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Fluids and Barriers of the CNS

REVIEW

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Research priorities for non-invasive therapies to improve hydrocephalus outcomes

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Abstract

The Hydrocephalus Association organized two workshops with the support of the Rudi Schulte Research Institute and Cincinnati Children's Hospital Medical Center entitled "Developing Non-Invasive Hydrocephalus Therapies: Molecular and Cellular Targets", held September 27–29, 2023, in Dallas, TX, and "Developing Non-Invasive Hydrocephalus Therapies: Advancing Towards Clinical Trials", held April 12–13, 2024, in Cincinnati, OH. The goal of these workshops was to explore the frontiers of ongoing research for non-invasive therapies for the treatment of hydrocephalus across all etiologies and to improve patient outcomes at all stages of diagnosis and management. During the consensus-building discussions throughout the research workshops, basic, translational, and clinical scientists aimed to identify the next steps to develop novel treatments for hydrocephalus. This detailed report discusses the research priorities that emerged from these workshops to inspire researchers and guide studies towards better treatment for people living with hydrocephalus.

Keywords Hydrocephalus, Pharmacotherapies, Adjuvant therapies, Preclinical models, Clinical trials, Collaboration

Background

Hydrocephalus is a complex clinical and neuroradiographic diagnosis characterized by excessive cerebrospinal fluid (CSF) and dysregulated pressure equilibrium within the cranium. The incidence of hydrocephalus exceeds 0.1–0.6% of children, making it as common as Down Syndrome [1]. Hydrocephalus in children is commonly associated with other comorbidities such as cerebral palsy, seizures, and developmental delays. Hydrocephalus can affect any age; as many as 1% of adults develop hydrocephalus in their lifetime, though underdiagnosis may mean that the actual rate is higher [2]. Unfortunately for patients and their families, there is no cure for hydrocephalus, and all treatment interventions require neurosurgical intervention. The clinical management of hydrocephalus varies, in part due to the differences in age at presentation, etiology, manifestation, and comorbidities. Management generally aims to optimize intracranial pressure (ICP) to improve intracranial compliance, thereby ameliorating pain, preserving vision and continence, and preventing stroke or brainstem compression, as well as potentially improving future cognitive and behavioral outcomes and other associated sequelae.

In the acute setting, hydrocephalus is a life-threatening clinical emergency and often requires permanent CSF diversion with shunting. However, shunts are among

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the highest-failing medical devices on the market [3]. In the pediatric population, an estimated 50% of shunts implanted fail within two years, often requiring repeated surgical interventions [4–6]. While the prevalence of shunt failure is lower in adults, approximately 32% of adult patients experience shunt failure [6, 7]. Shunt revisions due to shunt infection or failure have been associated with long-term physical and psychosocial burden [8, 9]. The long-term outcomes of even well-controlled hydrocephalus are variable and may include intellectual disability, motor impairment, and pain [8, 10]. Long-term management of hydrocephalus is limited by the current non-invasive diagnostic and therapeutic tools, resulting in continued sequelae and potential for complications following intervention.

The limitations of current hydrocephalus care highlight the urgent need to develop non-invasive or minimally invasive therapies to treat hydrocephalus and to prevent and repair the associated brain damage. Patient voices have further underscored the demand for non-invasive treatment strategies, ideally one-time treatments that do not require additional interventions for hydrocephalus, and therapies to prevent or repair hydrocephalus-associated brain damage [11]. Paramount to achieving these goals is the improved understanding of CSF physiology and the mechanisms that precipitate dysfunctional CSF dynamics. Advances in exploratory "omics" analyses, imaging tools, and materials science have spurred a new dawn in our understanding of hydrocephalus, challenging decades-old hypotheses. To this end, the Hydrocephalus Association, in conjunction with the Rudi Schulte Research Institute and Cincinnati Children's Hospital Medical Center, organized two workshops—"Developing Non-Invasive Hydrocephalus Therapies: Molecular and Cellular Targets" and "Developing Non-Invasive Hydrocephalus Therapies: Advancing Towards Clinical Trials", respectively—where a diverse group including clinical, translational, and basic science researchers and physicians discussed strategies to accelerate research in hydrocephalus therapies and translate novel developments into clinical practice. The goal of this report is to summarize the research priorities that emerged from these workshops by 1) providing a broad overview of hydrocephalus treatment targets, 2) exploring the utility of big data and machine learning to search for novel treatment strategies, and 3) discussing the ideal outcome measures and preclinical models to assess an intervention and facilitate translation into clinical trials.

