MARCH 2019

MIGRAINE RESEARCHER ROUNDTABLE SUMMARY

A MEETING OF CLINICAL AND BASIC SCIENCE RESEARCHERS WITH THE GOAL OF INITIATING IMPORTANT, UNMET RESEARCH PROJECTS IN THE FIELD OF MIGRAINE.

ASSOCIATION OF MIGRAINE DISORDERS

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Overview

On March 31, 2019, The Association of Migraine Disorders (AMD) convened an interdisciplinary scientific expert advisory panel in Providence, Rhode Island for a one-day roundtable meeting with the goal of building an inter- and multi-disciplinary research community to address unmet research needs in migraine. The expert advisory panel focused their discussions on five topic areas: clinical aspects in migraine, migraine mimicking rhinosinusitis, role of hormones and sex differences in migraine, lessons learned from chronic pain and preclinical models of migraine. The expert advisory panel members were basic and clinical researchers and represented diverse perspectives, experiences and thought leadership from the areas of migraine, chronic pain, otolaryngology, stroke and depression. Following the discussions on the five topic areas, the expert advisory panel members identified several recommendations pertaining to these areas.

About AMD

The Association of Migraine Disorders (AMD) is a non-profit organization founded in 2012 by otolaryngologists, with the mission to expand the understanding of migraine and its true scope by supporting research, education and awareness. In support of that mission, one of AMD's main goals is to build an inter- and multi-disciplinary research community to address unmet research needs in migraine by viewing it as a multisystem neurovascular disease involving multiple medical specialties.

To further that goal, AMD is interested in establishing a multi-year migraine research fund with a goal of 1) attracting non-migraine researchers to the migraine community, 2) encouraging cross-collaborations among basic and clinical researchers, 3) providing support to young investigators and 4) forming an inter-institutional and multi-disciplinary network of migraine researchers.

Acknowledgements

AMD would like to thank the expert panel for contributing to the robust discussions at the roundtable and their input during the preparation of this report. A special thanks to Dr. Monica Mallampalli for moderating the panel discussions and for preparing this report, AMD staff for organizing the meeting and Dr. Frederick Godley and Dr. Michael Teixido for critical input during the discussions. AMD would like to acknowledge Eli Lilly for their generous support towards the program meeting.

Introduction

Migraine is a complex neurobiological disorder impacting multiple systems within the body. The global burden of migraine is disproportionately higher in women (18.9 percent) compared to men (9.8 percent) and the US burden of migraine is highest among women aged 15 to 49 years [1-5]. Migraine is ranked as the second most common cause of disability impacting 1.04 billion individuals worldwide. Migraine is also associated with several comorbid conditions including cardiovascular, neurological, mental health and various other medical conditions contributing to its complex pathophysiology. However, the underlying biology between migraine and its comorbidities is still relatively unknown.

Migraine is characterized by distinct phases and different parts of the brain are activated during each of these phases. The first phase, called prodrome or premonitory, marks the beginning of a migraine attack lasting a few hours to days and is characterized by a number of symptoms including yawning, polyuria, mood and appetite changes, irritability, light sensitivity and neck pain. Changes in hypothalamus function is thought to cause polyuria, mood and appetite changes, whereas the light sensitivity has been shown to be correlated with increased activity in the occipital cortex and the nausea has been attributed to brainstem activation [6]. The second or the aura phase, experienced by some but not all people with migraine, immediately precedes or accompanies a headache (some patients could also experience only auras and no headache) and is characterized by several visual symptoms such as blurred vision and temporary vision loss which arise a slowly propagating depolarization of neurons and glia within grey matter structures initiating in the occipital lobe [7]. The neurological deficits such as numbness or tingling on the body including language disturbance are reversible and can last between 5-60 minutes before a migraine attack. Cortical spreading depression (CSD) is thought to be the underlying pathophysiological mechanism for this second phase [8]. The third phase, the headache phase, follows within an hour of resolution of the aura symptoms and is characterized by unilateral throbbing pain with moderate to severe intensity and is thought to originate from the activation of the trigeminovascular system centers in the brain stem [9, 10]. Other accompanying symptoms may include nausea and vomiting, as well as sensory amplifications: photophobia, phonophobia, osmophobia, hyperalgesia, and allodynia. These sensory amplifications are an important part of both the migraine attack and its chronification – they distinguish migraine from other pain disorders, and they are a significant part of disease burden [11]. The headache phase can last from 4-72 hours without treatment and can be aggravated by physical exertion. Activation of the trigeminovascular system is thought to cause the head pain in a migraine attack [9]. A network of nerves relating to the perception or sensation originate from the trigeminal ganglion in the TVS and innervate the scalp, skull, dura, and large intracranial blood vessels. The latter structures can be stimulated, as shown in animal studies, by mechanical, chemical, or electrical means, resulting in headache pain very similar to the pain in migraine. This stimulation also results in other symptoms associated with migraine, including nausea and photophobia resulting in behavioral and physiological phenotypes consistent with what is experienced in people with migraine [11, 9]. It is important to point out that repetitive attacks over the years can lead to functional and structural changes in the brain networks reflecting a change from symptoms of episodic to chronic. This transition is thought to involve a shift from the transient sensory amplifications of the migraine attack to a persistent state of sensitizations and sensory amplifications likely involving many mechanisms involved in network function [11]. During the final phase known as postdrome, symptoms from the prodrome phase are once again observed and it is unclear whether these symptoms initiate in the prodrome, persist through the headache phase and follow into the postdrome phase.

