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RNA-Seq investigations of human post-mortem trigeminal ganglia

Danielle M LaPaglia^{#1}, Matthew R Sapio^{#1}, Peter D Burbelo², Jean Thierry-Mieg³, Danielle Thierry-Mieg³, Stephen J Raithel¹, Christopher E Ramsden^{4,5}, Michael J Iadarola¹, and Andrew J Mannes¹

¹Department of Perioperative Medicine, Clinical Center, National Institutes of Health, Bethesda, MD, USA

²Dental Clinical Research Core, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA

³National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD, USA

⁴Lipid Mediators, Inflammation, and Pain Unit, Laboratory of Clinical Investigation, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

⁵Intramural Program of the National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA

These authors contributed equally to this work.

Abstract

Background: The trigeminal ganglion contains neurons that relay sensations of pain, touch, pressure, and many other somatosensory modalities to the central nervous system. The ganglion is also a reservoir for latent herpes virus 1 infection. To gain a better understanding of molecular factors contributing to migraine and headache, transcriptome analyses were performed on postmortem human trigeminal ganglia.

Methods: RNA-Seq measurements of gene expression were conducted on small sub-regions of 16 human trigeminal ganglia. The samples were also characterized for transcripts derived from viral and microbial genomes. Herpes simplex virus 1 (HSV-1) antibodies in blood were measured using the luciferase immunoprecipitation assay.

Results: Observed molecular heterogeneity could be explained by sampling of anatomically distinct sub-regions of the excised ganglia consistent with neurally-enriched and non-neural, i.e. Schwann cell, enriched subregions. The levels of HSV-1 transcripts detected in trigeminal ganglia correlated with blood levels of HSV-1 antibodies. Multiple migraine susceptibility genes were strongly expressed in neurally-enriched trigeminal samples, while others were expressed in blood vessels.

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Corresponding author: Michael J Iadarola, Department of Perioperative Medicine Clinical Center Building 10, Room 2C40I, 10 Center Drive, MSC 1510 National Institutes of Health Bethesda, MD 20892-1510, USA. miadarol@cc.nih.gov.

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Conclusions: These data provide a comprehensive human trigeminal transcriptome and a framework for evaluation of inhomogeneous post-mortem tissues through extensive quality control and refined downstream analyses for RNA-Seq methodologies. Expression profiling of migraine susceptibility genes identified by genetic association appears to emphasize the blood vessel component of the trigeminovascular system. Other genes displayed enriched expression in the trigeminal compared to dorsal root ganglion, and in-depth transcriptomic analysis of the *KCNK18* gene underlying familial migraine shows selective neural expression within two specific populations of ganglionic neurons. These data suggest that expression profiling of migraine-associated genes can extend and amplify the underlying neurobiological insights obtained from genetic association studies.

Keywords

RNA-Seq; transcriptome; trigeminal ganglion; herpes simplex virus; post-mortem; migraine

Introduction

Molecular analyses of nervous system and other tissues known to be involved in chronic headache are not readily attainable in the living subject. A variety of physiological methods such as brain imaging (1–3) and electroencephalography (4,5) can be employed to examine the human disorder or animal models, but human biochemical endpoints are usually confined to blood or post-mortem tissue. One of the main tissues hypothesized to be relevant to migraine and headache is the trigeminal ganglion; however, this tissue is not accessible to either biochemical or molecular analyses in the living subject. While some therapeutic manipulations such as dietary modification (6–9) have been successful in treating headache in humans, the molecular impact of these manipulations may be most apparent in tissues that can only be collected post-mortem. Such analyses can present problems in terms of coordination between researchers and the medical examiner, consistency of dissection techniques, standardization of post-mortem interval (PMI), and other quality control issues (10–12). In the present paper, we identify transcriptomic signatures of the trigeminal ganglia that may be useful for further genetic and pharmacological investigations. In human post-mortem tissue, we determine the molecular composition of trigeminal subregions and use the corresponding gene expression profiles to identify two different transcriptional profiles characterized by the presence or absence of neurons.

This report quantifies the transcriptome of 16 human trigeminal ganglia using an expanded target sequence that includes viral and microbial genomes to identify non-mammalian reads. The majority of non-human reads were contributed by bacteria, most likely due to contamination during autopsy procedures. The major virus detected was Herpes Simplex Virus 1 (HSV-1), which was indicative of infection in subjects in which this virus was detected, and may have important implications for ganglionic pathology. Previous RNA-Seq in rat trigeminal and DRG clearly shows abundant transcriptional signatures from Schwann cells within sensory ganglia (13), and we present human transcriptomes for trigeminal samples enriched or depleted for neurons. In parallel, the level and expression pattern of genetically identified migraine susceptibility genes was examined in the trigeminal subregions and compared to the Genotype-Tissue Expression (GTEx) database. Three

categories of expression emerged: trigeminal, vascular, and brain. Our analysis of multiple genes indicated that ~45% were related to vascular components, with many exhibiting enriched expression in blood vessels, supporting the idea of trigeminovascular involvement in migraine. We extensively explored one migraine susceptibility gene, *KCNK18*. This gene encodes a neuronal potassium channel highly enriched in trigeminal ganglia relative to both brain and DRG. Rare, dominant negative mutations in this gene resulting in loss of channel conductance have been proposed to cause familial migraine (14,15). Using multiple datasets, we determine that several populations of primary afferent neurons express the gene encoding this channel, including populations of neurons that also express the precursor gene for the proinflammatory neuropeptide calcitonin gene-related peptide (CGRP), which is involved in onset of migraine, and is itself a target for anti-migraine therapies (16–18).

The present datasets highlight the strengths of RNA-Seq for comprehensive investigation of multiple interacting elements that can impact the interpretation and use of results obtained from post-mortem tissue for headache and migraine research, as well as other pathological orofacial pain conditions such as trigeminal neuralgia or temporomandibular joint disorder, and extend observations from genetic association studies.

Materials and methods

Tissue collection and preparation

The initial patient population contained 22 organ donors. Trigeminal ganglia were obtained at autopsy and provided by the Human Brain Collection Core at the National Institute of Mental Health, National Institutes of Health Bethesda (NIH). The autopsy proceeded as follows: The internal organs were removed first, then the brain, and lastly the trigeminal ganglia bilaterally. Whole blood was obtained from the heart. Tissues were stored at -80°C until processing. To disrupt the tissue, frozen ganglia were wrapped in aluminum foil and mechanically broken apart on dry ice using a metal anvil and hammer, both also frozen on dry ice. Small fragments of tissue (20–25 mg each) from a trigeminal ganglion from each subject were used for RNA extraction.

RNA extraction

Frozen tissue was homogenized in 1 mL of Qiazol (Qiagen) in Lysing Matrix D (MP Biomedicals, Santa Ana, CA), which contains 1.4mm ceramic beads (Supplementary Figure S1) using a Fast Prep-24 Homogenizer, (MP Biomedicals) for 20 seconds at 4.5 m/s. The homogenate was incubated at room temperature for 5 min and was subsequently extracted following the protocol from the RNeasy Lipid Tissue Mini Kit with DNase digestion (Qiagen). RNA was eluted off the column in 50 μL of RNase-free water. RNA quantity and integrity were evaluated using a 2100 Bioanalyzer and the RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA).

Library preparation and next-gen sequencing

PolyA⁺ mRNA isolation, size fragmentation, cDNA synthesis, size selection, and next gen sequencing were performed at Beckman Coulter Genomics (Pasadena, CA) according to their standard protocols, as described previously (13). Briefly, non-stranded cDNA libraries

were prepared using the Illumina TruSeq RNA Library Preparation Kit v2 (San Diego, CA) with Biomek (Beckman Coulter) liquid handling automation, followed by sequencing on the Illumina HiSeq2500. Paired end unstranded sequence reads at 2×125 bp read length were obtained using v4 chemistry.

Alignment, quantification and quality control using MAGIC RNA-Seq software

Human trigeminal samples were aligned and quantified using the MAGIC pipeline (19, Supplementary File 1, pages 31–46) and a human genome target built using recent a recent RefSeq annotation (GRCh38.p7) and Aceview (20). Additional genomic targets for viral and microbial genomes were selected manually, and include non-redundant RefSeq genome sequences for viruses hosted by humans (21) (Figure 1). The major virus detected within human trigeminal ganglia was HSV-1 (Figures 1 and 2). The list of microbial accessions, fasta file and read counts in the 16 samples are available as supplemental files. Calculations of read coverage, alignment percentages, and metrics of read quality, and numerous other quality control metrics are generated after sequence alignment (Supplementary Figure S3). Normalization of gene counts was performed as described (19) and includes several refinements to absorb technical variations and batch effects. Briefly, only compatible read pairs mapping uniquely over their quasi-entire length contribute to the expression measures and to SNV calling. Expression measures are corrected for the length of the gene and the insert size of the library, for the 3' bias, and for the level of genomic contamination. Uniquely mapped bases are then normalized relative to the number of bases aligned in the protein coding genes, not counting the very highly expressed genes (above 2% of all mapped bases), as this latter gene set is more susceptible to variations in library preparation. These corrections yield an expression index which is comparable to the log2 of a corrected FPKM, called sFPKM (index = 10 for sFPKM = 1, index 20 for sFPKM 1024).

A method to more precisely define and score differential expression, even for genes that fail to be expressed in a fraction of the cohort, or that display a non-normal distribution of expression index, has been developed and shown to yield excellent correlation between RNA-Seq and microarray results (19). As a refinement, this method uses knowledge of the intrinsic measurability of each gene, as the gene-specific variance was measured from 400 replicate measures of four RNA samples (UHR cell lines and Brain) in the SEQC project (22). To achieve better sample clustering, correlation analysis was performed by comparing all samples or groups of samples to each other, using all genes expressed above sFPKM 1 (14700 genes on average) or four (11100 genes) in at least one of the two samples (Figure 3(e)). This method had allowed MAGIC to achieve an accurate classification of all samples in a blinded toxicogenomics study (23). Differential correlation, where the expression index of each gene in each sample is compared to the average index of the gene across all samples, reinforces the role of the non-coding genes, which are usually less expressed, and allows discrimination of sample relationships more finely, leading to an even better auto-classification and clustering. For comparison, a gene panel of markers of neuronal and non-neuronal cell types was compiled by literature review and used to estimate the abundance of various cell types within each sample (Figure 3(f)). Rat DRG and sciatic nerve samples were mined from a previously published dataset (13), and aligned using MAGIC and a genomic target based on the Rn6 annotations. This dataset was also used for determination of neural

vs. non-neural DRG genes (13), and a high level of concordance was observed between these two datasets (Figure 4).