Workshop organization

This report presents the research gaps and priorities that emerged from two workshops on developing non-invasive hydrocephalus therapies designed by a team of experts composed of experienced hydrocephalus clinicians, researchers, and funders. New researchers in the hydrocephalus field were invited to connect with established domestic and international colleagues spanning the spectrum of this condition. Participants of the two workshops included adult and pediatric neurosurgeons and neurologists; neuroscientists; biomedical and software engineers; physiologists; biologists; geneticists; critical care clinicians; neuroradiologists; neuropathologists; toxicologists; representatives from pharmaceutical and neurotherapeutic device industries; directors, advisors, and program managers from the National Institutes of Health, Food and Drug Administration and the Department of Defense; a former Centers for Medicare & Medicaid Services administrator; the CEO of the Cerebral Palsy Research Network; the executive director of the Theodore W. Batterman Family Foundation; the managing director of the Sontag Innovation Fund; patients and parents of patients with hydrocephalus; and members of the Hydrocephalus Association (Supplemental File 1). The Hydrocephalus Association and Rudi Schulte Research Institute workshop, "Developing Non-Invasive Hydrocephalus Therapies: Molecular and Cellular Targets", was held in Dallas, TX September 27–29, 2023. Nine plenary sessions consisted of formal presentations by invited speakers on topics including the use of big data to identify hydrocephalus targets and modes of treatment delivery, with each session ending with a synthesis discussion (Supplemental File 2). The Hydrocephalus Association and Cincinnati Children's Hospital workshop, "Developing Non-Invasive Hydrocephalus Therapies: Advancing Towards Clinical Trials", was held in Cincinnati, OH April 12–13, 2024 (Supplemental File 3).

A major goal of these workshops was to connect researchers and clinicians to facilitate therapeutic development and spark new ideas for non-invasive strategies. The Hydrocephalus Association has been proud to provide seed funding for the advancement of novel ideas. Over the last 15 years, researchers have capitalized on $16 million of seed funding and, as a result, have been awarded $91 million in large-scale grant-funded research projects. Another goal of these workshops was to build consensus around research priorities for the development of non-invasive strategies for treatment (Table 1). Discussions geared toward consensus building during the Cincinnati research workshop were held between and during seven plenary sessions in which invited speakers gave formal presentations on topics including ideal endpoint measurements and strategies for advancing into clinical trials (Supplemental File 2). Attendees were asked to share and discuss their top 2 ideal endpoint measurements. The responses were reviewed, and common themes, topics, and endpoint measurements were

Table 1 Research priorities for the development of non-invasive treatments for hydrocephalus

Theme 1: Discern mechanisms of CSF pathophysiology to advance hydrocephalus therapies	
Priority 1	Understand how tissues involved in CSF formation and secretion: (1.1) contribute to its composition and flow, (1.2) modulate inflammation, and (1.3) impact brain development in physiological and pathophysiological conditions
Priority 2	Invest research in small and large animal preclinical models to determine efficacy of therapies which: (2.1) treat the root cause of hydrocephalus, (2.2) minimize acute damage caused by CSF accumulation, and (2.3) prevent long-term damage induced by hydrocephalus
Theme 2: Leverage big data to identify novel hydrocephalus treatment targets	
Priority 3	Using multi-omics approaches, (3.1) identify key pathogenic changes associated with hydrocephalus, (3.2) perform large cohort, multi-site preclinical and clinical studies to guide diagnostics and therapeutics, (3.3) collect, compile, and share preclinical and clinical data within hydrocephalus research groups, and (3.4) train machine learning (ML) models for analysis of complex heterogenous datasets
Theme 3: Design preclinical studies with a focus toward translation	
Priority 4	Develop and rigorously cross-validate optimal in vitro and in vivo models of hydrocephalus, with an emphasis on age, etiology, pathophysiology of interest, outcomes measures, and endpoints
Priority 5	Design clinical trials with a unified approach including: (5.1) patient-driven objectives, (5.2) integration of the entire patient care team, and (5.3) collaboration between sites

