Review Article

Human tissue studies in primary headache disorders: A scoping review

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Abstract

Background: Despite the identification of structures with putative pathophysiological significance in primary headache disorders (e.g., posterior hypothalamus in cluster headache) there appears to be a paucity of human tissue studies examining the neuropathology of these regions.

Objective: To synthesize the extent and knowledge pertaining to direct human tissue analysis in primary headache disorders.

Methods: Scoping literature review.

Results: Of 2718 located articles, 15 were eligible for inclusion. These studies evaluated either migraine (9, 60%) or cluster headache (6, 40%). Approximately 75% were published before or during the era of the first edition of the International Classification of Headache Disorders. The most common study design was case-control (8, 53.3%), and the most commonly examined tissues equally included skin (3, 20%), muscle (3, 20%), and brain (3, 20%). Thematically, these manuscripts generally evaluated peripheral nervous and systemic pathology, as well as more targeted pathophysiological aspects, including mitochondrial and mast cell dysfunction.

Conclusions: While interest in this type of study design appears to be waning, histopathological evaluation of human tissue provides unparalleled opportunity to reveal novel pathophysiological insight. Considerations for future study design and reporting of work involving human tissue is suggested based on our review.

Keywords: Autopsy, biopsy, cluster, human tissue, migraine, neuropathology, primary headache

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INTRODUCTION

Primary headache disorders are defined as headache disorders not attributed to another disorder.[1] Primary headache disorders represent a diverse diagnostic group recognized by the International Classification of Headache Disorders, 3rd Edition (ICHD-3), typified by entities such as migraine, tension-type, and cluster headaches.[1] The pathophysiology of disorders such as a migraine and cluster headache has been established to have a neural mechanism on the basis of human neuroimaging and preclinical work,[2,3] with risk susceptibility conferred in the case of migraine based on genetic[4] and acquired mechanisms.[5] However, the pathophysiological significance of nearly all targets identified in genome-wide association studies of

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migraine remains to be elucidated, with only a variant in the transient receptor potential cation channel subfamily M member 8 having a known role in nociception.[6] In addition, despite numerous advantages, preclinical work has important associated pitfalls, including an inability to model the numerous complexities of headache disorders.[7,8]

The opportunity to gain direct and unbiased insights into primary headache disorders in humans exists in the form of direct human tissue analysis. However, the extent that this methodology has been utilized, as well as the quality and barriers encountered in executed studies has not been previously appraised. This approach has been utilized with success in allied fields, such as in the study of primary mood disorders and schizophrenia.[9,10] In many circumstances in the field of headache medicine, distinct structures of pathophysiological significance have been identified by functional imaging techniques (e.g., posterior hypothalamus in cluster headache[11]), but with completely unknown histopathological correlate. Examples of structural neuroimaging abnormalities with unknown pathology include the ubiquitous stroke-like lesions and white matter hyper-intensities seen in migraine, as well as the microscopic consequences of iron deposition in the periaqueductal gray.[12,13]

In this scoping review, we intended to synthesize the extent and knowledge pertaining to studies specifically analyzing human tissue with the intent to further understand primary headache disorders. The results are intended to document the current state-of-the-science with regard to the topic and establish a framework for future research directions.

METHODS

Overall design

The scoping review was not eligible for registration in the PROSPERO database due to the intent of synthesizing the extent, range, and characteristics of evidence on a particular topic. A core aim of a scoping-type review is to facilitate future study. The PRISMA extension for scoping reviews (PRISMA-ScR) was utilized in the preparation and execution of the current work before the initiation of the literature review.[14]

In contrast to a systematic review, a scoping review provides reporting independent of methodological quality or risk of bias.[14] However, limitations of the methodologies of included manuscripts will be commented upon in our reporting. Data reporting will be sub-divided by the corresponding primary headache diagnosis, using the categorization presented in the ICHD-3 as a framework.[1]

Search strategy and methods

An experienced medical librarian (DAD) conducted literature searches in the electronic databases PubMed (which includes Medline), Embase, Scopus, Web of Science, and the Cochrane Database of Systematic Reviews, and in ClinicalTrials.gov. A combination of keywords and subject headings were applied to retrieve broad results pertaining to analyzing human tissue of primary headache disorders. Representative search terms included "headache," migraine, "biopsy," "autopsy" and "physiopathology." Detailed search methodology is available upon request to the corresponding author. The number of papers retrieved from the databases was 2718. EndNote software was used to automatically and then manually de-duplicate the list of papers, which identified 139 duplicates resulting in a net 2579 total papers. The searches were limited to English-language and human subjects of all ages. Investigators (MAA, JHS) ran a series of hand-searches and identified 7 additional citations.