Histology and immunohistochemistry

For neuroanatomical evaluation, human trigeminal ganglia were embedded in paraffin and 6 mm sections were cut. The slides were warmed at 60° C for 20 minutes, deparaffinized in xylenes three times for 5 minutes each, and hydrated in a decreasing ethanol (EtOH) gradient (100%, 95%, 70%, 30%, dH₂O) for approximately 1 minute each. After the slides were deparaffinized, they were processed for either histology or immunohistochemistry.

For histology, the sections were stained with Multiple Stain Solution (MSS, Polysciences Inc., Warrington, PA) for 5 minutes in 5% MSS and rinsed in tap water. Slides were dehydrated in an increasing EtOH gradient (30%, 70%, 95%, 100%) for 1 minute each, allowed to air dry, cleared three times with xylenes, and again allowed to air dry. The slides were mounted using Permount (Fisher Scientific, Waltham, MA). Scans of all slides were taken with a Hamamatsu NanoZoomer 2.0HT Digital Slide Scanner (Hamamatsu Photonics, Naka-ku, Hamamatsu) or photographed using an Olympus BX60 microscope (DP80 camera).

For immunocytochemistry, trigeminal sections were subjected to antigen retrieval performed using citrate buffer (10mM citric acid, 0.05% Tween-20, pH6.0) in a 1200W microwave for 3 minutes at 100% power and 10 minutes at 30% power. Slides were washed in buffer containing 145mM NaCl, 5mM KCl, 1.8mM CaCl₂, 0.8 mM MgCl₂, and 10 mM HEPES. Tissue sections were blocked by incubation in horse serum (VECTASTAIN Elite ABC HRP Kit, Peroxidase, Mouse IgG, Vector Labs, Burlingame, CA) for 30 minutes. The slides were incubated in primary antibody (Neurofilament, 1:100, Dako, Carpinteria, CA) diluted in antibody diluent (1% BSA, 0.05% sodium azide, and 0.1% Tween-20 in PBS) for 1 hour. The slides were washed in buffer for 5 minutes and incubated in biotinylated secondary antibody from the VECTASTAIN Kit for 30 minutes. The slides were washed again for 5 minutes in buffer and incubated in the Vector ABC Reagent for 30 minutes. The slides were washed for 5 minutes in buffer and developed with the ImmPACT DAB kit (Vector, Carlsbad, CA) until optimal color developed (30 seconds). The slides were rinsed in tap water and counterstained with Hematoxylin (Sigma Aldrich, St. Louis, MO). TRPV1 staining was performed on paraffin embedded sections of human TG as described by Goswami (2014) (24). The slides were dehydrated in an increasing EtOH gradient and cleared with xylenes before mounting.

Luciferase immunoprecipitation systems (LIPS) assay for anti-HSV-1 antibody quantification in blood

Herpes Simplex Virus 1 (HSV-1) serological status was determined using a LIPS assay (25) by an experimenter blind to sample identity. One microliter of blood was assayed for antibodies against the gG-1 protein, the presence of which is indicative of HSV-1 infection (26,27). Briefly, the LIPS assay consists of expression of a recombinant *Renilla* luciferase gG-1 fusion protein in mammalian cells, incubation of the gG-1 fusion protein extract with the blood samples, and precipitation of the antigen-fusion-immunoglobulin complex with

protein A/G beads (26,27). After washing, light units (LU) were measured in a Centro LB960 luminometer (Berthold Technologies, Bad Wildbad, Germany) using coelenterazine mix. Light unit data are the average of duplicate assays.

Migraine susceptibility gene expression profiles

The relationship between expression profiles obtained in neurally-enriched and neurally-depleted trigeminal samples was determined for genes reported to be genetically associated with migraine or headache susceptibility. Susceptibility genes were obtained from literature sources (28,29) or the Online Mendelian Inheritance in Man (OMIM) database.

Heatmap

Gene expression from our trigeminal datasets was compared to transcriptome data available in the GTEx database. Gene expression values in Figure 5 were normalized so that each value in a row is expressed as a fraction of the maximum in that row as described previously (13,30). Enrichment of migraine genes in tissues was ranked from highest to lowest (left to right). Subsequently, the vascular and brain datasets were grouped together. Genes were loosely categorized into clusters based on enrichment in trigeminal, vascular tissue, or brain, and manually sorted to cluster those with the highest levels of enrichment in the trigeminal, vascular and brain datasets. These data are highly overlapping, and many of these genes are broadly expressed, indicating that some genes may have multiple roles. A literature review was performed to categorize some genes as those that act on neural cells or that cause vascular defects when mutated (purple and green labels, Figure 5).

Statistics

Statistical tests in Figures 1(a) and 1(b), 2(a) and 2(b), and Supplementary Figures S1 and S2 were performed using Prism (GraphPad, La Jolla, CA). A p -value < 0.05 was assigned to significance. Differential expression was performed using MAGIC, which reports a differential score indicating the separation of the distributions between two sample groups. This method does not assume a monomodal distribution, and has been shown to be highly reliable (19,22) (Supplementary file 1). The maximum score is 200, indicating complete separation of variance. We determined the cutoff differential score by converting these scores to false discovery rate, establishing that, for samples greater in the neural group, a cutoff of 83 or greater had a false discovery rate of less than 5%; for samples greater in the non-neural group, this cutoff for a 5% false discovery rate was 105 (Supplementary Figure S3). The final number of differentially expressed genes (DEGs, Figure 4(a)) also reflects a noise subtraction, which increases the score required to achieve significance for genes that show high differential scores in 80 random resamplings of the trigeminal samples.

Results

Post-mortem samples of trigeminal ganglia from 22 individuals were obtained from the Human Brain Collection Core, NIMH (National Institute of Mental Health); demographics are shown in Table 1. The PMI ranges from 22 to 63 hours, age ranges from 18 to 64 years old, and only two samples were from males. The manner of death for each individual is

classified as accidental, natural, or suicide. RNA-Seq was performed on 16 of the 22 samples representing a range of RIN values (8.4–5.5, Supplementary Figure S1).

After aligning reads from trigeminal samples to our standard human target, we observed a high percentage of residual high quality reads that failed to align to human genomic, mitochondrial, or ribosomal DNA (Figure 1(b)). Running BLAST searches on a subset of the unaligned reads identified contributions from bacteria. To address this, we added microbial and viral genomes to our genomic target, which improved the alignment (Figure 1(b)). Bacterial reads were anticorrelated with RIN (Figure 1(c)), presumably due to degradation of the tissue by the contaminating bacteria. Of these bacterial reads, the majority aligned most closely to *E. coli* and *Shigella* strains (Figure 1(d)). Because of the high degree of similarity between bacterial genomes we cannot determine substrains unequivocally using this method. Of the viral reads, HSV-1 was the most prevalent virus detected, and was expressed in five of 16 samples, with two samples showing high levels of HSV-1 transcripts (Figure 1(e), Figure 2(a)).

To date, reads for HSV-1 have not been directly measured in human trigeminal ganglia. To verify that reads from HSV-1 were indicative of HSV-1 infection, antibody levels for HSV-1 were measured with the LIPS assay using the highly-specific gG-1 HSV-1 target and whole cadaveric blood obtained from 13 of the 16 individuals sequenced (three were unavailable). LIPS showed that six of the 13 subjects were HSV-1 seropositive, including one patient with no detectable reads for HSV-1. There was a positive correlation between antibody levels determined by LIPS and HSV-1 reads determined by RNA-Seq (Figure 3(a), $p < 0.001$), demonstrating that HSV-1 is readily detectable by RNA-Seq in post-mortem human TG. The reads aligning to the HSV-1 genome come almost exclusively from the LAT transcript (Figure 2(c)), the main transcript produced while HSV-1 is latent, which is consistent with the idea that none of the tissue donors had actively replicating HSV-1 at the time of death.

The trigeminal ganglion is a heterogeneous tissue (Figure 3(a)) comprised of neuronal cell bodies (Figure 3(b)), satellite cells, myelinated axon tracts that contain Schwann cells (Figure 3(c)), and fibrous regions that form the structural scaffold (Figure 3(d)). The heterogeneity of this tissue presents an obstacle for RNA-Seq studies, as different percentages of cells within the tissue homogenate will produce vastly different gene expression profiles. Individual fragments of trigeminal collected from autopsy samples were segregated into discrete subtypes based on gene expression which indicated tissue composition. Marker genes were used to label these two discrete subgroups into neural and non-neural (Figure 3(f)).

In general, only about half of the tissue fragments showed a clear contribution from neurons, whereas the remainder showed a contribution mainly from Schwann cells (Figure 3(f)). To further assess the characteristics of the samples, we performed a differential correlation analysis on all genes expressed above 4 sFPKM, approximately 11,000 genes per sample, and segregated the samples into subgroups using the standard clustering algorithm of heatmap.2 (R, gplots) (Figure 3(e)).

Differential correlation revealed two clear subgroups and one sample (TG8) that was between these two subgroups. Based on gene-wise information (Figure 3(f)) we labeled these two subgroups as neural and non-neural based on the presence of neuronal markers almost exclusively in the eight samples in the neural category. Marker genes for the gene-wise correlation were corroborated in previous reports (13). The eight most neurally-enriched samples (TG3, 11, 5, 14, 9, 16, 13,1) showed high levels of marker genes for neurons, such as *TRPM8*, while the second broad class of samples (TG 12, 22, 7, 4, 6, 2, 10) showed very low expression of these marker genes. By contrast, markers of Schwann cells and fibroblasts are present in all samples. One sample was not included in tabulations of either the neural or non-neural subclasses due to an intermediate gene expression profile between these two groups (TG8). The neural subgroup contains more genes, and more highly significant differentially expressed genes, because these samples contain the unique neural signature genes, whereas both samples contain supportive cells. *TRPV1* is highlighted as a highly differential gene in the neural group because of its importance in thermal nociception and thermal sensation in the oral cavity. A subset of trigeminal neurons stains for TRPV1, with no staining detectable in large diameter neurons (usually proprioceptors) or in non-neural cells (Figure 4(b),(c)). Dense staining is observed in small diameter neurons, which have been shown to be thermonociceptive C-fibers (31) (Figure 4(c)).