identified. These findings were organized into categories based on the recurring themes and the frequency with which they appeared in the responses (Table 2). Generally, the more frequently an endpoint measurement or topic appeared in the responses, the higher its priority within the table. A writing committee composed of participants from both workshops wrote this report, which was circulated to all attendees and the Hydrocephalus Association's Medical Advisory Board before submission.

Main text

Theme 1: elucidate mechanisms of CSF pathophysiology to advance hydrocephalus therapies

To expand treatment strategies for hydrocephalus, we must first understand the mechanisms of CSF physiology and pathophysiology and the damage caused by CSF accumulation (*Theme 1*, Table 1). Hydrocephalus has numerous etiologies, presentations, comorbidities, and complications. As such, therapies should target the root cause of CSF pressure and volume disequilibrium, modulate CSF production or reabsorption, and/or prevent or repair the acute and chronic damage caused by such imbalance. The current standard of care treatments—shunt placement and endoscopic third ventriculostomy (ETV) with or without choroid plexus (ChP) cauterization (CPC)—largely provide a means of CSF volume control. Research on the mechanisms of impaired CSF volume homeostasis and brain damage has led to novel treatment strategies currently in preclinical stages of development that target the cause of hydrocephalus and minimize or prevent the resulting damage. In this section, we provide an overview of the tissues that contribute to the formation and composition of CSF, including the ChP, the ependymal cells lining the ventricle walls, and other barrier cells, and summarize therapies currently in development.

Emergence and homeostatic roles of the CSF-brain interface

The choroid plexus

The ChP is a barrier epithelium composed of mature, multiciliated columnar epithelial cells with tight junction proteins surrounding a fenestrated capillary bed perfused by the choroidal arteries and accompanied by a matrix of stromal tissue replete with dynamic immune cells. As such, the ChP is uniquely poised to control the composition of CSF and its rate of production [12]. Summarized in recent review articles [13–15], hydrocephalus researchers have historically trialed therapies that were thought to control fluid secretion by the ChP via inhibition of ion transporters to control ventricular size and prevent neurosurgical intervention. Although

Table 2 Ideal endpoint measurements for hydrocephalus

Category	Endpoint Measurement	Description
Imaging Metrics	Ventricular Volume	*Measurement of ventricle size; Test for reduction of ventriculomegaly*
	White Matter Integrity, Neuro-connectivity	*Use of DTI to assess white matter tracts and injury; Understand impact of treatments on brain structure*
	Brain Volume and cerebral blood flow characteristics	*General brain/CSF volume and cerebral blood flow measurements* via *MRI*
Cognitive and Behavioral Outcomes	Cognitive Functioning	*Cognitive outcomes such as memory/attention, reasoning, learning, and motor functions*
	Behavioral Tests	*Specific references to executive functions and motor abilities*
	Pain	*Nociceptive pain (peripheral neuropathic pain); Headaches*
	Neurodevelopmental Outcomes	*Particularly in children, the focus is on maximizing brain development, with an emphasis on functional rather than structural changes alone*
	Motor Analysis	*Particularly in normal pressure hydrocephalus, gait is a practical and quantifiable outcome measure*
Physiological Measurements	Intracranial Pressure	*Changes in ICP and CSF dynamics; Opening pressure in rodent models*
	Markers of Neuronal and Axonal Injury	*Biochemical markers, histology, and specific imaging findings related to neuronal health and recovery*
	Histopathology and Molecular Changes	*Assessment of the cellular and molecular changes post-treatment, providing insights into the mechanisms of action and potential side effects*