Inclusion and exclusion criteria

The following inclusion and exclusion criteria were utilized in the screening of abstracts and then full texts:

Inclusion

- All study designs
- Human studies
- All ages
- The study must include a headache diagnosis corresponding to and established by current ICHD-3,[1] or prior versions[15,16]
- Studies predating the 1988 first edition of the ICHD will be included if a reasonable attempt to classify and/or describe the headache disorder is provided, or reference to an alternative diagnostic template. The inclusion of these studies will require agreement by two study investigators
- For primary headache disorders, any tissue study was considered relevant that focused on understanding the biology of the disorder.

Exclusion

- Animal studies
- Human biochemical and genetic studies without histopathological analysis
- Studies of cellular components from human blood samples[17]
- Clinicopathological correlations of headache disorders not recognized by ICHD[18]
- Human tissue studies of structures relevant to headache disorders, but sampled from healthy controls[19]
- Studies in which the headache diagnosis cannot be confirmed by consensus agreement according to contemporary criteria.[20]

Screening and abstracting identified manuscripts

Following the application of our search strategy, two reviewers independently screened all titles and abstracts for more detailed review for study inclusion. Disagreements were resolved between reviewers by discussion, and involvement of a third reviewer, if needed. The agreed upon studies were each reviewed by two investigators for more in-depth evaluation of content to determine final study eligibility. A standardized data charting form will be utilized by the investigators abstracting the included manuscripts.

Abstracted data elements included: Publication year, country of origin, study design, patient age(s) and sex distributions, headache diagnosis, histopathological findings, and a qualitative description of study findings.

RESULTS

Characteristics of included studies

Manual review of full texts for eligibility resulted in 15 manuscripts for analysis [Figure 1]. The details of the manuscripts are summarized in Table 1.

Headache diagnoses were established with reference to ICHD-1 (6, 40%), ICHD-2 (3, 20%), or ICHD-3 (1, 6.7%). Five (33.3%) of the manuscripts were published before the first publication of ICHD in 1988, where inclusion was felt to be reasonable by agreement by the authors.

Diagnoses included migraine (9, 60%) and cluster headache (6, 40%). Among reports of migraine, diagnoses included migraine with and without aura (5, 55.5%), familial hemiplegic migraine (3, 33.3%), and chronic migraine (1, 11.1%). Among reports of cluster headache, 2 (33.3%) included patients with chronic cluster headache. All tissue studies of cluster headache included patients during an active cluster bout.

The study design was most commonly case-control (8, 53.3%), but also included case reports (3, 20%), case series (3, 20%), and one randomized controlled trial[21] with a histopathological primary endpoint.

Sources of samples included: Research volunteer (10, 66.6%), clinical samples (4, 26.6%), and necropsy/autopsy material (2, 13.3%).

Manuscripts evaluated biopsied or necropsy tissue from skin (3, 20%), muscle (3, 20%), brain (3, 20%), gastrointestinal mucosa (2, 13.3%), pericranial nerve (1, 6.7%), periosteum (1, 6.7%), nasal mucosa (1, 6.7%), and temporal artery (1, 6.7%).

Findings in human tissue studies of primary headache disorders

Migraine

In patients with migraine, mild abnormalities in muscle reflecting mitochondrial oxidative dysfunction were observed.[22,23] One of these series exclusively included 9 patients with migraine with aura, including 5 who had a presumed migrainous infarction.[23] In patients with familial hemiplegic migraine, muscle pathology was suggestive of mitochondrial oxidative dysfunction in two cases,[24] as well as demonstrating potential noninflammatory small vessel arteriopathy in another.[25]

In a case-control study, abnormalities predominantly in myelin were reported in a descriptive fashion in the zygomaticotemporal nerve obtained during so-called migraine surgeries.[26] Samples were obtained at least 5-mm from reported compression sites, and compared to nerve obtained from patients without migraine undergoing brow lift procedures.