We hypothesized that genes differentially expressed in these eight trigeminal samples represent the transcriptomic signature of trigeminal neurons versus supporting tissue. We reanalyzed previously published transcriptomics data (13) on rat DRG and sciatic nerve to parse differential gene expression between neurons and non-neural supportive cells. The sciatic nerve transcriptome shows a strong Schwann cell signature, with presence of some connective, vasculature, and adipose tissue (13). The top 125 expressed genes in neural and non-neural subsets of trigeminal samples were plotted and colored according to enrichment of the same gene in rat DRG relative to rat sciatic nerve (Figure 4(d)). In general, neural trigeminal samples showed a greater than three-fold enrichment for genes found in DRG relative to genes from sciatic nerve samples, confirming that these are neural markers. Significance values also corresponded between these two datasets (Supplementary Figure S4). Similarly, genes enriched in non-neural cells showed a greater than three-fold enrichment for genes found in the sciatic nerve, indicating that these samples are enriched mainly in trigeminal Schwann and supportive cells.

Transcriptome data for selected genes and gene families are highlighted in Table 2. Several important considerations need to be taken into account when making comparative examinations of the trigeminal genes in Table 2 to other ganglionic datasets. First, while the table emphasizes neurally enriched genes, there is not a complete neural/non-neural dichotomy between the two sets of trigeminal sub-regions. Second, the trigeminal ganglion, relative to the DRG, contains much more connective tissue and nerve bundles, resulting in generally lower levels for a particular neural transcript than measured in the DRG. For comparison and confirmation, we provide the values for fold neurally enriched for each entry from rat DRG versus rat sciatic nerve in which Schwann cells are a major component (13).

Using these datasets, we mapped putative headache and trigeminal pain disorder genes to the expression profile in the anatomically distinct neural and non-neural samples of human trigeminal. This analysis reveals the distribution of headache susceptibility genes within cellular subpopulations (Table 3). We also make a comparison to our previous study examining DRG and sciatic nerve (mainly Schwann cells) transcriptomics (13), and extend this analysis to body-wide expression mapping using the publicly-available GTEx database (32). The GTEx database is composed of RNA-Seq expression values for 50 human tissues, whole blood and two transformed cell lines, and includes 12 brain regions and spinal cord. In Figure 5, GTEx values from the 32 tissues with the highest level of enrichment for migraine-related genes are plotted. Skeletal muscle and whole blood are included for comparison, but are not among the most enriched tissues. The vascular and brain region datasets are grouped side by side. Genes are loosely categorized into four clusters based on enrichment in TG, vascular tissues from GTEx (various arteries), or central nervous system regions. A fourth group below the horizontal white line consists of genes whose expression profile shows depletion in neural, trigeminal and vascular tissues relative to another somatic tissue. This subgrouping includes the estrogen receptors *ESR1*, *ESR2*, as well as the receptor for follicle stimulating hormone (*FSHR*). Whole blood shows very little enrichment for any migraine gene. Twelve genes of the 53 genes support neural functions and their names are shown in green; of these, six are exclusively expressed in the sensory ganglia (top part of the heat map). Eighteen of the 53 genes (34%), when mutated, cause vascular defects or abnormalities (names shown in purple).

Comparison of rat trigeminal and DRG samples showed that several nociceptive signal-transducing ion channels are highly enriched in the trigeminal ganglia, including *Tpm8* and *Trpa1* (Figure 6). In general, many peptide precursors are expressed at lower levels in the trigeminal relative to the DRG, including the mRNA (*Calca*) encoding the precursor to the calcitonin gene-related peptide (CGRP), which has been implicated in migraine (33). Of the genes in the list in Table 3, the migraine susceptibility gene *KCNK18* was highly enriched in the trigeminal ganglia (Figure 6). Due to its strong association with migraine and neural pattern of expression, this gene was examined in greater detail.

The potassium channel, *KCNK18*, is neurally and trigeminally enriched, and is more highly expressed in the DRG relative to the sciatic nerve (Figure 6, 7(a),(b)). In aggregate, this suggests a predominantly neural distribution, with little expression in non-neural ganglia cells such as Schwann cells and fibroblasts (13). In the mouse DRG, *Kcnk18* is expressed in several populations of neurons, previously classified using single-cell RNA-Seq (34). Two subclasses of neurons express both *Kcnk18* and *Calca*, which encodes the precursor to the calcitonin gene-related peptide (CGRP, top panel, Figure 7(c); subclasses marked by the bracket). These cells, which encode the potassium channel mutated in some patients with familial migraine, and which release CGRP, a peptide that has been implicated in migraine, are potentially the subpopulation of cells by which this mutation causes migraine. Two GPCRs, *Mrgprd* and *Mrgpra3*, are additional markers of the cells co-expressing these two genes. These cells also contain broader non-specific markers (middle panel, Fig 7C) such as *Trpa1*, *Scn9a*, and *P2rx3*, whereas *Trpv1*, *Tac1* (coding for the Substance P precursor) and *Oprm1* (μ opiate receptor) are largely in a separate population of cells (bottom panel, Figure 7(c)). In an independent analysis using a second single-cell RNA-Seq dataset of mouse DRG

neurons (35), we observed colocalization of *Calca* and *Kcnk18* in a loose cluster of neurons, potentially representing several related subclusters. Gene expression for *Calca* is high across many cells (Figure 7(d)), compared to the more restricted expression of *Kcnk18* (Figure 7(e)).

Raw counts and normalized expression values (sFPKM) can be found in Supplementary Tables S1–S4. FASTQ files containing the primary data are accessible in the Sequence Read Archive (SRA, Projects PRJNA313202 and PRJNA384203) (13).

Discussion

Trigeminal ganglia were examined to gain a better understanding of biochemical and molecular factors that may contribute to migraine or chronic headache. This tissue contains primary sensory neurons that respond to a wide variety of nociceptive and somatosensory modalities (36). Trigeminal neurons release neuropeptides such as CGRP and Substance P, which have vasodilatory actions that may contribute to some of the pathophysiology of migraine and headache (37,38). In the present experiment, we were able to take advantage of the anatomical heterogeneity that exists within the trigeminal (Figure 3, Supplementary Figure S1) to assess neuronal and non-neuronal transcriptomes; this type of local heterogeneity has been explored in other human post-mortem studies of, for example, the substantia nigra (39). Among the most differentially expressed genes were ion channels related to neuronal excitability, electrophysiological and metabotropic transduction of nociceptive signals, and action potential generation and conduction. The neuron-specific selectivity is evident within some, but not all, members of the gene families that are explored in Table 2. This theme of neuronal, non-neuronal, or broader spectrum expression is further examined and exemplified in our analysis of migraine susceptibility genes (Table 3, Figure 5 and additional discussion below).

In RNA-Seq experiments, alignment to a comprehensive and well-annotated genome is critical (22). The presence of a substantial portion of unaligned reads of high quality in our first pass alignment suggested the presence of non-human transcripts. Consequently, we realigned the reads to a new genomic target that included microbial and viral genomes, which led to a higher percentage of identified reads (Figure 1(b)). This strategy can be considered a general approach to solving the problem of missing alignments. If a large percentage of reads do not align to a target genome, individual unaligned reads can be sampled and queried against broad databases to understand their origin. Once the potential targets are identified, all reads are aligned against the complete genome of these new targets, and the new alignments are kept if they gain at least 20 bases relative to the previous best alignment. This two-pass alignment system is different from traditional multipass alignment, where further alignment is performed only on the unaligned reads (40) and utilizes outside genomic databases to expand the genomic target to include other organisms.

The addition of microbial and viral genomic target sequences improves read mapping, as these sequences are frequently detected in many biological samples. The presence of bacterial reads is additionally informative as an indicator of quality control issues (Supplementary Figure S2) and may be related, in part, to the stringency of the poly A +

selection. In fact, it is interesting to consider that stringent poly A + selection may mask the presence of transcriptomes from non-polyadenylating species. Due to the small size of these genomes, their addition does not substantially impact the time or computational resources for alignment. The vast majority of bacterial reads in the trigeminal likely came from enteric gut bacteria. We subsequently ascertained that, during the autopsy, the trigeminals had been harvested last, and were most likely contaminated by blood and other substances from previously dissected tissue during the autopsy. Overall, we agree with other researchers who have been adamant that quality control must be “proactive and comprehensive” (41) and we believe that the addition of bacterial and viral genomes to quality control pipelines adds a further layer of scientific rigor to the alignment and aids in the discovery process. Quality control was comprehensively addressed by correlating RIN with several metrics from MAGIC (Supplementary Figure S2), and we did not exclude any samples based on quality control.

HSV-1 infects trigeminal neurons, and the transcripts produced by these cells are present at detectable quantities in the trigeminovascular system after infection (43,44) or in post-mortem tissue as detected by in situ hybridization (45). Sili et al. reported that out of 106 patients with herpes simplex virus encephalitis (HSVE), 70% presented with headache among other symptoms (42). Thus, we propose that further investigation of the relationship between HSV and headache could be addressed using transcriptomics and LIPS for HSV-1 antibodies. Indeed, we observed good correspondence between transcriptomic detection of human HSV-1 and LIPS detection of serum anti-HSV-1 reactivity (Figure 2) suggesting that RNA-Seq of viral transcripts is a valid methodology to quantify HSV-1 viral load in post-mortem trigeminal.

Another critical element of data analysis is to obtain an unbiased classification of all samples by expression profile. In this project, we first clustered trigeminal ganglia samples using differential correlation analysis, considering expression of all genes expressed at >1 sFPKM (Figure 3(e)). This resulted in two well-separated clusters of samples, and one sample intermediate between the two clusters (TG8). Subsequently, we used a list of marker genes from a previous study of neural and non-neural DRG genes (13) to identify the composition of each cluster. This method allowed us to distinguish neuronally-enriched trigeminal samples (containing neuronal perikarya and support cells) from trigeminal nerve bundles (containing Schwann cells, fibroblasts, and other support cells but few neuronal perikarya) (Figure 3(f)). This analysis, in conjunction with our previous comparison between the DRG and sciatic nerve (13) reinforces the validity of this approach and the identity of the clusters (Figure 5(d), Table 2, Supplementary Figure S4).