Having a better understanding of these various phases leading to migraine pathophysiology can help a clinician treat the patient specifically during each phase. Furthermore, defining the underlying pathophysiological mechanisms of these different phases can allow for the development of preventive therapeutics. It is also important to conceptualize

the disease in all of its aspects – through time as described above, but also in terms of all of its characteristics – head pain, but also the prominent sensory amplifications that accompany the pain – migraine is truly a network disorder, likely involving the whole brain and whole body [9, 11].

Migraine disease is characterized by changes within an individual throughout their life span and also by the influence of external factors, such as chronic stress, on these changes within the individual. Understanding sex and gender differences is critical to studying migraine pathophysiology as the disease disproportionately impacts women and men. Sex differences refer to the biological and physiological differences between women and men, where sex hormones and sex chromosomes contribute to these differences at cellular, organ and system levels. Gender differences refer to the influence of a combination of environmental, social and cultural influences on the biological factors in women and men Medicine [12]. Since migraine has a sex-specific prevalence, both female and male hormones are thought to have an influence on the pathophysiology of migraine and at least in part responsible for the sex differences [13]. Other sex-related factors that can play a role, and have not been extensively explored, are the effects of sex hormones on development (so-called organizational effects of sex hormones) and sex chromosome differences [14, 15].

AMD hosted a one-day interdisciplinary expert advisory panel discussion on March 31, 2019 in Providence, Rhode Island, as part of its efforts toward establishing a migraine research fund. The members of the expert advisory panel were carefully selected for their diverse perspectives, ability to provide thought leadership and represented basic and clinical researchers from the areas of migraine, chronic pain, otolaryngology, stroke and depression **(Table 1).** The clinical researchers on the panel had diverse backgrounds that included specific expertise in treating and managing migraine and co-morbidities in women.

Table 1: ***Scientific Expert Advisory Panel, Names and Affiliations***

Amynah Pradhan, PhD, Assistant Professor, Psychiatry, University of Illinois, Chicago, IL
Angela O'Neal, MD, Assistant Professor, Neurology, Brigham and Women's Hospital, Boston, MA
Carl Saab, MD, Associate Professor, Neuroscience, Brown University, Providence, RI
Dan Levy, PhD, Associate Professor, Anesthesia, Harvard Medical School, Boston, MA
David Witsell, MD, Professor, Otolaryngologist, Duke University, Durham, NC
Georgia E. Hodes, PhD, Assistant Professor, Neuroscience, Virginia Tech, Blacksburg, VA
Gregory Dussor, PhD, Associate Professor, Behavioral and Brain Sciences, University of Texas at Dallas, Richardson, TX
Jeff Mogil, PhD, Professor, Psychology, McGill University, Montreal, Canada
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Paul L. Durham, PhD, Distinguished Professor, Biology, Missouri State University, Springfield, MO
Shivang Joshi, MD, **MPH, RPh**, Headache Physician, Neurology, DENT Neurologic Institute, Amherst, NY
Stephen Silberstein, MD, Professor, Neurology, Thomas Jefferson University, Philadelphia, PA

The scope of the discussion at the one-day in-person roundtable focused on the following five areas: 1) clinical aspects of migraine, 2) migraine mimicking recurring rhinosinusitis 3) role of hormones and sex differences in migraine, 4) animal models, pain and lessons learned, and 5) preclinical models of migraine. Within each area, the expert advisory panel discussed existing research gaps, potential challenges and future opportunities in the migraine field. The highlights of the one-day roundtable meeting are discussed below.

Clinical Aspects of Migraine

The journey of a migraine patient is very tedious and can take several years before they get appropriate diagnosis and care from a headache specialist. The challenges during this journey include visits to several specialists, missed opportunities in life, financial difficulties and not receiving appropriate treatments. These challenges pose an opportunity to educate physicians on patient's struggles in many medical specialties. The current International Classification of Headache Disorders (ICHD-3) diagnostic criteria only describe the core migraine symptoms, but omit other symptoms such as osmophobia, allodynia, imbalance and cognitive dysfunction as diagnostic criteria for migraine [16, 17]. Recognition of these through patient engagement and making an accurate migraine diagnosis is important. Ruling out alternate etiologies and secondary causes, ordering appropriate tests and addressing headache's impact are other important factors leading to appropriate headache treatment. Unfortunately, there is not enough research data to actually incorporate these symptoms as a part of a diagnostic criteria, but awareness can help a clinician to provide appropriate migraine relief to patients who are suffering.