In a more recent study of periosteal biopsy of the calvarium in patients with chronic migraine undergoing occipital nerve decompression, targeted transcriptomic analysis was compared with samples obtained from Parkinson's disease controls undergoing deep brain stimulation. The study controlled for medication exposures and Parkinson's – related genes. An upregulation of pro-inflammatory genes was identified, as well as a concomitant down-regulation of immune regulatory transcripts, suggesting an impact of extra-cranial pathophysiology in chronic migraine.[27]

Pradalier *et al*. evaluated duodenal biopsy samples in patients with migraines either self-reporting a food trigger or not, and did not identify differences in overall or Ig-subtyped plasmacytes.[28] Finally, in a larger case-control study, *Helicobacter pylori* was more common on gastric biopsies in patients with migraine (57.1%) versus control (33.3%).[29]

In a single case report of a patient with hemiplegic migraine, diagnostic brain and dural biopsies were obtained in the case also involving brain edema. Nonspecific reactive changes were observed in the brain.[30]

Cluster headache

In patients with cluster headache, mast cell degranulation was variably observed to be more common[31,32] or not[33,34] on the affected side, and mast cells to be overall more numerous[33] or not.[31] Data on preferential mast cell localization by nerve fibers were also conflicting based on qualitative observation alone.[31,33] A case report describing

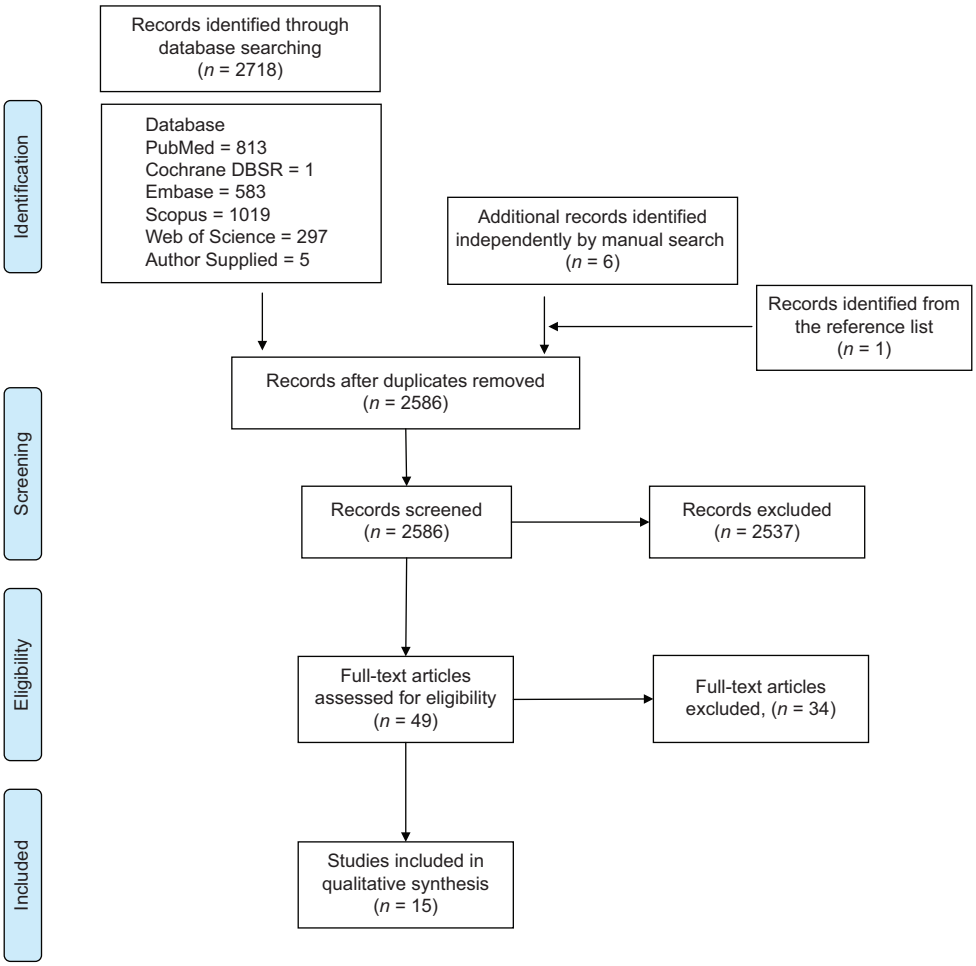

Figure 1: PRISMA flowchart

necropsy of a patient cluster headache was generally unrevealing, noting an incidental (subacute) hypothalamic infarct without further characterization.[35] Finally, a single-blind randomized controlled trial of hyperbaric oxygen in cluster headache demonstrated semi-quantitative reduction in substance P immunoreactivity in the nasal mucosa as a primary outcome.[21]