The present paper reports the most extensive human trigeminal transcriptome, and is the first report of the transcriptome of the supportive and Schwann cells within the trigeminal. Previous studies have shown similarities between DRG and trigeminal ganglion (24,46,47), and have also characterized individual cell types or populations within the DRG (24,35,48,49). Despite several tissue-level transcriptomic differences between DRG and trigeminal, it is generally appreciated that the same or similar cell types are present in these two sensory ganglia. These data can be utilized to gain further understanding of differential expression patterns of target genes of potential therapeutic importance for headache, many

examples of which are shown in Table 2. One usage of the present datasets is to confirm that a gene of interest is indeed expressed in the human trigeminal. This is an essential element for translational studies (50). Beyond this, these data can be extended to understanding the genetics of cephalic pain disorders such as migraine. Using the anatomically distinct neural and non-neural trigeminal samples, we characterized the expression of susceptibility genes in ganglionic neurons or non-neural cells (Table 3). The expression of many of these genes in other cells throughout the nervous system, and indeed in other bodily organs, suggests that neurological deficits or other organ involvement may be present. One example of this is familial hemiplegic migraine, in which patients present with unilateral paresis. The genes causing familial hemiplegic migraine are expressed in the central nervous system, and motoric aspects of this disease are presumably attributable to their action centrally.

Our trigeminal subpopulation analyses indicated that many of the migraine susceptibility genes can be broadly characterized as affecting neurons or blood vessels (29). Of note, many of these genes are weakly associated with migraine, and in many cases are only the closest gene to a low-penetrance intergenic variant. Higher penetrance genes with a stronger relationship to migraine are described in Table 3, while lower incidence genes are marked as “migraine susceptibility” (29). In the heatmap (Figure 6), most of the genes implicated in migraine disorders are enriched in neuronal tissues (either trigeminal or brain), the vasculature, or both. Of note, many of the genes in the TG and brain directly affect neuronal excitability and firing characteristics (Figure 6, gene symbols in green) including voltagegated sodium channels and potassium channels. Many of the non-neural vascular genes have been shown to underlie vascular patterning or growth, or produce other types of vessel defects when mutated (Figure 5, purple.) Multiple genes within the Jagged/Notch pathway are represented and well expressed, including *JAG1* (Jagged) and its receptors *NOTCH3* and *NOTCH4*, all of which participate in vascular patterning and development (51–53). *PRDM16*, a PR-domain zinc finger transcription factor, may also be involved in the regulation of this pathway (54). The expression characteristics and functional data provided from mutation studies strongly suggest that most migraine genes are either expressed in vasculature to regulate the size, function, development, or patterning of blood vessels, or are expressed in neurons where they regulate neuronal activity (Figure 5). This conclusion is further supported by several recent genome-wide association studies which also identify gene contributions from the vascular compartment (55).

The trigeminal ganglia has mainly the same neural subtypes as the DRG, with some key differences in gene expression likely representing the specific adaptations of the sensory afferents contained within this ganglion (46,47). We examined the relationship between DRG and trigeminal in the rat, revealing enrichment of several migraine genes in the trigeminal, including the migraine-associated potassium channel gene, *Kcnk18* (Fig 6). The *Trpm8* and *Tpa1* transcripts were also highly enriched in the trigeminal, which is of interest given that *TRPM8* mutations have been identified as causing susceptibility to migraine (56,57), and drugs targeting each of these ion channels have been proposed for treatment of migraine (58).

Out of the genes examined, the potassium channel gene *KCNK18* stood out because it is (a) highly enriched in the trigeminal ganglion relative to all other tissues examined, including

the DRG (Figures 5, 6), (b) it is highly enriched in the neurons of the trigeminal ganglia relative to non-neural cells (Figure 7), and (c) dominant negative mutations in this gene have been shown to be associated with familial migraine, although other loss of function mutations are not associated with migraine (14,15). While it remains unclear how mutations in *KCNK18* cause migraine, and with what penetrance, it is generally accepted that migraine is a complex multifactorial disease. Nevertheless, understanding the basic biology of *KCNK18* may shed light on the underlying mechanisms of migraine susceptibility. For example, mutations in this channel likely activate a subset of neurons that may increase migraine susceptibility in affected individuals by activating nociceptive afferents that release vasodilatory transmitters. Using single-cell RNA-Seq we identified that several subpopulations of neurons express this channel, including two populations that co-express the CGRP precursor *Calca* (Figure 7(c)–(e)). Based on this finding, we expect that activation of this population by the mutation in *KCNK18* in patients with migraine underlies the clinical presentation. Conversely, other populations such as one population of non-peptidergic A-fibers (neurofilament 1) are unlikely to contribute to migraine. We submit these findings as an example of the utility of the present datasets for reaching comprehensive, data-driven conclusions about susceptibility genes in humans. These analyses allow us to identify anatomical distribution of genes of interest, which allows for hypothesis testing of how these genes may be related to migraine.

The sequence-based nature of RNA-Seq provides objective information that is less ambiguous or subject to misinterpretation compared to other methods such as immunocytochemistry, PCR, or microarray (50). While single-cell studies allow for subpopulations to be defined, they rely on interpolation of data points that are not directly measurable in every cell within the population. Differential expression between anatomical subdivisions allows for the depth and accuracy of deep sequencing to be preserved and provides a middle ground between the respective advantages of single cell and traditional whole tissue RNA-Seq. In this regard, the present report is an *in silico* resource for neural and non-neural trigeminal gene discrimination in headache disorder research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Kurth T, Mohamed S, Maillard P, et al. Headache, migraine, and structural brain lesions and function: Population based Epidemiology of Vascular Ageing-MRI study. *BMJ* 2011; 342: c7357. [PubMed: 21245119]
2. De Benedittis G, Lorenzetti A, Sina C, et al. Magnetic resonance imaging in migraine and tension-type headache. *Headache* 1995; 35: 264–268. [PubMed: 7775189]
3. Calandre L, Hernandez-Lain A and Lopez-Valdes E. Benign Valsalva's maneuver-related headache: An MRI study of six cases. *Headache* 1996; 36: 251–253. [PubMed: 8675432]
4. Sand T EEG in migraine: A review of the literature. *Funct Neurol* 1991; 6: 7–22. [PubMed: 2055554]
5. Bjork M, Stovner LJ, Hagen K and Sand T. What initiates a migraine attack? Conclusions from four longitudinal studies of quantitative EEG and steady-state visualevoked potentials in migraineurs. *Acta neurologica Scandinavica Supplementum* 2011; (191): 56–63. [PubMed: 21711258]
6. Ramsden CE, Faurot KR, Zamora D, et al. Targeted alterations in dietary n-3 and n-6 fatty acids improve life functioning and reduce psychological distress among patients with chronic headache: A secondary analysis of a randomized trial. *Pain* 2015; 156: 587–596. [PubMed: 25790451]
7. Ramsden CE, Faurot KR, Zamora D, et al. Targeted alteration of dietary n-3 and n-6 fatty acids for the treatment of chronic headaches: A randomized trial. *Pain* 2013; 154: 2441–2451. [PubMed: 23886520]
8. Ramsden CE, Mann JD, Faurot KR, et al. Low omega-6 vs. low omega-6 plus high omega-3 dietary intervention for chronic daily headache: Protocol for a randomized clinical trial. *Trials* 2011; 12: 97. [PubMed: 21496264]
9. Ramsden CE, Zamora D, Makriyannis A, et al. Dietinduced changes in n-3- and n-6-derived endocannabinoids and reductions in headache pain and psychological distress. *J Pain* 2015; 16: 707–716. [PubMed: 25958314]
10. Tomita H, Vawter MP, Walsh DM, et al. Effect of agonal and postmortem factors on gene expression profile: Quality control in microarray analyses of postmortem human brain. *Biol Psychiatry* 2004; 55: 346–352. [PubMed: 14960286]
11. Weis S, Llenos IC, Dulay JR, et al. Quality control for microarray analysis of human brain samples: The impact of postmortem factors, RNA characteristics, and histopathology. *J Neurosci Methods* 2007; 165: 198–209. [PubMed: 17628689]
12. Bennett DA, Wilson RS, Arvanitakis Z, et al. Selected findings from the Religious Orders Study and Rush Memory and Aging Project. *J Alzheimer's Dis* 2013; 33: S397–S403. [PubMed: 22647261]
13. Sapio MR, Goswami SC, Gross JR, et al. Transcriptomic analyses of genes and tissues in inherited sensory neuropathies. *Exp Neurol* 2016; 283: 375–395. [PubMed: 27343803]
14. Lafreniere RG, Cader MZ, Poulin JF, et al. A dominantnegative mutation in the TRESK potassium channel is linked to familial migraine with aura. *Nat Med* 2010; 16: 1157–1160. [PubMed: 20871611]
15. Andres-Enguix I, Shang L, Stansfeld PJ, et al. Functional analysis of missense variants in the TRESK (KCNK18) K channel. *Sci Rep* 2012; 2: 237. [PubMed: 22355750]
16. Dodick DW, Goadsby PJ, Spierings EL, et al. Safety and efficacy of LY2951742, a monoclonal antibody to calcitonin gene-related peptide, for the prevention of migraine: A phase 2, randomised, double-blind, placebo-controlled study. *Lancet Neurol* 2014; 13: 885–892. [PubMed: 25127173]
17. Hewitt DJ, Aurora SK, Dodick DW, et al. Randomized controlled trial of the CGRP receptor antagonist MK-3207 in the acute treatment of migraine. *Cephalalgia* 2011; 31: 712–722. [PubMed: 21383045]
18. Lassen LH, Haderslev PA, Jacobsen VB, Iversen HK, Sperling B and Olesen J. CGRP may play a causative role in migraine. *Cephalalgia* 2002; 22(1): 54–61. [PubMed: 11993614]
19. Zhang W, Yu Y, Hertwig F, et al. Comparison of RNAseq and microarray-based models for clinical endpoint prediction. *Genome Biol* 2015; 16: 133. [PubMed: 26109056]
20. Thierry-Mieg D and Thierry-Mieg J. AceView: A comprehensive cDNA-supported gene and transcripts annotation. *Genome Biol* 2006; 7: S12. [PubMed: 16925834]

