translates the 325 aa protein from the AUG codon in exon 2 through exons 2–8 including the 33 nts in-frame initial region of the 257 nt insert, followed by a stop codon (UGA); e – this mRNA also translates the 682 aa protein from the terminal hexanucleotide in the 257 nt insert containing a putative alternative start codon (AUG) and glutamine codon (CAG), and then through exons 9–25, without exons 16 and 17, up to UGA codon. Thus, the BC143819.1 mRNA isoform contain non-protein-coding spliced exons from the opposite parts of the translated transcript and have different AUG start codons: one in exon 2 and the other in the 257 nt insert.
forms by others have been described in previous reports (Bidaux et al., 2007, 2012). Thus, the TRPM8 mRNAs produced by alternative splicing not only expand the protein diversity, but may also increase the range of post-translational regulation mechanisms.

The Figure illustrates a variety of TRPM8 mRNA isoforms expressed only from the H1 gene variant; however, TRPM8 mRNA isoform diversity may be far greater, given the functional coupling of the molecular machinery involved in transcription and alternative splicing (Montes et al., 2012; Kelemen et al., 2013). The results of this analysis indicate that mRNA isoforms generated by alternative splicing allow the potential synthesis of various proteins differing in the lengths of their N-terminal domains. Splicing may also generate alternative translation initiation zones, in addition to alternative mechanisms of post-translational regulation of TRPM8 activity.

From the data in Tables 3 and 4, it follows that 419Asn variants of the TRPM8 gene are more common in Asian populations compared with Russians. Their compositions are heterogeneous, probably, due to the recurrent emergence of the minor alleles of polymorphisms rs28901637 and rs11562975 (exon 7) in different 419Asn variants. It is possible that the minor alleles of these SNPs from exon 7 may influence on the features of alternative splicing of the 419Asn TRPM8 pre-mRNA and, as a consequence, the composition of mRNA isoforms in Asians. These results demonstrate the need for research of TRPM8 expression in individuals with different haplotype variants, to obtain direct confirmation of the forces underlying their selection.

Conclusion

In summary, it appears that the prevalent fixation of the 419Asn TRPM8 gene variants carrying minor alleles SNPs in codons 249P and 250L (exon 7) in Asians is a Eurasian acquisition, which is presumably more characteristic for Eastern than Western Asians. The surrounding conditions probably favored this selection.

References

Babes A., Ciobanu A.C., Neacsu C., Babes R.-M. TRPM8, sensor for mild cooling in mammalian sensory nerve endings. Curr. Pharm. Biotechnol. 2011;12:78-88.

Bidaux G., Beck B., Zholos A., Gordinenko D., Lemmoner L., Flourakis M., Roudbaraki M., Borowiec A.-S., Fernandez J., Delcourt P., Lepage G., Shuba Y., Skryma R., Prevarskaia N. Regulation of activity of transient receptor potential melastatin 8 (TRPM8) channel by its short isoforms. J. Biol. Chem. 2012;288(5):2948-2962. DOI 10.1074/jbc.M112.072026.

Bidaux G., Flourakis M., Thebault S., Zholos A., Beck B., Gkika D., Roudbaraki M., Bonnai J.-L., Mauroy B., Shuba Y., Skryma R., Prevarskaia N. Prostate cell differentiation status determines transient receptor potential melastatin member 8 channel subcellular localization and function. J. Clin. Invest. 2007;117:1647-1657. DOI 10.1172/JCI30168.

Kelemen O., Convertini P., Zhang Z., Wen Y., Shen M., Falaleeva M., Stamms S. Function of alternative splicing. Gene. 2013;514:1-30. DOI 10.1016/j.gene.2012.07.083.

Kühn F.J.P., Winking M., Kühn C., Hoffman D.C., Lückhoff A. Surface expression and channel function of TRPM8 are cooperatively by transmembrane segments S3 and S4. Eur. J. Physiol. 2013;465:1599-1610. DOI 10.1007/s00424-013-1302-4.

Latorre R., Brauchi S., Madrid R., Oro P. A cool channel in cold transduction. Physiology. 2011;26:273-285. DOI 10.1152/physiol.00004.2011.

Lis A., Wissenbach U., Philipp S.E. Transcriptional regulation and processing increase the functional variability of TRPM channels. Naunyn-Schmiedebergs Arch. Pharmacol. 2005;371:315-324. DOI 10.1007/s00210-005-1050-x.

Mahieu F., Owssianik G., Verbert L., Janssens A., Smedt Y.D., Nilius B., Voets T. TRPM8-independent menthol-induced Ca2+ release from endoplasmic reticulum and Golgi. J. Biol. Chem. 2007;282(5):3325-3336. DOI 10.1074/jbc.M605213200.

Montes M., Becerra S., Sánchez-Alvarens M., Suñët C. Functional coupling of transcription and splicing. Gene. 2012;501:104-117. DOI 10.1016/j.gene.2012.04.006.

Pedretti A., Marconi C., Bettinelli I., Vistoli G. Comparative modeling of the quaternar structure for the human TRPM8 channel and analysis of its binding features. Biochim. Biophys. Acta. 2009;1788:973-982. DOI 10.1016/j.bbagen.2009.02.007.

Pertusa M., Gonzales A., Hardy P., Madrid R., Viana F. Bidirectional modulation of thermal and chemical sensitivity of TRPM8 channel by the initial region of the N-terminal domain. J. Biol. Chem. 2014;289:21828-21843. DOI 10.1074/jbc.M114.565994.

Phelps C.B., Gaudet R. The role of the N terminus and transmembrane domain of TRPM8 in channel localization and tetramerization. J. Biol. Chem. 2007;282(50):36474-36480. DOI 10.1074/jbc.M707205200.

Potapova T.A., Babenko V.N., Kobsev V.F., Romashchenko A.G., Maksimov V.N., Voevod M.I. Associations of cold receptor TRPM8 gene nucleotide polymorphisms with blood lipids and anthropometric parameters in Russian population. Bul. Exp. Biol. Med. 2014;157(6):757-761. DOI 10.1007/s10517-014-2660-4.

Ramsey S., Delling M., Clapham D.E. An introduction to TRP channels. Annu. Rev. Physiol. 2006;68:619-647. DOI 101016/j.crcc.2007.04.004.

Sabnis A.S., Shadd M., Yost G.S., Reilly C.A. Human lung epithelial cells express a functional cold-sensing TRPM8 variant. Am. J. Respir. Cell Mol. Biol. 2008;39:466-474. DOI 10.1165/rcmb.2007-0440OC.

Stewart J.R., Stringer C.B. Human evolution out of Africa: role of refugia and climate change. Science. 2012;335:1317-1321. DOI 10.1126/science.1215627.

Stoneking M., Delfin F. The human genetic history of East Asia: weaving a complex tapestry. Curr. Biol. 2010,20(R188-R193. DOI 10.1016/j.cub.2009.11.052.

Tsvaler L., Shapero M.H., Morkowski S., Laus R. Trp-p8, a novel potential calcium channel proteins. Cancer Res. 2001;61:3760-3769. DOI 10.1126/science.1215627.

Yee N.S. Role of TRPM8 ion channels in cancer: proliferation, survival and invasion. Cancers. 2015;7:2134-2146. DOI 10.3390/cancers7090882.