DISCUSSION

In our scoping-type review, diverse manuscripts were identified focusing on the diagnostic entities of migraine and cluster headache. Thematically, these manuscripts generally focused on peripheral and systemic headache pathology, as well as more targeted pathophysiological aspects, including mitochondrial and mast cell dysfunction. Interest in this type of study design appears to be waning, as nearly three-quarters of the identified publications were in the pre-ICHD or ICHD first edition era. The level of evidence was variable, with 6 (40%) of the studies lacking a control group. However, notable exceptions existed, most recently including a well-controlled evaluation of the periosteal transcriptome in patients with chronic migraine,[27] as well as a single-blinded randomized control trial in cluster headache with a histopathological primary endpoint.[21] The value of human tissue studies as identified in our review is highlighted in the following examples, directly demonstrating peripheral pathology and mitochondrial abnormalities in migraine.

The relative roles of central and peripheral nervous system pathology in migraines remain controversial. Central trigeminovascular neuronal activation may follow cortical spreading depression,[36] and hypothalamic activation is seen in the premonitory phase of migraine,[37] findings both indicative of centrally-driven pathways. In a preclinical model, lignocaine injection into the trigeminal ganglion did not prevent activation of central trigeminal neurons following cortical spreading depression, indicating a dependence on a purely central pathway.[38] However, on the basis of anatomic work demonstrating branching of intracranial nociceptors into extracranial tissue through calvarial sutures, as well as the efficacy of onabotulinumtoxinA in chronic migraine

Table 1: Human tissue studies in primary headache disorders

Reference (year)	Headache diagnosis (criteria)	Study design	Sample size for tissue analysis	Tissue studied (by biopsy, unless otherwise stated)	Histopathological findings
Appenzeller *et al*. (1981)	Episodic cluster headache (Pre-ICHD)	Case-control	6 cases (3 during a cluster period) 3 controls	Bilateral skin of temples	Light microscopy: Increased number of mast cells overall with additional localization near cutaneous nerves as compared to controls Electron microscopy: Degranulated mast cells not confirmed to be more common on side of attacks; degranulation occasionally observed in controls
Cevoli *et al*. (2010)	Migraine without aura (ICHD-2)	Case series of patients without the MELAS mutation (from within two families carrying the mutation)	11	Muscle (vastus lateralis)	Immunohistochemistry: Mild abnormalities of oxidative enzymes in 9/11 (81%), with ↑ subsarcollemal SDH and COX
Cha *et al*. (2007)	Familial hemiplegic migraine (ICHD-2)	Case report	1	Brain (temporal lobe) and dura (age 48) Brain (temporal lobe) and dura (age 50) Both biopsies during attacks	Light microscopy: Reactive lymphocytosis and astrogliosis (age 48), and marked astrocytosis and microgliosis (age 50)
Cuypers *et al*. (1980)	Cluster headache (pre-ICHD)	Case-control	6 cases (3 during a cluster period) 9 controls	Bilateral skin of temples (*n*=3) or affected side (*n*=3)	Light microscopy: No appreciable differences between patients and controls, or between affected and unaffected sides Electron microscopy: No morphological changes in the mast cells of the patients
Di Sabato *et al*. (1996)	Episodic cluster headache (ICHD-1)	Randomized, single-blinded, placebo-controlled trial of hyperbaric oxygen	14 (all during a cluster period)	Nasal mucosa of the middle turbinate	Immunohistochemistry: Semi-quantitatively assessed decrement in substance P immunoreactivity in patients treated with hyperbaric oxygen
Dimitriadou *et al*. (1990)	Episodic and chronic cluster headache (ICHD-1)	Case-control	19 cases (13 episodic, 6 chronic), all during a cluster period 10 controls	Temporal artery on the affected side	Light microscopy: Similar density of mast cells in cases and controls Electron microscopy: Mast cells similarly localizing to adventitial nerve fibers in cases and controls. Mast cell degranulation commonly observed in cases, but not in any controls
Guyuron *et al*. (2014)	Migraine (ICHD-2)	Case-control	15 cases 15 control	Trigeminal nerve, zygomaticotemporal branch	Electron microscopy: Labeling with myelin basic protein and neurofilament demonstrated a more linear organization, diminished and discontinuous myelination, and variable axonal abnormalities. Disorganized and excessive myelination of axons, constricting the axon
Krabbe *et al*. (1987)	Cluster headache (pre-ICHD)	Case report	1 (death at age 61 secondary to *Aspergillus pneumonia*)	Brain autopsy	Autopsy: Entire brain microscopically normal, apart from an estimated 2-3 week old microinfarct in the hypothalamus, and état de lacunaire of the small arterioles. Pituitary was not dissected
Liberski *et al*. (1984)	Cluster headache (pre-ICHD)	Case-control	13 cases (9 "sporadic," 4 "chronic"), all during a cluster period 10 headache controls (details not specified)	Skin of temporal area, on affected side	Electron microscopy: Degranulation pattern observed in 10/13 cases, involving 57%-100% of observed mast cells, but never in control samples. Findings most consistent with "piecemeal-type" degranulation
Montagna *et al*. (1988)	Migraine with aura (ICHD-1)	Case series	9, including 5 with migrainous stroke	Muscle (triceps or deltoid)	Light microscopy: Normal apart from one case where ragged red fibers were observed, along with occasional COX-negative fibers Electronic microscopy: Subsarcolemmal clusters of giant mitochondria, with concentric cristae and paracrystalline inclusions in the single abnormal case Muscle biochemistry: Depression of mitrochondrial respiratory chain enzyme activity in all migraine patients, as compared to controls