21. Brister JR, Ako-Adjei D, Bao Y, et al. NCBI viral genomes resource. *Nucleic Acids Res* 2015; 43: D571–D577. [PubMed: 25428358]
22. Consortium SM-I. A comprehensive assessment of RNAseq accuracy, reproducibility and information content by the Sequencing Quality Control Consortium. *Nat Biotechnol* 2014; 32: 903–914. [PubMed: 25150838]
23. Wang C, Gong B, Bushel PR, et al. The concordance between RNA-seq and microarray data depends on chemical treatment and transcript abundance. *Nat Biotechnol* 2014; 32: 926–932. [PubMed: 25150839]
24. Goswami SC, Mishra SK, Maric D, et al. Molecular signatures of mouse TRPV1-lineage neurons revealed by RNA-Seq transcriptome analysis. *J Pain* 2014; 15: 1338–1359. [PubMed: 25281809]
25. Burbelo PD, Ching KH, Klimavicz CM, et al. Antibody profiling by Luciferase Immunoprecipitation Systems (LIPS). *J Vis Exp* 2009; 32.
26. Burbelo PD, Hoshino Y, Leahy H, et al. Serological diagnosis of human herpes simplex virus type 1 and 2 infections by luciferase immunoprecipitation system assay. *Clin Vaccine Immunol* 2009; 16: 366–371. [PubMed: 19129469]
27. Zubair A, Burbelo PD, Vincent LG, et al. Microfluidic LIPS for serum antibody detection: Demonstration of a rapid test for HSV-2 infection. *Biomed Microdevices* 2011; 13: 1053–1062. [PubMed: 21826483]
28. Tolner EA, Houben T, Terwindt GM, et al. From migraine genes to mechanisms. *Pain* 2015; 156: S64–S74. [PubMed: 25789438]
29. Gormley P, Anttila V, Winsvold BS, et al. Meta-analysis of 375,000 individuals identifies 38 susceptibility loci for migraine. *Nature Gen* 2016; 48: 856–866.
30. Burbelo PD, Iadarola MJ, Alevizos I, et al. Transcriptomic segregation of human autoantigens useful for the diagnosis of autoimmune diseases. *Mol Diagn Ther* 2016; 20: 415–427. [PubMed: 27259330]
31. Caterina MJ, Schumacher MA, Tominaga M, et al. The capsaicin receptor: A heat-activated ion channel in the pain pathway. *Nature* 1997; 389: 816–824. [PubMed: 9349813]
32. Consortium GT. The Genotype-Tissue Expression (GTEx) project. *Nat Gen* 2013; 45: 580–585.
33. Doods H Development of CGRP antagonists for the treatment of migraine. *Curr Opin Investig Drugs* 2001; 2: 1261–1268.
34. Usoskin D, Furlan A, Islam S, et al. Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat Neurosci* 2015; 18: 145–153. [PubMed: 25420068]
35. Li CL, Li KC, Wu D, et al. Somatosensory neuron types identified by high-coverage single-cell RNA-sequencing and functional heterogeneity. *Cell Res* 2016; 26: 83–102. [PubMed: 26691752]
36. Goadsby PJ. Pathophysiology of cluster headache: A trigeminal autonomic cephalgia. *Lancet Neurol* 2002; 1: 251–257. [PubMed: 12849458]
37. Goadsby PJ and Edvinsson L. The trigeminovascular system and migraine: Studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. *Ann Neurol* 1993; 33: 48–56. [PubMed: 8388188]
38. Tajti J, Szok D, Majlath Z, et al. Migraine and neuropeptides. *Neuropeptides* 2015; 52: 19–30. [PubMed: 26094101]
39. Iadarola MJ, Ofri D and Kleinman JE. Enkephalin, dynorphin and substance P in postmortem substantia nigra from normals and schizophrenic patients. *Life Sci* 1991; 48: 1919–1930. [PubMed: 1708847]
40. Engstrom PG, Steijger T, Sipos B, et al. Systematic evaluation of spliced alignment programs for RNA-seq data. *Nat Methods* 2013; 10: 1185–1191. [PubMed: 24185836]
41. Hartley SW and Mullikin JC. QoRTs: A comprehensive toolset for quality control and data processing of RNASeq experiments. *BMC Bioinf* 2015; 16: 224.
42. Sili U, Kaya A, Mert A, et al. Herpes simplex virus encephalitis: Clinical manifestations, diagnosis and outcome in 106 adult patients. *J Clin Virol* 2014; 60: 112–118. [PubMed: 24768322]
43. Stroop WG, Rock DL and Fraser NW. Localization of herpes simplex virus in the trigeminal and olfactory systems of the mouse central nervous system during acute and latent infections by in situ hybridization. *Lab Invest* 1984; 51: 27–38. [PubMed: 6330452]

44. Kristensson K, Nennesmo L, Persson L, et al. Neuron to neuron transmission of herpes simplex virus. Transport of virus from skin to brainstem nuclei. *J Neurol Sci* 1982; 54: 149–156. [PubMed: 6281393]
45. Croen KD, Ostrove JM, Dragovic LJ, et al. Latent herpes simplex virus in human trigeminal ganglia. *New Eng J Med* 1987; 317: 1427–1432. [PubMed: 2825014]
46. Flegel C, Schobel N, Altmüller J, et al. RNA-Seq analysis of human trigeminal and dorsal root ganglia with a focus on chemoreceptors. *PloS One* 2015; 10: e0128951. [PubMed: 26070209]
47. Mantziotis S, Lehmann R, Flegel C, et al. Comprehensive RNA-Seq expression analysis of sensory ganglia with a focus on ion channels and GPCRs in Trigeminal ganglia. *PloS One* 2013; 8: e79523. [PubMed: 24260241]
48. Usoskin D, Furlan A, Islam S, et al. Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nature Neurosci* 2015; 18: 145–153. [PubMed: 25420068]
49. Chiu IM, Barrett LB, Williams EK, et al. Transcriptional profiling at whole population and single cell levels reveals somatosensory neuron molecular diversity. *Elife* 2014; 3: e04660.
50. Goswami SC, Thierry-Mieg D, Thierry-Mieg J, et al. Itch-associated peptides: RNA-Seq and bioinformatics analysis of natriuretic precursor peptide B and gastrin releasing peptide in dorsal root and trigeminal ganglia, and the spinal cord. *Mol Pain* 2014; 10: 44. [PubMed: 25123163]
51. Xue Y, Gao X, Lindsell CE, et al. Embryonic lethality and vascular defects in mice lacking the notch ligand Jagged1. *Hum Mol Genet* 1999; 8: 723–730. [PubMed: 10196361]
52. Ruchoux MM, Domenga V, Brulin P, et al. Transgenic mice expressing mutant Notch3 develop vascular alterations characteristic of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Am J Pathol* 2003; 162: 329–342. [PubMed: 12507916]
53. Uyttendaele H, Ho J, Rossant J, et al. Vascular patterning defects associated with expression of activated Notch4 in embryonic endothelium. *Proc Nat Acad Sci USA* 2001; 98: 5643–5648. [PubMed: 11344305]
54. Beerens M, Craps S, MacRae C, et al. Abstract 19545: Prdm16 deficiency leads to arterial vascular defects by modulating the activity of the notch signaling cascade. *Circulation* 2016; 134: A19545–A.
55. Nyholt DR and van den Maagdenberg AMJM. Genomewide association studies in migraine: Current state and route to follow. *Curr Opin Neurol* 2016; 29: 302–308. [PubMed: 26967494]
56. Dussor G and Cao YQ. TRPM8 and migraine. *Headache* 2016; 56: 1406–1417. [PubMed: 27634619]
57. Chasman DI, Schurks M, Anttila V, et al. Genome-wide association study reveals three susceptibility loci for common migraine in the general population. *Nature Genet* 2011; 43: 695–698. [PubMed: 21666692]
58. Dussor G, Yan J, Xie JY, et al. Targeting TRP channels for novel migraine therapeutics. *ACS Chem Neurosci* 2014; 5: 1085–1096. [PubMed: 25138211]

Article highlights

- The full transcriptome of human trigeminal ganglion was determined by comprehensive RNA–Seq in multiple subjects, providing an *in silico* research resource.
- Both neurally–enriched and depleted sub–regions were sequenced and contrasted to reveal neuronal–specific genes.
- Herpes simplex 1 viral sequences were identified in trigeminal ganglia; implied viral infection was verified by measurement of antibody levels in blood.
- The expression profiles of migraine susceptibility genes were examined for enrichment in trigeminal ganglion, vascular tissue, and brain.
- Detailed body–wide and neural cell–type specific expression pattern is described for the migraine susceptibility gene, *KCNK18*.

Figure 1.

Experimental design and alignment to bacterial and viral genomes in human trigeminal ganglia. (a) Overall schema of experimental design. Trigeminal ganglia were sequenced, and these data were integrated with existing datasets to analyze migraine-related genes. (b) Initial alignment to the standard human genomic target sequence showed a high incidence of reads that were of good quality but failed to align to any queried sequence. The incidence of these high quality unaligned reads was inversely correlated with RIN, indicating the presence of contamination in the low RIN samples from sequences not contributed by the

human genome. After the addition of additional target sequences, including microbial and viral genomes, the correlation was abolished and the percentage of these reads was substantially reduced. (c) The percentage of reads aligning to bacterial genomes inversely correlates with RIN, with as much as ~ 12% of RNA in some samples contributed by bacteria. (d) Approximately 76% of bacterial reads were contributed by *E. coli*, which is normally present within the human enteric gut. (e) A small number of reads aligning to viral genomes were detected, with 14.9% contributed by human endogenous retrovirus, and 80.3% contributed by Human herpesvirus 1.

Figure 2.

Human herpes virus 1 (HSV-1) antibody levels from whole blood and transcript levels of HSV-1 from human post-mortem trigeminal ganglia. Antibody levels for gG-1, a serological HSV-1 target, were measured using the supernatants from whole cadaveric blood obtained from 13 patients. Transcript levels for HSV-1 were determined from trigeminal RNA-Seq data for each subject. (a) The dotted line represents the cutoff value for HSV-1 seropositivity based on our previously published study (26). Six of the 13 were HSV-1 seropositive, and five of those six had detectable HSV-1 reads via RNA-Seq. The limit of quantitation was 1 read. ND = not detectable for HSV-1 reads. There was a significant correlation between HSV-1 reads and antibody levels from LIPS ($r_s = 0.833$, $p < 0.001$, Spearman Correlation). (b) There was a significant difference in average antibody levels between the HSV-1-negative and HSV-1-positive groups as determined by RNA-Seq ($p = 0.002$, Mann Whitney U Test). Error bars represent standard error of the mean. The reads aligning to the HSV-1 genome come almost exclusively from the LAT transcript (c) which is the main transcript

produced while HSV-1 is latent in the trigeminal ganglia. This suggests that none of the tissue donors had actively replicating HSV-1 at the time of death.