DTI Diffusion Tensor Imaging, *MRI* Magnetic Resonance Imaging; *ICP* Intracranial Pressure; CSF: Cerebrospinal Fluid

unsuccessful in clinical practice, emerging work has renewed interest in this area of study and identified several novel drug therapies for the treatment of hydrocephalus [14]. Furthermore, recent research has highlighted the unique role of the ChP in modulating immune cell entry and exit into the CSF spaces [16–19]. This function of the ChP may serve as a focus for new therapies to treat the inflammation associated with certain types of hydrocephalus, particularly from hemorrhage, infection, and trauma. Identifying the key transporters and signaling molecules in the ChP that regulate CSF production and composition and gate immune cell entry across the lifespan would significantly advance the field (*Research Priority 1*, Table 1).

The ependymal cells of the ventricular lining

Distinct in structure and function from the ChP, the ependyma is a multiciliated, glial-epithelial barrier that is thought to play a role in sustaining laminar flow at the ventricular surface, contributing to the maintenance of CSF flow throughout the ventricular system. The ciliated ependymal border also forms a barrier against toxin entry and, sitting at the CSF-brain interface, modulates brain homeostasis through its role as a gatekeeper, controlling influx and efflux and responding to signals secreted in the CSF. Damage to the ependyma is commonly seen in animal models of hydrocephalus and human autopsy studies [20], but the question of whether this represents the cause or impact of hydrocephalus remains contentious [21, 22]. Like the ChP, understanding the contributions of the ependymal cells to CSF signaling and flow across development is a key opportunity for the development of therapies to treat hydrocephalus (*Research Priority 1.1*, Table 1). Further, determining the similarities and differences between physiological and pathological immune cell breaching of the epithelial barriers of the ChP and ependyma in hydrocephalus is likely to be valuable in the search for novel therapies (*Research Priority 1.2*, Table 1).

Other CSF-contacting barrier cells

Though understudied, the physiological role of CSF-contacting neural-glial populations, particularly their role in brain development, may shed new light on how CSF can impact brain development in the context of hydrocephalus (*Research Priority 1.3*, Table 1). The immediate CSF-contacting parenchyma in the dorsal-lateral border of the lateral ventricles houses a neurogenic niche, the subventricular zone (SVZ), home of the neural progenitor stem cells (NPSCs) that give rise to new neurons and glia, including astrocytes and oligodendrocytes, which migrate to populate the developing cortex and support white matter tracts. In the third ventricle, a population of specialized glial-ependymal cells called tanycytes

can signal to the hypothalamic structures, influencing states of arousal, feeding, and behavior [23]. Throughout the ventricular system, various regions of the deep gray parenchyma-ependymal CSF border form circumventricular organs (CVOs), which are free of traditional blood–brain barriers (BBBs) and create leaky sites of communication between the CSF, brain, and blood. Damage to the SVZ is particularly implicated in pediatric hydrocephalus, but the contributions of *all* ependymal barriers to hydrocephalus pathophysiology across *all* ages are poorly understood.

The remaining CSF-contacting spaces include the barriers of the meningeal spaces, including the epithelia of the pia and arachnoid maters, which sandwich the subarachnoid CSF and form the primary framework for CSF drainage and absorption into the blood, lymph, and glial cells of the parenchyma. This lymphatic and glial-mediated lymphatic exchange site is indeed implicated in hydrocephalus across all ages [24, 25]. A key research priority is therefore to understand the mechanisms of CSF clearance and drainage pathways targeting the restoration of CSF outflow in hydrocephalus (*Research Priority 1.1*, Table 1).