Contd...

Table 1: Contd...

Reference (year)	Headache diagnosis (criteria)	Study design	Sample size for tissue analysis	Tissue studied (by biopsy, unless otherwise stated)	Histopathological findings
Nelligan P *et al*. (1977)	Familial hemiplegic migraine (pre-ICHD)	Case report	1 (death at age 41 secondary to respiratory failure of uncertain etiology)	Brain autopsy	Autopsy: No lesions observed in any brainstem section. Widespread loss of Purkinje cells in the cerebellum, and gliosis of dentate nuclei. Cystic infarctions (small) in the basal ganglia. Small sulcal infarcts in the parietal and occipital lobes, sparing lamia 5 and 6. Small arteries largely normal, but some with variable changes in the elastic lamina, including reduplication or loss
Perry *et al*. (2016)	Chronic migraine (ICHD-3)	Case-control	18 cases undergoing occipital nerve decompression surgery 7 headache-free controls with Parkinson's disease undergoing deep brain stimulation surgery	Calvarial periosteum	Targeted transcriptome analysis: Upregulation of pro-inflammatory genes and downregulation of genes responsible for suppression of inflammatory process and immune cell differentiation. Putative four gene biomarker set for chronic migraine (*CLEC4E, IL1R2, TNFAIP3, NF-κLEC*)
Pradalier *et al*. (1994)	Migraine without aura (ICHD-1)	Case-control	11 with self-reported food- induced migraine (cases) 9 without (controls)	Duodenum	Light microscopy: No differences Immunohistochemistry: No difference in total plasmacytes, or for Ig subtypes
Tunca *et al*. (2004)	Migraine with and without aura (ICHD-1)	Case-controls	70 cases 60 controls	Gastric	Light microscopy with Giemsa stain: *Helicobacter Pylori* found in 40 (57.1%) of migraine patients, as compared to 20 (33.3%) controls
Uncini *et al*. (1995)	Familial hemiplegic migraine (ICHD-1)	Case series	2	Muscle (biceps brachialis)	Light microscopy: Rare ragged red fibers in 1 case, which were COX negative. Normal in the other. Mitochondrial enzyme activities were normal in both patients Electron microscopy: Subsarcollemal giant mitochondria

ICHD: International Classification of Headache Disorder, MELAS: Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes, SDH: Succinate dehydrogenase, COX: Cytochrome-c oxidase

prevention, an extracranial "origin" of migraine has been hypothesized.[39] Further, neuroimaging using an inflammatory radiotracer has demonstrated extra-axial inflammatory signals in the meninges in migraine, also consistent with long-standing preclinical work modeling peripheral neurogenic inflammation.[40] In our review, human tissue studies were consistent with the involvement of peripheral trigeminovascular pathways;[26,27] however, a temporal sequence of recruitment and contribution relative to central pathways cannot be deduced.