Migraine is a bio-psycho-social disease state; therefore, treatment should be based up on integration of acute, behavioral and prevention approaches to manage migraine. Interestingly, migraine prevention treatment is underutilized as only 1 in 5 migraine sufferers use preventive therapy according to the American Migraine Prevalence and Prevention (AMPP) study [18]. One of the many challenges with migraine treatment is efficacy and tolerability of current preventive medicines. Based on current U.S. guidelines, it is not advisable to use some of these preventive medications or acute medications in certain populations due to safety concerns. Currently, there are a wide class of non-migraine selective preventive medications available, but several new ones are in development [19].

Migraine Mimicking Recurring Rhinosinusitis

Otolaryngologists routinely come across middle-aged women complaining of recurring rhinosinusitis. The sinus pain in these patients can be relieved with antibiotics for a few days before the pain reoccurs. These patients present a clear and normal CT scan indicating that the condition is most likely neurological rather than an infection. Several clinical studies have documented evidence that patients with rhinosinusitis symptoms meet the criteria for migraine disease and were likely misdiagnosed [20]. Surprisingly, these patients respond to migraine treatments suggesting that recurring rhinosinusitis may be another presentation of migraine [21-23].

There are pathological explanations for how the symptoms are generated and trigeminovascular system is known to be involved in generating the sinus pressure; however, it is unclear which of the nerve sensors are measuring this pressure. It is further known that parasympathetic nerve is creating the nasal congestion and the runny nose that is often mistaken for rhinosinusitis. Some patients also complain of middle ear pressure, which most physicians mistake it for eustachian tube dysfunction, but tympanograms in these patients show that middle ear pressures are normal suggesting that several unanswered questions still remain. There is a need for greater understanding of how migraine may be an underlying cause of rhinosinusitis since this has implications on how otolaryngologists prescribe antibiotics

and overuse surgery in these patients. Although the evidence is limited, there is enough to support the theory that recurring rhinosinusitis could be an atypical migraine [20]. Vestibular migraine, on the other hand, has defined diagnostic criteria and has been recognized by the neurology community. Therefore, there is now a need for new clinical guidelines, diagnostic criteria, diagnostic code and a formal name for migraine mimicking recurring rhinosinusitis.

A recent cross-sectional study was performed to evaluate the prevalence of migraine in an otolaryngologic cohort utilizing the CHEER (Creating Healthcare Excellence through Education and Research) Network [24]. This Network is comprised of 30 (academic and community-based clinics) across the country with 200 otolaryngologists. Patients were recruited through 14 CHEER sites between June 2015 and March 2017 and were first screened for migraine using Migraine Assessment Tool (MAT). If tested MAT positive (MAT+), they were further required to fill out validated and custom questionnaires for sinonasal, otologic and migraine-specific symptoms. The key findings from this study were that 1) MAT+ (16.1 percent) were greater than general population (13 percent), 2) two-thirds of the patients screened positive for migraine (MAT+) were previously undiagnosed, 3) the MAT+ patients were predominantly women and middle-aged, and 4) they reported ear and sinus related symptoms such as tinnitus, ear pressure, balance problems, facial pressure and rhinorrhea. The high rate of missed diagnosis among otolaryngologists highlights the need for the symptoms to be addressed in ICHD-3 and the need for education and training among otolaryngologists for recognition of this atypical migraine [24].

Role of Hormones and Sex Differences in Migraine

There is clearly a relationship between sex hormones and migraine across a woman's lifespan. For example, the rate of change of migraine incidence is highest during adolescence in girls [25]. The prevalence rate quickly deaccelerates closer to age 30 but picks up again closer to ages 40 and 50 during the perimenopausal transition [26]. Earlier age of menarche predicts an increased risk of developing migraine by age 25 suggesting puberty contributes to the risk of developing migraine [27]. Girls had a seven percent increased risk of developing migraine by age 25 if they had menarche at an earlier age. Migraine in women also correlates with changing levels of female hormones. In menstrual-related migraine, the migraine occurs two days before the onset of bleeding and remains for 3-5 days into bleeding during the menstrual cycle when both estrogen and progesterone levels decline. Also, during the menopausal transition there is a significant change in hormone levels when progesterone levels starts waning and estrogen levels start fluctuating. These changes correlate with an increase in headache frequency among women already diagnosed with migraine [28]. Pregnancy is protective for migraine due to steadily rising estrogen and progesterone and stable hormone levels. However, in some women a new onset of migraine can happen in a small percent of women during pregnancy and a chance of developing a new aura is common in these women since aura is stimulated by high estrogenic states. Furthermore, pregnant women with migraine have a higher risk of other complications including preeclampsia/eclampsia and preterm birth as well as low birth weight [29, 30]. At delivery, there is a sudden drop in hormone levels with the headaches frequently returning to the pre-existing baseline, however breastfeeding can delay ovulation and ameliorate the common postpartum worsening in migraine. The role of estrogen in affecting migraine was proposed by BW Somerville in 1972 when he proposed the "estrogen withdrawal hypothesis" [31]. When women were treated with estradiol, there was a delay in normal menstrual cycle during the late luteal phase along with the delay in onset of migraine attacks. Interestingly, estrogen during the menstrual cycle in women with migraine withdraws twice – once during the periovulatory and then during the late luteal phase. The SWAN (Study of Women Across the Nation) Daily Hormone study further expanded on the "estrogen withdrawal