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Figure 3. Segregation of trigeminal ganglion samples containing primarily neuronal, Schwann cell/axonal, or connective tissues based on marker gene expression and correlation analysis. (a) Human TG was stained for Neurofilament (scale bar represents 5 mm). There are three distinct cell types present in the tissue section: (b) neuronal, (c) Schwann, and (d) other (scale bar represents 100 μ m). This represents the high inhomogeneity of trigeminal ganglion and presents a clear problem when extracting RNA from only a few small pieces of the larger tissue. (e) Differential covariance analysis was performed using all genes expressed over 1 sFPKM, using the MAGIC pipeline to generate correlation coefficients, subsequently sorted using heatmap.2 in R, resulting in two well-separated clusters of samples with one outlier (TG8). (f) A panel of selective marker genes were chosen to identify the cell types within each cluster of samples. All of the neuronal marker genes were enriched in the eight samples in the first cluster (Neural cluster), while the non-neural

markers of Schwann cells (such as *MPZ* and *MBP*) were present in both clusters (Non-neural cluster). TG9 and TG14 are highly correlated, and both express high levels of neuronal marker genes.

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Figure 4.

Differential expression of genes in neural enriched trigeminal ganglia vs. trigeminal containing mostly non-neural cells. The eight most neurally-enriched samples (Neural: TG1, TG3, TG5, TG9, TG11, TG13, TG14, TG16) were compared to the samples that showed enrichment for Schwann cell markers without a strong neural transcriptomic signature (Non-neural: TG2, TG4, TG6, TG7, TG10, TG12, TG22). Differential expression was plotted for strongly differentially expressed neural genes with scores ≥ 83 , as well as significantly differential non-neural genes with scores > 105 (a) showing several known markers of neurons enriched in the neural sample subset relative to the non-neural subset. *TRPV1* is highlighted as one such strongly differential gene (score = 134). Staining for the TRPV1 protein shows strong expression in a subset of neurons, with no expression in non-neural cells (b); scale bar represents 250 μm). Dense staining is observed in small diameter

neurons, which are likely thermosensitive C-fibers ((c); scale bar represents 100 μ m). Based on the observation that the five selected “non-neural” containing trigeminal samples expressed high levels of Schwann cell markers, we compared the top 125 genes in neural and non-neural subsets to expression in DRG tissue homogenate and sciatic nerve tissue homogenate as described by Sapio, et al. 2016 (d). Genes highly enriched in the DRG relative to the sciatic nerve are mostly neural genes, whereas sciatic nerve enriched genes are largely markers of Schwann cells and connective tissue. The majority of genes enriched in the neural subset were also enriched in the DRG relative to the sciatic nerve. Conversely, the majority of genes enriched in the non-neural subset were also enriched in the sciatic nerve relative to the DRG.

Figure 5. Expression patterns and enrichment of migraine and trigeminal pain genes. Gene expression from trigeminal datasets (sFPKMs) was compared to the data available in the GTEx database (RPKMs, divided by vertical white space). Data were normalized so that each value in a row is expressed as a fraction of the maximum in that row, and colored according to the flame scale (bottom). Enrichment of migraine genes in tissues was ranked from left to right. Subsequently, datasets were reordered so that the vascular and brain datasets were grouped together. The top 32 enriched datasets are plotted, along with skeletal muscle and whole

blood, which are included for comparison. Whole blood shows very little enrichment for any migraine gene. Genes were loosely categorized into clusters based on enrichment in trigeminal, vascular tissue, or brain (right labels). A literature review was performed to categorize the genes as directly acting on neural cells (green) versus those genes that, when mutated, cause vascular defects or abnormalities (purple).

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Figure 6.

Selected differentially expressed genes between rat trigeminal and dorsal root ganglia.

Trigeminal (TG) and dorsal root ganglia (DRG) transcriptomic datasets were compared to

look for highly enriched genes in each. Several neural ion channels responsible for

conducting nociceptive inputs are differentially enriched in trigeminal ganglia relative to

DRG (*Trpm8*, *Trpa1*, *Kcnk18*, *Nos1*, *Oprd1*), while several neuropeptides, including the mRNA encoding the Calcitonin

Gene-related Peptide precursor, are enriched in DRG (*Calca*, *Calcb*, *Sst*). Several other

proteins are equal in both datasets (*Tac1*, *Trpv1*).

Figure 7.

Expression profiling of the migraine susceptibility gene *KCNK18* in human, rat and mouse sensory ganglia. The trigeminally-enriched potassium channel, *KCNK18* is more highly expressed in the neural-enriched human trigeminal samples (a), and in the rat DRG relative to the sciatic nerve (b), suggesting a highly neural distribution with little expression in non-neural ganglia cells. In the mouse DRG, *Kcnk18* is expressed in several populations of neurons, previously classified in Usoskin et al. (2015). Two subclasses of neurons express both *Kcnk18* and *Calca*, which encodes the precursor to the calcitonin gene related peptide (CGRP) (top panel, (c); subclasses demarked by bracket). These cells, which encode both the potassium channel mutated in some patients with familial migraine, and which release CGRP peptide, which has been implicated in migraine, are potentially the subpopulation of cells by which this mutation causes migraine. *Mrgprd* and *Mrgpra3* are additional markers of the cells co-expressing these two genes. These cells also contain broader non-specific markers (middle panel, (c)) such as *Trpa1*, *Scn9a*, and *P2rx3* whereas *Trpv1*, *Tac1* and

Oprm1 are largely in a separate population of cells (bottom panel, (c)). Using t-distributed stochastic neighbor embedding (t-SNE) plots (D-F), we show the colocalization of *Kcnk18* and *Calca* transcripts in mouse DRG neurons sequenced in Li et al. (2016). Points represent cells in the database, and cells with similar gene expression are clustered together in the plot. Gene expression for *Calca* is high across many cells (d), compared to the more restricted expression of *Kcnk18* (e). A cluster of cells ((f), gold cells) have high expression of *Calca* (100 FPKM) and also express *Knck18* (5 FPKM), further implicating this population of neurons in migraine.

Table 1.

Demographics of the 22 individuals included in this study.

Sample Number	PMI (h)	Manner of Death	Gender	Age (y)
TG1	22.5	Suicide	F	18
TG2	40.5	Suicide	F	26
TG3	20.0	Suicide	M	27
TG4	31.0	Accident	F	35
TG5	32.5	Suicide	F	43
TG6	26.5	Accident	F	43
TG7	35.5	Suicide	F	50
TG8	29.0	Natural	F	61
TG9	26.0	Suicide	F	18
TG10	47.0	Accident	F	24
TG11	32.5	Suicide	M	25
TG12	27.0	Natural	F	35
TG13	36.0	Suicide	F	44
TG14	25.0	Accident	F	44
TG15	29.5	Suicide	F	50
TG16	57.5	Natural	F	64
TG17	–	Suicide	F	31
TG18	63.0	Suicide	F	40
TG19	55.5	Suicide	F	44
TG20	22.0	Accident	F	31
TG21	26.5	Natural	F	37
TG22	24.0	Suicide	F	44

Table 2.

Selected genes from the human trigeminal transcriptome. The hTG samples designated Neural are enriched for neurons and contain a high proportion of neuronal genes, whereas the Non-neuronal samples are comparatively neuronally-depleted. The correspondence to a similar analysis from rat is shown in the Rat DRG/SN (sciatic nerve) column. Genes highly enriched in the DRG relative to the sciatic nerve are mostly neural genes, whereas sciatic nerve enriched genes are largely markers of Schwann cells and connective tissue, and these can be found in both the DRG and the sciatic nerve. The majority of genes enriched in the neural trigeminal samples were also enriched in the DRG relative to the sciatic nerve. Conversely, the majority of genes enriched in the non-neural TG samples were also enriched in the sciatic nerve relative to the DRG.

Gene symbol	Gene name	hTG Neural	hTG Non-neural	hTG Neural/non	Rat DRG/SN
Calcium binding proteins					
<i>CALM3</i>	Calmodulin 3	379.6	131.3	2.9 *	2.7 *
<i>CALM1</i>	Calmodulin 1	319.3	144.8	2.2 *	3.0 *
<i>PVALB</i>	Parvalbumin	210.1	32.5	6.5 *	82.7 *
<i>RCAN2</i>	Regulator of calcineurin 2	82.7	17.5	4.7 *	3.7 *
<i>RCAN1</i>	Regulator of calcineurin 1	43.0	76.2	0.6	1.1
<i>RCAN3</i>	RCAN family member 3	5.5	3.7	1.5	1.4
<i>VSNL1</i>	Visinin like 1	78.6	7.9	9.9 *	5.7 *
<i>NCS1</i>	Neuronal calcium sensor 1	73.2	15.5	4.7 *	5.5 *
<i>HPCAL1</i>	Hippocalcin like 1	43.0	20.6	2.1 *	5.9 *
<i>NCALD</i>	Neurocalcin delta	20.5	7.7	2.7 *	5.1 *
<i>CALB2</i>	Calbindin 2	7.6	0.9	7.4 *	396.3 *
<i>CALB1</i>	Calbindin 1	4.8	0.3	10.8 *	10.3 *
<i>CASQ2</i>	Calsequestrin 2	6.1	2.5	2.4	0.1 *
<i>CASQ1</i>	Calsequestrin 1	1.5	0.9	1.6 *	0.4 *
Growth factor receptors and other kinases					
<i>NGFRAP1</i>	Brain expressed X-linked 3, BEX3	305.0	180.6	1.7 *	2.2 *
<i>NGFR</i>	Nerve growth factor receptor	225.9	144.0	1.6	50.3 *
<i>NTRK2</i>	TRKB	68.7	77.6	0.9	1.3
<i>NTRK1</i>	TRKA	50.1	3.5	13.9 *	222.6 *
<i>NTRK3</i>	TRKC	43.7	32.9	1.3	0.5 *
<i>GFRA1</i>	GDNF family receptor α 1	62.9	61.7	1.0	0.8
<i>GFRA3</i>	GDNF family receptor α 3	59.4	33.9	1.8	30.9 *
<i>GFRA2</i>	GDNF family receptor α 2	4.7	1.8	2.6 *	1.5
<i>JAK1</i>	Janus kinase 1	61.3	43.4	1.4	1.6
<i>RET</i>	Ret proto-oncogene	41.7	5.7	7.2 *	42.0 *
<i>KIT</i>	KIT proto-oncogene RTK	7.1	5.7	1.2	3.4 *
G-protein Coupled Receptors (GPCRs)					