Prior work has also implicated a mismatch in cerebral metabolism and antioxidant capacity as a homeostatic imbalance that may trigger migraine.[41] This work has often implicated mitochondria as a putative substrate, consistent with identified spectroscopic abnormalities in brain mitochondrial metabolism and clinical efficacy of suggestive nutraceuticals (e.g., coenzyme Q10) in migraine prevention.[41] However, a recent mitochondrial genome-wide association study looking at 4021 migraine patients and 14,288 controls did not support a role for mitochondrial genetic variation in migraine pathophysiology.[42] In our review, the value of human tissue work is highlighted as being able to directly visualize mitochondrial abnormalities in patients with migraine.[22-24]

Strengths of human tissue research as suggested by identified references include the elimination of assumptions inherent in preclinical work, as well as the possibilities for study designs to be both hypothesis-generating[26] without a clear end-point, as well as hypothesis-testing, which was the case in the majority of studies. However, among hypothesis-testing studies, clearly defined primary outcome measures were rarely present, and not well defined, making the evaluation of the results challenging. Outcomes were often qualitative or semi-quantitative, likely susceptible to reporting biases. Putative abnormalities, such as mast cell degranulation, were noted in control samples in one study, highlighting the importance of including a control group.[33]

The source of samples and controls was also variable, most often derived from patient volunteers. Sources of samples come with advantages and disadvantages that investigators must consider, weighing ease and ethics of

Table 2: Additional recommendations for reporting human tissue studies

	Recommendation(s)
Reporting (general)	Reporting should follow existing reporting guidelines, as appropriate, for the study design (eg., STROBE), where the same core reporting principles should still apply (eg, sample-size estimation).
Title	Identify the study by design (e.g., cross-sectional), as well as by use of "human tissue"
Abstract	Identify the source(s) of case and control tissue
	Identify the corresponding clinical diagnosis of included cases
Introduction	
Background	Cite any relevant preclinical work justifying use of human tissue
Objectives	Clearly describe the primary and any secondary objectives of the study, as well as the specific hypotheses being tested. Otherwise, the study should be defined as exploratory
Methods	
Ethics	A statement should be made indicating that the study protocol was in accordance with the ethical standards of Helsinki declaration, as well as other responsible committees on human experimentation
Source identification	The source(s) of case and control tissue should be identified, and described in detail, with elaboration of a rationale for selection
Case definition(s)	Diagnostic criterion should be referenced in the description of included cases. In instances where diagnosis is unknown, the authors should report as much relevant clinical description as-is appropriate
Resource sharing	Authors should indicate whether tissue samples are available for sharing to qualified investigators with appropriate institutional approvals
Results	
Cohort description	Relevant clinical covariates particular to the diagnostic entity should be reported in order for the reader to best understand the generalizability of the pathologic studies being performed
Adverse events	In studies involving volunteers consenting to provide a tissue sample, any incident adverse events in the course of those procedures should be reported
Description of findings	A systematic description of relevant histopathological findings in qualitative, semi-quantitative and/or quantitative means, as appropriate to the prespecified outcome measures
Discussion	
Generalizability	Relevant confounding comorbidities related to the clinical sample source, as well as confounders inherent to the study methodology should be elaborated upon

access, as well as the introduction of confounding biology. The voluntary use of superficial biopsies in controlled populations allows for a more rigorously controlled study design, whereas the use of clinical/surgical specimens may be more ethically acceptable, at the cost of some control over clinical covariates. Autopsy allows for in-depth evaluation of otherwise inaccessible targets, however, clinical correlates may be inadequate, and medical and neurologic confounders may be present. Krabbe[35] provides an indication of the potential yield of reviewing autopsy specimens, noting the death of 16/337 (5%) cluster headache patients over a 10-year interval. International brain banks exist with searchable databases that researchers can access. Notable examples include the National Institutes of Health NeuroBioBank (https://neurobiobank.nih.gov/specimens/) and UK Brain Bank Networks (https://mrc.ukri.org/research/facilities-and-resources-for-researchers/brain-banks/).

Limitations of this current manuscript include the probability that despite our best efforts, our search may not have been completely exhaustive given the broad scope of our review. In an effort to be methodologically rigorous, we also may have excluded manuscripts of potential relevance. For example, certain publications included relevant histopathological analysis, but case definitions could not be confirmed allow inclusion in our review, such as in a basic science study including analysis of a dorsal root ganglion from a possible migraine case.[43] In these cases, more deliberate efforts at inter-disciplinary collaboration may remedy such pitfalls. Finally, our prespecified inclusion/criteria did not allow the inclusion of human studies examining individual cellular elements from hematological samples.[17]

The analysis of human tissue with the intention of understanding headache neurobiology is ripe for future work. Our current review suggests that in addition to standardized reporting guidelines, additional requirements in the realm of human tissue studies would be relevant and useful [Table 2].