hypothesis" by demonstrating that women with migraine had a faster drop in luteal estradiol compared to women without migraine [32]. This accelerated drop in estradiol was specific to phase on menstrual cycle (only late luteal) and to the first two days from peak. There was no difference in the estrogen peak levels and the rate of estrogen withdrawal following the periovulatory estrogen [32]. Furthermore, this was independent of headache occurring in that particular cycle, suggesting that this was an endogenous trait of women with migraine that might confer neuroendocrine vulnerability to this population.

Neuroimaging has contributed significantly to our understanding of the pathophysiology of migraine disease. Magnetic resonance imaging (MRI) can distinguish healthy versus migraine brain (using functional MRI) and also episodic versus chronic (using structural MRI) with high accuracy (86.1 and 84.2 percent) [33, 34]. Neuroimaging findings on reduced hippocampal volume in migraine patients with high versus low frequency of migraine attacks and the associated functional activity and connectivity differences led to the hypothesis that repeated attacks by stressors on the brain due to the chronicity of the attacks may shift the brain to a maladaptive state due to the inherent inability of the migraine brain to adapt well to these stressors [35-37]. Neuroimaging studies have also shown that there are sex differences across the brains of women and men with migraine. For example, cortical thickness differences in the insula and the precuneus regions of the brain of women with migraine are different with that of men with migraine and age-matched healthy subjects of both sexes [36]. Furthermore, women with migraine showed a greater activation in brain regions involved in emotional processing and lack of insular thinning with age indicating that probably the insular region is linked with migraine pathophysiology [36, 38, 39].

Women with migraine have a two-fold increased risk of stroke [40, 41]. Importantly, women who have migraine with aura have two- to three-fold higher risk of stroke compared with women who have migraine without aura. The ICHD-3 criteria for migraine-related stroke is that typical aura should be greater than 60 minutes with neuroimaging showing an infarct in the relevant area. The American Heart Association and the American Stroke Association 2014 guidelines for prevention of stroke in women list migraine with aura as a risk factor for stroke in women only. Although the underlying biological mechanism is not really clear, many factors have been implicated including vasospasm, neuronal glutamatergic hyperexcitability, release of prothrombotic factors, genetic predisposition, prolongation of cortical spreading depression, increased platelet aggregability, endothelial abnormalities and abnormal activity of serotonergic cells [42]. Migraine is also associated with other stroke risk factors as demonstrated in several studies such as EVA, PRIMA, MIST and CADASIL trials.[43-46] Certain metabolic conditions such as the MELAS (mitochondrial and encephalopathy lactate acidosis syndrome), Lupus, Livedo reticularis, and Sneddon syndrome also pose an increased risk for migraine related stroke due to the higher prevalence of migraine in these individuals [42, 47]. Unfortunately, the relationship and implications between these metabolic conditions, migraine and stroke are not known [47-49].

Migraine and depression are linked; with people with migraine having a 25 percent higher rate of lifetime risk of depression compared to 16 percent in the general population [50]. There is a correlation between migraine-related disability and depression [51]. There is also an increased risk of depression in relatives of patients with migraine and vice versa suggesting that there is an overlap in biology [52]. The risk for perinatal depression in patients with migraine is high [53, 54]. As discussed before, there is a higher risk of migraine with earlier menarche, similarly there is a higher risk of depression with earlier menarche [55]. Just like migraine, depression is suppressed during pregnancy likely related to the change in hormone levels. Right at the end of pregnancy there is an increased risk of developing depression and some women show symptoms beforehand. There is also a suppression of the immune system

occurring during this time period and then a sudden increase in the immune system or inflammatory markers right before delivery [56].

Gamma-aminobutyric acid (GABA) is a neurotransmitter that blocks certain signals and decreases activity in the central nervous system. There are two classes of receptors that respond to GABA – $GABA_A$ and $GABA_B$. In patients with postpartum depression, the brain's inhibitory $GABA_A$ receptors may not recover in time following their reduced numbers during pregnancy [57]. Allopregnanolone, a neurosteroid synthesized from progesterone, can act on $GABA_A$ and can increase progesterone via a feedback loop eventually leading to downregulation of $GABA_A$ receptors. Brexanolone, a synthetic allopregnanolone was recently approved by the FDA for postpartum depression. It is possible that hormonal modulation of $GABA_A$ receptors might contribute to migraine and there is a research opportunity to explore the relationship between $GABA_A$, allopregnanolone and progesterone in migraine pathophysiology. Additionally, there is scientific evidence that potentially links $GABA_A$, migraine and inflammation. For example, there is altered $GABA_A$ receptor function in people with familial hemiplegic migraine type 1 [58]. Migraine prophylactics like Topamax and valproate actually act on $GABA_A$ receptors [59, 60] and $GABA_A$ receptor subunit (GABRA3) was down regulated in leukocytes of patients with migraine [61]. Stress can lead to an increase in monocytes and leukocytes and increases in monocytes has also been reported in patients with migraine [62]. An interesting hypothesis to pursue is trying to target progesterone and $GABA_A$ receptors in the monocytes to change inflammatory state given that migraine is a neurovascular condition and how these disorders can be treated in the body, outside the brain. Perhaps these signals can be stopped from getting into the brain and there may be a potential with much easier drug delivery to modulate some of the inflammatory activity in these patients.