Gene symbol	Gene name	hTG Neural	hTG Non-neural	hTG Neural/non	Rat DRG/SN
<i>HTR3A</i>	5-HT receptor 3A	27.4	5.3	5.1 *	195.7 *
<i>HTR1D</i>	5-HT receptor 1D	2.5	0.5	4.5 *	103.5 *
<i>HTR7</i>	5-HT receptor 7	1.7	0.3	4.1 *	1.0
<i>HTR1B</i>	5-HT receptor 1B	0.9	0.0	6.4 *	2.4 *
<i>HTR5A</i>	5-HT receptor 5A	0.7	0.0	6.5 *	2.8
<i>P2RY12</i>	Purinergic receptor P2Y12	20.8	14.4	1.4	0.5
<i>P2RY1</i>	Purinergic receptor P2Y1	12.5	9.2	1.4	1.7
<i>P2RY2</i>	Purinergic receptor P2Y2	6.1	4.3	1.4	0.2 *
<i>P2RY14</i>	Purinergic receptor P2Y14	5.9	8.1	0.7	0.1 *
<i>P2RY13</i>	Purinergic receptor P2Y13	2.2	1.8	1.2	0.3 *
<i>P2RY6</i>	Pyrimidinergic receptor P2Y6	1.0	2.1	0.5	0.4 *
<i>LPAR3</i>	Lysophosphatidic acid receptor 3	13.8	1.5	8.9 *	2.2 *
<i>PTGER3</i>	Prostaglandin E receptor 3	11.0	0.7	13.1 *	0.7
<i>PTGER4</i>	Prostaglandin E receptor 4	5.2	4.6	1.1	1.4
<i>PTGER2</i>	Prostaglandin E receptor 2	1.8	4.1	0.4 *	7.4 *
<i>OPRM1</i>	Opioid receptor mu 1	6.7	0.6	10.1 *	56.9 *
<i>OPRK1</i>	Opioid receptor kappa 1	4.0	0.8	4.6 *	0.7
<i>OPRL1</i>	Opioid related nociceptin receptor 1	1.8	0.6	2.7 *	174.6 *
<i>OPRD1</i>	Opioid receptor delta 1	0.2	0.0	2.4	9.8 *
<i>S1PR1</i>	Sphingosine-1-phosphate receptor 1	5.2	20.6	0.3 *	0.2 *
<i>FPR1</i>	Formyl peptide receptor 1	4.7	47.7	0.1 *	4.4 *
<i>FPR3</i>	Formyl peptide receptor 3	2.0	5.7	0.4	0.5
<i>CNR1</i>	Cannabinoid receptor 1	4.0	2.2	1.8	2.0 *
<i>CNR2</i>	Cannabinoid receptor 2	0.0	0.0	1.0	0.9
<i>ADORA3</i>	Adenosine A3 receptor	3.7	8.7	0.4	0.9
<i>ADORA1</i>	Adenosine A1 receptor	4.7	0.7	6.2 *	3.8 *
<i>ADORA2B</i>	Adenosine A2b receptor	2.3	3.5	0.7	1.7
<i>NPY1R</i>	Neuropeptide Y receptor Y1	1.9	1.0	1.9	0.7
Synaptic vesicle proteins					
<i>SYT11</i>	Synaptotagmin 11	168.4	56.1	3.0 *	5.9 *
<i>SYT2</i>	Synaptotagmin 2	41.9	3.2	12.9 *	1050.8 *
<i>SYT1</i>	Synaptotagmin 1	29.9	4.2	7.0 *	119.1 *
<i>SYT4</i>	Synaptotagmin 4	24.5	3.4	7.1 *	380.4 *
<i>SYT7</i>	Synaptotagmin 7	19.4	2.7	7.1 *	4.8 *
<i>SYT6</i>	Synaptotagmin 6	10.4	1.2	8.0 *	82.1 *
<i>SYT9</i>	Synaptotagmin 9	8.2	1.1	6.9 *	76.3 *
<i>SYT5</i>	Synaptotagmin 5	5.6	0.7	7.0 *	7.7 *

Gene symbol	Gene name	hTG Neural	hTG Non-neural	hTG Neural/non	Rat DRG/SN
<i>SYT12</i>	Synaptotagmin 12	2.7	0.5	4.8 *	24.1 *
<i>SYT15</i>	Synaptotagmin 15	0.6	0.4	1.5	0.2 *
<i>SYNPR</i>	Synaptoporin	12.1	2.9	4.0 *	727.0 *
Ligand and voltage-gated ion channels, subunits and interacting proteins					
<i>GRINA</i>	NMDAR subunit associated protein 1	122.9	98.2	1.3	2.5 *
<i>GRIN1</i>	NMDAR subunit 1	24.2	1.9	12.0 *	265.3 *
<i>GRIK3</i>	kainate receptor subunit 3	68.5	30.5	2.2 *	2.2 *
<i>GRIK2</i>	kainate receptor subunit 2	22.0	14.2	1.5	1.1
<i>GRIK5</i>	kainate receptor subunit 5	8.0	4.0	2.0 *	1.0
<i>GRIK1</i>	kainate receptor subunit 1	3.1	0.6	4.6 *	287.1 *
<i>GRIA2</i>	AMPA 2	11.2	8.2	1.4	9.6 *
<i>GRIA4</i>	AMPA 4	11.0	1.8	5.9 *	2.2 *
<i>GRIA3</i>	AMPA 3	2.9	1.1	2.4 *	1.7
<i>SCN4B</i>	Na ⁺ VGC β 4, Nav β 4	102.5	14.8	6.9 *	808.3 *
<i>SCN7A</i>	Na ⁺ VGC α 7, Nav2.1, Nav2.2	95.1	74.9	1.3	0.6
<i>SCN9A</i>	Na ⁺ VGC α 9, Nav1.7	41.7	13.4	3.1 *	20.0 *
<i>SCN11A</i>	Na ⁺ VGC α 11, Nav1.9	38.3	8.2	4.6 *	1019.5 *
<i>SCN10A</i>	Na ⁺ VGC α 10, Nav1.8	21.9	1.8	11.8 *	1483.8 *
<i>SCN8A</i>	Na ⁺ VGC α 8, Nav1.6	18.3	1.6	11.1 *	160.3 *
<i>SCN2B</i>	Na ⁺ VGC β 2	16.6	4.4	3.7 *	7.3 *
<i>SCN1B</i>	Na ⁺ VGC β 1	15.9	8.3	1.9 *	9.1 *
<i>SCN3B</i>	Na ⁺ VGC β 3	12.6	2.4	5.0 *	7.8 *
<i>SCN1A</i>	Na ⁺ VGC α 1, Nav1.1	8.4	0.5	14.0 *	533.3 *
<i>SCN2A</i>	Na ⁺ VGC α 2, Nav1.2	3.5	0.5	6.5 *	2.3 *
<i>SCN5A</i>	Na ⁺ VGC α 5, Nav1.5	2.5	0.7	3.4 *	4.5 *
<i>PIEZO2</i>	Piezo mechanosensitive channel 2	34.2	9.5	3.6 *	5.7 *
<i>PIEZO1</i>	Piezo mechanosensitive channel 1	15.7	24.6	0.6	0.1 *
<i>TRPV1</i>	TRP cation channel V1, VR1	27.7	10.1	2.7 *	12.9 *
<i>TRPV2</i>	TRP cation channel V2, VRL1	17.1	6.3	2.7 *	10.6 *
<i>TRPM3</i>	TRP cation channel M3	20.1	13.3	1.5 *	1.7
<i>TRPM7</i>	TRP cation channel M7	13.5	18.4	0.7	0.6
<i>TRPM2</i>	TRP cation channel M2	7.4	5.3	1.4	7.0 *
<i>TRPM8</i>	TRP cation channel M8	5.2	0.1	23.9 *	238.9 *
<i>TRPM4</i>	TRP cation channel M4	5.0	4.1	1.2	2.1 *
<i>TRPA1</i>	TRP cation channel A1	12.9	3.2	3.9 *	92.5 *
<i>TRPC1</i>	TRP cation channel C1	10.7	10.9	1.0	1.5