CONCLUSIONS

In our scoping review, we identified a role for human tissue studies in primary headache disorders in providing direct evidence for the involvement of trigeminal nerves, as well as mitochondrial and mast cell dysfunction in headache pathophysiology.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd edition. Cephalalgia 2018;38:1-211.
2. Brennan KC, Pietrobon D. A systems neuroscience approach to migraine. Neuron 2018;97:1004-21.
3. May A, Schwedt TJ, Magis D, Pozo-Rosich P, Evers S, Wang SJ. Cluster headache. Nat Rev Dis Primers 2018;4:18006.
4. Gormley P, Kurki MI, Hiekkala ME, Veerapen K, Happola P, Mitchell AA, *et al*. Common variant burden contributes to the familial aggregation of migraine in 1,589 families. Neuron 2018;99:1098.
5. Buse DC, Greisman JD, Baigi K, Lipton RB. Migraine progression: A systematic review. Headache 2019;59:306-38.
6. Gormley P, Anttila V, Winsvold BS, Palta P, Esko T, Pers TH, *et al*. Meta-analysis of 375,000 individuals identifies 38 susceptibility loci for migraine. Nat Genet 2016;48:856-66.
7. Akerman S, Holland PR, Hoffmann J. Pearls and pitfalls in experimental *in vivo* models of migraine: Dural trigeminovascular nociception. Cephalalgia 2013;33:577-92.
8. Harriott AM, Strother LC, Vila-Pueyo M, Holland PR. Animal models of migraine and experimental techniques used to examine trigeminal sensory processing. J Headache Pain 2019;20:91.
9. Harrison PJ. The neuropathology of schizophrenia. A critical review of the data and their interpretation. Brain 1999;122(Pt 4):593-624.
10. Harrison PJ. The neuropathology of primary mood disorder. Brain 2002;125:1428-49.
11. Goadsby PJ, May A. PET demonstration of hypothalamic activation in cluster headache. Neurology 1999;52:1522.
12. Kruit MC, van Buchem MA, Launer LJ, Terwindt GM, Ferrari MD. Migraine is associated with an increased risk of deep white matter lesions, subclinical posterior circulation infarcts and brain iron accumulation: The population-based MRI CAMERA study. Cephalalgia 2010;30:129-36.
13. Palm-Meinders IH, Koppen H, Terwindt GM, Launer LJ, van Buchem MA, Ferrari MD, *et al*. Iron in deep brain nuclei in migraine? CAMERA follow-up MRI findings. Cephalalgia 2017;37:795-800.
14. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, *et al*. PRISMA extension for scoping reviews (PRISMA-ScR): Checklist and explanation. Ann Intern Med 2018;169:467-73.
15. Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. Headache Classification Committee of the International Headache Society. Cephalalgia 1988;8 Suppl 7:1-96.
16. Headache Classification Subcommittee of the International Headache S. The International Classification of Headache Disorders: 2nd edition. Cephalalgia. 2004;24 Suppl 1:9-160.
17. D'Andrea G, Welch KM, Riddle JM, Grunfeld S, Joseph R. Platelet serotonin metabolism and ultrastructure in migraine. Arch Neurol 1989;46:1187-9.
18. Cutrer FM, Sandroni P, Wendelschafer-Crabb G. Botulinum toxin treatment of cephalalgia alopecia increases substance P and calcitonin gene-related peptide-containing cutaneous nerves in scalp. Cephalalgia 2010;30:1000-6.
19. Eftekhari S, Salvatore CA, Calamari A, Kane SA, Tajti J, Edvinsson L. Differential distribution of calcitonin gene-related peptide and its receptor components in the human trigeminal ganglion. Neuroscience 2010;169:683-96.
20. Selby G, Fryer JA. Fatal migraine. Clin Exp Neurol 1984;20:85-92.
21. Di Sabato F, Giacovazzo M, Cristalli G, Rocco M, Fusco BM. Effect of hyperbaric oxygen on the immunoreactivity to substance P in the nasal mucosa of cluster headache patients. Headache 1996;36:221-3.
22. Cevoli S, Pallotti F, La Morgia C, Valentino ML, Pierangeli G, Cortelli P, *et al*. High frequency of migraine-only patients negative for the 3243 A>G tRNALeu mtDNA mutation in two MELAS families. Cephalalgia 2010;30:919-27.