Animals Models, Pain, and Lessons learned

Animal models used for pain research can provide important insights for migraine research. Also, important insights from mechanistic studies of sex differences in animal models can be applied to migraine research. For instance, behavioral responses in common animal pain models are typically not different between males and females. However, the underlying mechanisms by which those behavioral mechanisms are mediated are dramatically different between sexes. For decades, the pain field has been focused on microglia (primary immune cells of the central nervous system) as a contributor to pain since growing evidence showed that there can be extensive crosstalk between microglia and neurons that contributes to pain i.e. neuronal hyperactivity can cause microglial activation and activated microglia can activate/sensitize neurons. However, the microglia-mediated pain mechanisms seem to only be relevant in males [63]. Interestingly the number of microglia activated is the same in males and females which means that the observed difference is actually in the mechanisms by which activated microglia communicate bi-directionally with neurons. Furthermore, a number of drugs that target microglia in a dose dependent manner have been shown to inhibit the microglia in males but not in females, once again suggesting that microglia are ideal drug targets in males but not in females [64]. Furthermore, immune system has been implicated to play a role in the morphine mediated analgesia. Mice deficient in T cells show increased pain and decreased opioid analgesia and when mice are treated with opioids, sex differences are observed in a dose-dependent manner [65]. The key takeaway from these examples are that pre-clinical migraine research should more extensively evaluate sex differences in models and furthermore evaluate the contribution of the immune system to the migraine-related pathophysiology, particularly the contribution of different kinds of immune cells to neuronal function and how that might be very different between females and males.

There are also innovative behavioral models for pain in development that have potential to be new models for migraine research. One such novel model is based on the technique called optogenetics whereby a mouse self-reports the detection of pain versus tactile stimuli (on-going research). Transgenic mice are made to artificially express a light-sensitive protein in a sub-specific population of neurons. These mice have a gene which is light-sensitive in TRPV1 C fiber sub-populations and when the light is shined using a laser probe placed on the foot or anywhere on the body, only C fibers will be activated in a time-locked fashion. This then leads to a pain specific licking behavior in mice, which is like generating a verbal report in humans (unpublished data Saab/Borton labs). More importantly this method is non-invasive as there is no surgical procedure or no chemicals injected into the mice. Similarly, another novel model using selective meningeal C-fiber activation can be validated for migraine and this is a touch-evoked optogenetic stimulation. Both these models have the necessary temporal resolution and cellular specificity required to define neural circuits of pain. The field of somatic, head and visceral pain would greatly benefit from the development of more specific preclinical models and pain assessment methods.

It is also important to understand what mouse model to use for research, whether it is an inbred or an outbred mouse. Several advantages have been attributed to both the outbred and the inbred strains; however, variance has been cited as a positive attribute to the inbred mice. For this reason, these mice have been used for particular genetic or immunological purposes. Dr. Jeffrey Mogil's lab found that outbred mice had no variability compared to inbred mice based on the tail withdrawal test [66]. Overall, outbred mice do not have more variability than inbred mice and this is observed in relation to behavioral, non-behavioral, trait classes etc. The reasons cited for this are that environmental variance is much bigger than genetic variance and swamps the genetic variance and inbreeding might prevent mice from resisting the effects of external influences. Normal mice are able to buffer against changes and get back to some homeostatic level, but inbred mice would be unable to do so. There are environmental factors in a laboratory setting related to animal husbandry that have been shown to affect pain sensitivity [67]. The implications for this are tremendous as the majority of pain related scientific evidence has been based on a flawed mouse model.

Preclinical Animal Models of Migraine

Migraine-associated symptoms are referred to as endpoints in preclinical animal models and can be used to assess therapeutics or study pathology. All the endpoints are broadly classified as behavioral or non-behavioral (cellular and molecular) **(Table 2)**. Some of the very common endpoints used in the headache field are related to pain signaling such as mechanical or thermal stimulation in the head and face and occasionally in the periphery. In recent times, there have been attempts to consider more cognitive or reasoning type endpoints such as conditioned place preference or aversion, where the animal is exposed to one particular type of chamber with a particular stimulus or a pain relieving drug or pain to see if they develop a preference or aversion to that state afterwards. Several limitations exist to using these endpoints: 1) validating a novel target in relation to positive controls (such as sumatriptan or anti-CGRP therapeutics) can be seen as a problem or not with respect to the mechanisms being studied, 2) addressing migraine attacks that are not responsive to migraine medications, 3) limiting behavioral endpoints to a certain kind of circuit processing – cephalic allodynia (mechanical mostly), which only reflects trigeminal central sensitization and not ongoing pain, 4) difficulty in capturing the affective component of pain as the headache conditioned place preference model is limited to work with, 5) using grimace as a non-evoked endpoint, 6) using decreased wheel running model which is used by few researchers and needs further validation, and 7) increasing diversity of animal models and the limitations on what is being measured.