Gene symbol	Gene name	hTG Neural	hTG Non-neural	hTG Neural/non	Rat DRG/SN
<i>TRPC3</i>	TRP cation channel C3	1.4	1.0	1.4	4.1 *
<i>KCNIP3</i>	K+ VGC interacting protein 3	25.9	4.4	5.8 *	43.4 *
<i>KCNIP1</i>	K+ VGC interacting protein 1	5.2	1.2	3.9 *	20.8 *
<i>KCNIP4</i>	K+ VGC interacting protein 4	3.9	1.1	3.3 *	80.8 *
<i>HCN1</i>	Hyperpolarization activated K+ channel 1	25.3	12.7	2.0 *	4.8 *
<i>HCN2</i>	Hyperpolarization activated K+ channel 2	16.9	1.7	9.5 *	14.5 *
<i>HCN3</i>	Hyperpolarization activated K+ channel 3	2.5	1.3	1.9 *	39.3 *
<i>P2RX7</i>	Purinergic receptor P2X 7	12.3	10.8	1.1	0.5 *
<i>P2RX3</i>	Purinergic receptor P2X 3	11.9	1.4	7.9 *	141.6 *
<i>P2RX4</i>	Purinergic receptor P2X 4	7.7	8.1	0.9	0.9
<i>P2RX6</i>	Purinergic receptor P2X 6	6.2	1.1	5.4 *	1.9
<i>ACCN2</i>	Acid sensing ion channel 1	11.6	1.7	6.5 *	47.5 *
<i>ACCN1</i>	Acid sensing ion channel 2	1.3	0.4	2.9 *	12.7 *
<i>AQP1</i>	Aquaporin 1 (Colton blood group)	134.9	159.1	0.8	0.2 *
<i>AQP11</i>	Aquaporin 11	3.0	1.2	2.5 *	5.4 *
<i>AQP7</i>	Aquaporin 7	1.5	0.9	1.7	0.0 *
Neuropeptides and granins					
<i>SST</i>	Somatostatin	138.4	30.3	4.6 *	395.1 *
<i>TAC1</i>	Tachykinin precursor 1	127.5	27.2	4.7 *	1454.3 *
<i>PCSK1N</i>	ProSAAS	107.3	22.5	4.7 *	0.9
<i>SCG5</i>	Secretogranin V	79.2	12.2	6.5 *	187.4 *
<i>SCG2</i>	Secretogranin II	77.8	17.3	4.5 *	845.4 *
<i>SCG3</i>	Secretogranin III	24.2	4.4	5.3 *	21.0 *
<i>CALCA</i>	Calcitonin related polypeptide α	77.9	11.2	6.9 *	1099.0 *
<i>NMB</i>	Neuromedin B	47.4	11.6	4.1 *	47.5 *
<i>CHGB</i>	Chromogranin B	42.1	6.3	6.6 *	615.9 *
<i>CHGA</i>	Chromogranin A	35.7	3.4	10.1 *	248.8 *
<i>ADCYAP1</i>	AC activating polypeptide 1	7.3	1.7	4.1 *	127.5 *
<i>NPPC</i>	Natriuretic peptide C	2.2	1.4	1.5	1.9
<i>CARTPT</i>	CART prepropeptide	1.8	0.4	3.9 *	22.5 *
Toll-like receptors					
<i>TLR4</i>	Toll like receptor 4	9.3	14.4	0.6	0.6
<i>TLR2</i>	Toll like receptor 2	5.1	21.9	0.2 *	0.4 *
<i>TLR3</i>	Toll like receptor 3	3.4	4.1	0.8	0.3 *
<i>TLR5</i>	Toll like receptor 5	1.6	2.9	0.6	0.1 *

Gene symbol	Gene name	hTG Neural	hTG Non-neural	hTG Neural/non	Rat DRG/SN
<i>TLR10</i>	Toll like receptor 10	1.1	0.7	1.5 *	0.8
<i>TLR8</i>	Toll like receptor 8	0.3	1.1	0.3 *	0.3 *

* Significantly neurally-enriched (differential score > 83 in human, > 108 in rat) or non-neurally enriched (differential score > 105 in human and rat)

AC: adenylate cyclase; AMPAR: AMPA receptor; NMDAR: NMDA receptor; RTK: receptor tyrosine kinase; TRP: transient receptor potential; VGC: voltage-gated channel; hTG: human trigeminal; SN: sciatic nerve.

Table 3.

Migraine-related genes expressed in human trigeminal ganglion. The entries were extracted from the literature and/or the OMIM subsection of the NCBI PubMed web site. The hTG samples designated Neural are enriched for neurons and contain a high proportion of neuronal genes, whereas the Non-neuronal samples are comparatively neuronally-depleted.

Gene symbol	Gene name	Role in headache, migraine or trigeminal neuralgia	hTG Neural	hTG Non-neural	hTG Neural/non
<i>CALCA</i>	Calcitonin related polypeptide alpha (CGRP)	Elevated in serum post-ictal	77.9	11.2	6.9 *
<i>TNF</i>	Tumor necrosis factor α	Elevated in serum post-ictal	0.2	0.3	0.7
<i>PTGS1</i>	Prostaglandin-endoperoxide synthase 1, COX1	Prostaglandin synthesis	2.5	4.1	0.6
<i>PTGS2</i>	Prostaglandin-endoperoxide synthase 2, COX2	Prostaglandin synthesis	3.4	7.8	0.4
<i>CACNA1A</i>	Calcium voltage-gated channel subunit α 1A	Familial hemiplegic migraine	12.5	5.7	2.2 *
<i>ATPIA2</i>	ATPase Na ⁺ /K ⁺ + transporting subunit α 2	Familial hemiplegic migraine	309.0	204.7	1.5
<i>SCN1A</i>	Na ⁺ + voltage-gated channel α 1, Nav1.1	Familial hemiplegic migraine	8.4	0.5	14.0 *
<i>KCNK18</i>	K ⁺ + two pore domain channel K, 18	Familial hemiplegic migraine	4.8	0.9	4.9 *
<i>MTDH</i>	Metadherin	Migraine susceptibility	36.7	43.5	0.8
<i>LRP1</i>	LDL receptor related protein 1	Migraine susceptibility	196.3	278.7	0.7
<i>MEF2D</i>	Myocyte enhancer factor 2D	Migraine susceptibility	16.0	17.0	0.9
<i>ASTN2</i>	Astrotactin 2	Migraine susceptibility	32.7	22.4	1.5
<i>PRDM16</i>	PR/SET domain 16	Migraine susceptibility	11.7	9.7	1.2
<i>FHL5</i>	Four and a half LIM domains 5	Migraine susceptibility	6.6	4.3	1.5
<i>PHACTR1</i>	Phosphatase and actin regulator 1	Migraine susceptibility	13.5	4.9	2.7 *
<i>TGFB2</i>	Transforming growth factor β receptor 2	Migraine susceptibility	76.4	104.2	0.7
<i>SUGCT</i>	Succinyl-CoA:glutarate-CoA transferase, C7orf10	Migraine susceptibility	1.6	1.1	1.4
<i>MMP16</i>	Matrix metalloproteinase 16	Migraine susceptibility	0.6	0.5	1.1
<i>TSPAN2</i>	Tetraspanin 2	Migraine susceptibility	8.3	1.5	5.4 *
<i>AJAP1</i>	Adherens junctions associated protein 1	Migraine susceptibility	0.9	0.4	2.0 *
<i>TRPM8</i>	Transient receptor potential channel M8	Migraine susceptibility	5.2	0.1	23.9 *
<i>SLC24A3</i>	Solute carrier family 24 member 3	Migraine susceptibility	6.8	7.3	0.9
<i>FGF6</i>	Fibroblast growth factor 6	Migraine susceptibility	0.0	0.0	1.0
<i>PLCE1</i>	Phospholipase C ϵ 1	Migraine susceptibility	21.5	27.1	0.8
<i>KCNK5</i>	K ⁺ + two pore domain channel K, 5	Migraine susceptibility	4.3	4.6	1.0
<i>MRV11</i>	Murine retrovirus integration site 1 homolog	Migraine susceptibility	5.5	4.4	1.3
<i>HPSE2</i>	Heparanase 2 (inactive)	Migraine susceptibility	0.0	0.0	1.3
<i>CFDP1</i>	Craniofacial development protein 1	Migraine susceptibility	61.8	56.1	1.1
<i>RNF213</i>	Ring finger protein 213	Migraine susceptibility	69.0	60.5	1.1
<i>NRP1</i>	Neuropilin 1	Migraine susceptibility	30.4	44.8	0.7
<i>GPR149</i>	G protein-coupled receptor 149	Migraine susceptibility	1.5	0.1	9.1 *
<i>JAG1</i>	Jagged 1	Migraine susceptibility	44.8	33.1	1.4
<i>ZCCHC14</i>	Zinc finger CCHC-type containing 14	Migraine susceptibility	10.6	13.6	0.8
<i>GJA1</i>	Gap junction protein α 1	Migraine susceptibility	31.5	83.8	0.4

Gene symbol	Gene name	Role in headache, migraine or trigeminal neuralgia	hTG Neural	hTG Non-neural	hTG Neural/non
<i>ITPK1</i>	Inositol-tetrakisphosphate 1-kinase	Migraine susceptibility	57.6	44.6	1.3
<i>YAP1</i>	Yes associated protein 1	Migraine susceptibility	48.5	72.4	0.7
<i>CARF</i>	Calcium responsive transcription factor, ALS2CR8	Migraine susceptibility	6.8	6.6	1.0
<i>IGSF9B</i>	Immunoglobulin superfamily member 9B	Migraine susceptibility	1.5	2.1	0.7
<i>MPPED2</i>	Metallophosphoesterase domain containing 2	Migraine susceptibility	9.5	3.3	2.9 [*]
<i>NOTCH4</i>	Notch 4	Migraine susceptibility	7.6	8.6	0.9
<i>ESR1</i>	Estrogen receptor 1	Migraine susceptibility	0.5	0.8	0.7
<i>ESR2</i>	Estrogen receptor 2	Migraine susceptibility	0.3	0.5	0.6
<i>FSHR</i>	Follicle stimulating hormone receptor	Migraine susceptibility	0.0	0.1	0.8
<i>HCRTR2</i>	Hypocretin receptor 2	Association w/ cluster headache	0.0	0.0	1.0
<i>EDNRA</i>	Endothelin receptor type A	Migraine resistance	7.5	7.8	1.0
<i>PRRT2</i>	Proline rich transmembrane protein 2	Complex disorders w/ migraine	22.9	10.2	2.2 [*]
<i>CSNK1D</i>	Casein kinase 1 delta	Familial Sleep Phase Syndrome	46.8	42.0	1.1
<i>TREX1</i>	Three prime repair exonuclease 1	RVCL	9.5	5.9	1.6
<i>NOTCH3</i>	Notch 3	CADASIL	29.2	32.5	0.9
<i>COL4A1</i>	Collagen type IV alpha 1 chain	HIHRATL	57.2	108.6	0.5
<i>SCN9A</i>	Na + voltage-gated channel α subunit 9, Nav1.7	Trigeminal neuralgia	41.7	13.4	3.1 [*]
<i>SCN3A</i>	Na + voltage-gated channel α subunit 3, Nav1.3	Trigeminal neuralgia	0.7	0.6	1.2
<i>SCN10A</i>	Na + voltage-gated channel α subunit 10, Nav1.8	Trigeminal neuralgia	21.9	1.8	11.8 [*]

^{*} Significantly neurally-enriched (differential score = 83)

RVCL: retinal vasculopathy with cerebral leukodystrophy; CADASIL: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; HIHRATL: hereditary infantile hemiparesis, retinal arteriolar tortuosity and leukoencephalopathy