23. Montagna P, Sacquegna T, Martinelli P, Cortelli P, Bresolin N, Moggio M, *et al*. Mitochondrial abnormalities in migraine. Preliminary findings. Headache 1988;28:477-80.
24. Uncini A, Lodi R, Di Muzio A, Silvestri G, Servidei S, Lugaresi A, *et al*. Abnormal brain and muscle energy metabolism shown by 31P-MRS in familial hemiplegic migraine. J Neurol Sci 1995;129:214-22.
25. Neligan P, Harriman DG, Pearce J. Respiratory arrest in familial hemiplegic migraine: A clinical and neuropathological study. Br Med J 1977;2:732-4.
26. Guyuron B, Yohannes E, Miller R, Chim H, Reed D, Chance MR. Electron microscopic and proteomic comparison of terminal branches of the trigeminal nerve in patients with and without migraine headaches. Plast Reconstr Surg 2014;134:796e-805.
27. Perry CJ, Blake P, Buettner C, Papavassiliou E, Schain AJ, Bhasin MK, *et al*. Upregulation of inflammatory gene transcripts in periosteum of chronic migraineurs: Implications for extracranial origin of headache. Ann Neurol 2016;79:1000-13.
28. Pradalier A, de Saint Maur P, Lamy F, Launay JM. Immunocyte enumeration in duodenal biopsies of migraine without aura patients with or without food-induced migraine. Cephalalgia 1994;14:365-7.
29. Tunca A, Türkay C, Tekin O, Kargili A, Erbayrak M. Is helicobacter pylori infection a risk factor for migraine? A case-control study. Acta Neurol Belg 2004;104:161-4.
30. Cha YH, Millett D, Kane M, Jen J, Baloh R. Adult-onset hemiplegic migraine with cortical enhancement and oedema. Cephalalgia 2007;27:1166-70.
31. Dimitriadou V, Henry P, Brochet B, Mathiau P, Aubineau P. Cluster headache: Ultrastructural evidence for mast cell degranulation and interaction with nerve fibres in the human temporal artery. Cephalalgia 1990;10:221-8.
32. Liberski PP, Mirecka B. Mast cells in cluster headache. Ultrastructure, release pattern and possible pathogenetic significance. Cephalalgia 1984;4:101-6.
33. Appenzeller O, Becker WJ, Ragaz A. Cluster headache. Ultrastructural aspects and pathogenetic mechanisms. Arch Neurol 1981;38:302-6.
34. Cuypers J, Westphal K, Bunge S. Mast cells in cluster headache. Acta Neurol Scand 1980;61:327-9.
35. Krabbe AA, Laursen, H. The structure of the brain in a cluster headache patient. An autopsy study. Cephalalgia 1987;7:338.
36. Zhang X, Levy D, Kainz V, Noseda R, Jakubowski M, Burstein R. Activation of central trigeminovascular neurons by cortical spreading depression. Ann Neurol 2011;69:855-65.
37. Maniyar FH, Sprenger T, Monteith T, Schankin C, Goadsby PJ. Brain activations in the premonitory phase of nitroglycerin-triggered migraine attacks. Brain 2014;137:232-41.
38. Lambert GA, Truong L, Zagami AS. Effect of cortical spreading depression on basal and evoked traffic in the trigeminovascular sensory system. Cephalalgia 2011;31:1439-51.
39. Burstein R, Blake P, Schain A, Perry C. Extracranial origin of headache. Curr Opin Neurol 2017;30:263-71.
40. Hadjikhani N, Albrecht DS, Mainero C, Ichijo E, Ward N, Granziera C, *et al*. Extra-axial inflammatory signal in parameninges in migraine with visual aura. Ann Neurol 2020;87:939-49.
41. Gross EC, Lisicki M, Fischer D, Sándor PS, Schoenen J. The metabolic face of migraine – From pathophysiology to treatment. Nat Rev Neurol 2019;15:627-43.
42. Børte S, Zwart JA, Skogholt AH, Gabrielsen ME, Thomas LF, Fritsche LG, *et al*. Mitochondrial genome-wide association study of migraine – The HUNT study. Cephalalgia 2020;40:625-34.
43. Landry M, Aman K, Dostrovsky J, Lozano AM, Carlstedt T, Spenger C, *et al*. Galanin expression in adult human dorsal root ganglion neurons: Initial observations. Neuroscience 2003;117:795-809.