Table 2: ***Different Endpoints Used in Migraine Research***

BEHAVIORAL ENDPOINTS	NON-BEHAVIORAL ENDPOINTS
• Pain signaling – mechanical and thermal stimulation in head and face • Cognition/reasoning (Conditioned Place Preference and Conditioned Place Aversion) • Anxiety/stress • Photophobia • Facial grimace • Wheel running	• Trigeminal ganglion, spinal cord, and brain tissues • Cultures, thin tissue sections, slices, in vivo • Study channels, receptors, intracellular signaling molecules, transcription factors, inflammatory molecules, genes • Electrophysiology and calcium imaging • Meningeal blood flow, plasma protein extravasation • Protein expression (western blot, immunostaining, ELISA, arrays) • mRNA (qPCR, Northern blot, in situ hybridization) • Transgenic and knockout animals • Drugs in development, biologics, inhibitory molecules, nutraceuticals

There are several types of preclinical models developed to study migraine and headaches. Various aspects of these models and their strengths are discussed below.

Nitroglycerin Model: Nitroglycerin is a known migraine trigger and causes vasodilation in humans leading to headaches. Migraine-like effects are observed in rodents and more severe headache occurs in the migraine mouse lasting several hours compared to a non-migraine mouse [68]. Nitroglycerin in mice will cause delayed hyperalgesia similar to humans, as well as photophobia, increased meningeal blood flow and conditioned place aversion [69-72]. This model can be used to study acute, episodic and chronic headaches and causes dose-dependent acute and chronic hyperalgesia. Two hours following an injection with nitroglycerin, these mice will develop severe pain compared to control animals and when treated chronically with nitroglycerin overtime, these mice develop basal hypersensitivity. There is plenty of pharmacological evidence to show that acute hyperalgesia in these animals is blocked by acute migraine medications such as sumatriptan and prophylactic migraine medications such as topiramate or propranolol will block both the development of this basal hypersensitivity as well as the post-treatment response. Post-treatment response results in essentially the same level of pain in these animals almost nine days post-treatment suggesting chronic hyperalgesia [72, 73]. Furthermore, female mice seem to be slightly more sensitive to nitroglycerin, although not by a lot compared to male mice and this difference could be dose related [72, 73]. Nitroglycerin also causes conditioned place aversion suggesting an emotional response to ongoing pain, a component of migraine. Animals that were conditioned with nitroglycerin tend to avoid the chamber where they experienced the pain, while control animals do not exhibit this behavior. Once again, use of anti-migraine therapies reversed the conditioned place aversion [74].

Dural Stimulation Model: There is data from human studies to suggest that stimulating the meninges causes headaches due to the activation of the nociceptors in the meninges [75]. When meningeal nociceptors are stimulated by applied stimuli, the mechanical threshold of meningeal nociceptors is lowered due to the activation and

sensitization of the afferent neurons. Several laboratories have conducted studies in these models using in vivo electrophysiology in anesthetized animals and demonstrated that dural afferents respond to a wide variety of stimuli including mechanical probing, capsaicin, inflammatory soup, mustard oil, low pH, cytokines, interleukins, protease activated receptors agonists, nitric oxide, reactive oxygen species etc. Furthermore, these sensitizations can be blocked by naproxen and sumatriptan, which are CGRP-directed therapeutics. These models have also shown that sensitizations can lead to expansion of receptive fields in the trigeminal cervical complex allowing hypersensitivity of the facial skin. Limitations of these models is that the experiments are conducted in an anesthetized animal, requiring a complex surgical procedure to gain access to the meninges and a behavioral measure is not measured.

The dural stimulation behavioral models have been used to test the effectiveness of anti-migraine agents and to observe whether the behavior was blocked by sumatriptan, naproxen, CGRP-receptor antagonists and NK1 substance P receptor antagonists [76]. Except for the NK1 antagonist, all the other agents were successful in blocking the behavior. Interestingly, this result has relevant translational implications as NK1 antagonists were not effective in humans and have failed clinical trials. However, other drugs have been shown to be successful therapeutics [76]. Behavioral responses in this model have been activated by a number of different kinds of chemical stimuli and the observed behavior has been blocked by naproxen, sumatriptan, CGRP antagonists as well as non-pharmacological therapies such as vagus nerve stimulation. A limitation of this model is that repeated dural stimulation can cause a persistent change in behavioral outputs. Although these animals provide the ability to induce the headache component, these animals are being injured and subjected to invasive processes [77].

Craniotomies are typically required in rats but not mice due to their thicker skulls, hence dural stimulation can be done non-invasively in mice. With the updated newer dural stimulation and behavioral mouse models there are certainly advantages such as removing the need for a craniotomy, using the conditioned preference place as a way to assay for headache, and the use of genetically-modified animals. Furthermore, the behavioral responses in mice were similar to those of rats with craniotomies adding to the advantage of these updated models. These newer models have certainly begun to address limitations of prior models which did not measure “headache” and required significant injuries.

Cortical Spreading Depression (CSD) Model: Both the nitroglycerin and the dual stimulation models use invasive approaches and the drawback with these preclinical models is that they cannot address the question of how migraine originates, what are its triggers, what causes the pain in our brain to begin. Currently, the CSD model is the best way to answer the origination question, because it models the aura that precedes the attack. One limitation is that formally it only models migraine with aura, but it may still provide clues relevant to the development of all migraine attacks. Basically, CSD is a wave of cortical depolarization that involves neurons, glia, blood vessels followed by synaptic depression that can last probably up to an hour [8, 11, 78]. CSD primarily relates to the aura and visual aura. Different neuroimaging techniques have shown spreading blood flow changes correlating with CSD during the aura phase in awake humans, including humans tracing their aura while corresponding imaging activity was recorded across the cortex, essentially linking CSD to the aura [79-82].

Preclinical work has tried to establish a link between migraine aura and headache by demonstrating that CSD activates trigeminal meningeal afferents [83]. Further studies have proposed that CSD acts as a nociceptive stimulus and activates the peripheral and the central trigeminovascular neurons that underlie the headache of migraine with aura [83-86]. CSD is also thought to modulate the processing of pain in nucleus caudalis and different areas of the brain

that can either enhance or inhibit the processing [87]. The hypothesis is that inflammatory mediators are released in the cortex that reach the meninges and activates CSD and this again activates the trigeminal ganglia and nucleus caudalis [88]. CSD also has effects on cortical network function and thus may directly affect sensory processing and thus contribute to the sensory amplifications of the migraine attack [89]. Several prophylactic drugs can block CSD or reduce the number of events [90, 91]. CSD is emerging as treatment target and also as a preclinical model to understand migraine with aura [11]. CSD occurs in a broad range of neurological diseases such as epilepsy, traumatic brain injury, stroke, and subarachnoid hemorrhage and has been considered as a therapeutic target in these conditions [92].

Medication Overuse Headache (MOH) model: Both sumatriptan or morphine are known to cause medication overuse headache. Chronic multiple injections of sumatriptan or opioids cause cephalic allodynia. The MOH models can be used as a tool to screen for drugs that might cause headache [93 94]. These models allow researchers to screen for drugs that may be effective specifically in MOH but they are not frequently used and require more characterization.

Post-traumatic Headache (PTH) Model: PTH primarily resembles migraine, suggesting a similar or shared etiology. Studying PTH mechanisms preclinically may lead to new insights on endogenous migraine generators. PTH affects a large population (~80 percent) of soldiers and veterans returning from combat missions in Iraq and Afghanistan that have suffered a traumatic brain injury (TBI). PTH is also diagnosed (20-70 percent) in many non-military TBI (e.g. sport concussions, domestic abuse, primarily in females). These models gives the ability to learn about migraine to understand how migraine headaches start, where they originate and how changes related to concussions result in a migraine phenotype.

Currently preclinical studies on migraine resulting from a TBI are still at an early stage [95]. To be able to study these headaches in mice, the closed-head injury models are preferred, and these are more clinically-relevant as they do not involve craniotomy. Current findings relevant to the headache pathway in mice and rats include: 1) biochemical changes in trigeminal nucleus caudalis, 2) behavioral endpoints observed such as trigeminal mechanical and thermal allodynia, hypersensitivity to nitroglycerine including nitroglycerine evoked allodynia and conditioned place aversion, 3) treatments validated include sumatriptan and anti-CGRP antibodies, 4) neuroinflammatory mechanisms such as increased dura mast cell degranulation and blocking mast cell action ameliorates nitroglycerine hypersensitivity, and 5) sex differences in pain and females exhibit a more pronounced pain phenotype and do not respond as well as males to CGRP antibodies. Interestingly, this difference was not observed with sumatriptan. The caveats are that it is not clear if the model is mimicking migraine-like PTH and/or TTH and also likely relevant only to acute PTH (less than 3 months).

Based on all the animal models discussed above, there are a number of potential targets in different stages of preclinical development and this list is growing **(Table 3).**

Role of Sex Hormones in Migraine using Preclinical Models: Several laboratories have used preclinical animal models in an attempt to understand the underlying mechanisms that contribute to sex differences in migraine and the role of sex hormones in mediating these differences [96-105]. Having a clear understanding of these mechanisms can allow for the development of potential therapeutic targets for a personalized treatment approach to migraine. The existing scientific literature clearly implicates the role sex hormones in the observed sex differences; however, the exact mechanistic role of sex hormones in mediating sex differences in migraine still remains unclear. For

example, estrogen has shown to be both pro-nociceptive and anti-nociceptive suggesting that different factors could be influencing estrogen in these experiments leading to the complexities of interpreting the true role of estrogen. Furthermore, it is also important to understand the effect of testosterone in migraine as it has been shown exert reciprocal effects to female hormones on CSD susceptibility [99]. Testosterone has been shown to play a protective role in the development of temporomandibular joint pain in rats [106]. It would be interesting to determine whether testosterone has a similar protective role in migraine. Clearly there is definitely a need for further work in this area to understand the role of sex hormones in mediating sex differences in migraine.

Table 3: ***Potential Targets in Different Stages of Clinical Development***

POTENTIAL TARGETS	MODELS	CLINICAL TRIAL STAGES
PACAP receptor	vascular, immune (mast cells), TNC electrophysiology	Phase II Clinical Trials (PAC1R Ab) and Preclinical (PACAP Ab)
5HT1F agonist		Phase III
Delta Opioid Receptor Agonist	NTG, CSD, MOH, PTH	Phase I
Kappa Opioid Receptor Antagonist	Stress-triggered model	Phase II for emotional disorders
Vagus nerve stimulation	CSD, chronic dural stimulation, human data exists	
SP/NK-1 revisiting -Mas-related GPCRs (Mrgprs)	(new finding-no data in migraine/headache models)	
ASICs	Dural stimulation, CSD, NTG, Small human trial with amiloride	
TRPA1	Dural stimulation, Intranasal admin, Human data with natural irritants	
Cannabinoids	There is a THC and CSD study, some in vivo electrophysiology from Akerman et al, also THC with wheel running.	Extensive anecdotal and retrospective human data that is not blinded and placebo controlled.
nNOS	MOH model, dural neuropeptide release	Phase II

TNC = Trigeminal Nucleus Caudalis; **THC** = Tetrahydrocannabinol; **PACAP** = Pituitary adenylate cyclase-activating peptide; **NK-1** = Neurokinin; **SP** = Substance P; **TRPA1** = Transient receptor potential ankyrin 1; **ASICs** = Acid-sensing ion channels; **nNOS** = Nitric oxide synthase;

Recommendations

AMD challenged the expert advisory panel to identify recommendations related to clinical aspects in migraine, role of hormones in migraine, preclinical models and other areas. These recommendations were proposed based on existing knowledge gaps in the migraine field and the discussions among the expert advisory panel members **(Table 4).** The recommendations broadly fell under the overarching themes of research, education and awareness.

Table 4: ***Proposed Recommendations by the AMD Expert Advisory Panel***

CLINICAL ASPECTS
• Develop a diagnostic tool or a set of tools to make better more informed diagnosis, especially ENTs • Develop migraine disease classification for sub-phenotyping migraine • Develop artificial intelligence (AI) or machine learning tools to assist in diagnosis and classification • Address drug interaction and adverse reactions • Develop better ICHD-3 diagnostic criteria to define migraine sub-types • Educate physicians on sinonasal symptoms and vertigo nomenclature • Raise awareness to bridge different medical disciplines for treatment and research • Conduct prescription drug utilization studies
HORMONAL INFLUENCE
• Address hormonal effects among different age groups • Develop gender specific treatments • Generate specific data for best migraine treatment to reduce stroke risk & complications • Expand understanding of co-morbidities to develop new treatments • Educate women, Ob/Gyns and primary care physicians about the timing of menstrual related migraines
PRECLINICAL
• Raise awareness on the value of sex differences in preclinical models • Develop better preclinical models that address all aspects of the migraine attack, including sensory amplifications that accompany pain and aura • Increase development of chronic migraine models and models of the chronification process • Develop a new mouse migraine model using optogenetics whereby a mouse self-reports the detection of pain versus tactile stimuli (based on ongoing research) • Evaluate immune system contribution to migraine pathophysiology • Address migraine attacks not responsive to medications • Cross validate promising drug targets
OTHERS
• Advocate and develop more dedicated migraine federal and non-federal funding • Educate and engage new researchers in migraine research • Encourage pharmaceutical companies to assess the drug trial data by sex

Conclusions

Migraine is a complex neurobiological disease with several co-morbidities and impacting multiple systems within the body. There is a need for increased research, education and awareness among several medical specialties beyond the traditional field of neurology. There is also a need for increased awareness of sex and gender differences in migraine as the disease disproportionately impacts women. An interdisciplinary scientific expert advisory panel assembled by AMD has identified several knowledge gaps and proposed recommendations to address these gaps following a one-day roundtable discussion. An established program such as the AMD's Migraine Research Fund may be the platform needed to address and advance these proposed recommendations. The migraine research fund can also serve as a platform for AMD to address the challenges that exist in otolaryngology clinical setting to prevent, diagnose and treat atypical migraine mimicking as rhinosinusitis.

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