The current state of the development of hydrocephalus treatments targeting CSF-contacting tissues

Several treatments for hydrocephalus have been investigated over the past century, summarized in these reviews [14, 26, 27]. A current key research priority is to assess the efficacy of these proposed therapies to control ventricular size, minimize acute damage, prevent long-term damage, and impact neurological outcomes in appropriate preclinical models of hydrocephalus *(Research Priority 2*, Table 1*).* Because interventions targeting a single pathway may be insufficient on their own, preclinical studies designed to test combination therapies, such as a combination of drugs targeting the genesis of hydrocephalus and the consequence of neuroinflammation, will likely be needed in the future.

Therapies to treat the genesis of hydrocephalus

Therapies targeting hydrocephalus-relevant ion channels and transporters to control CSF volume are a potential nonsurgical management strategy for hydrocephalus *(Research Priority 2.1*, Table 1*).* In adults, the ChP is responsible for ~ 80% of CSF production, but in early development and postnatal critical periods, its contribution is less understood. The apical surface of the ChP contains solute and water transporters needed for CSF production, including acid–base transporters, cation-chloride co-transporters, voltage-gated channels, ATPase pumps, and water pores [28, 29]. Past clinical trials that investigated pharmaceutical strategies to regulate the function of these transporters as treatments for hydrocephalus failed to control ventricular size [14], perhaps because these transporters are dysregulated as a consequence rather than a cause of hydrocephalus.

Promising new research has demonstrated that targeting the stress-response channel, transient receptor potential vanilloid 4 (TRPV4), can inhibit the progression of ventriculomegaly in a congenital rat model of hydrocephalus by controlling CSF production in ChP epithelial cells [30–32]. Another ion channel implicated in both normal CSF production and in hydrocephalus pathology is the sodium, potassium, chloride cotransporter, NKCC1 [8, 33–37]. Therapies targeting the function of this NKCC1 transporter or its downstream kinase to modulate CSF production have been investigated in rodents. Interestingly, there appears to be a functional link between TRPV4 activation and NKCC1-mediated hypersecretion in the context of hemorrhage; the increased concentration of the bioactive blood lipid lysophosphatidic acid (LPA) in CSF activates TRPV4, which results in downstream phosphorylation of NKCC1 and contributes to CSF hypersecretion [35]. Importantly, LPA and other blood components associated with cell lysis, including iron metabolites, have been linked to the development of posthemorrhagic hydrocephalus (PHH). In human samples and rodent studies, iron metabolites in CSF were a predictor of transition from neonatal intraventricular hemorrhage (IVH) to PHH, and transferrin concentrations appeared to be a marker of the functionality of the ChP and overall brain health [38–40]. In recent rodent studies, treatment with a low-dose iron chelator, deferoxamine, was remarkably effective at treating ventriculomegaly following IVH [41]. Excitingly, some therapies targeting TRPV4, NKCC1, and LPA are currently undergoing preclinical trials in large gyrencephalic animal models.

CSF clearance and drainage are clearly implicated in the pathophysiology of many etiologies of hydrocephalus [42, 43]. Indeed, patients across differing etiologies of hydrocephalus exhibit aberrant CSF flow, circulation, and drainage, and, in the case of idiopathic normal pressure hydrocephalus (iNPH), several key CSF biomarkers associated with CSF clearance have been identified to predict the development of clinical signs [44]. Exciting new research manipulating the CSF drainage pathways via ion channel agonists reduced hydrocephalus in a rodent model [45]. Further, physical manipulations such as focused ultrasound technologies may be leveraged to facilitate clearance of waste through CSF and improve penetration of drugs into the brain parenchyma, which holds promise for neurorestorative therapies to treat hydrocephalus-associated sequelae [46]. Additional validation of these modes of therapy to control CSF volume

via improved absorption is an underexplored research avenue.

Therapies to prevent acute brain damage associated with hydrocephalus

Common across many etiologies of hydrocephalus are changes to plasma and CSF molecular signatures that could precipitate a rise of ventricular pressure and inflammation (*Research Priority 2.2*, Table 1). Importantly, inflammatory pathways can be activated in both genetic hydrocephalus and acquired hydrocephalus [47–49]. Given the high incidence rate of PHH following IVH, many research efforts have focused on this condition. Therapies intended to limit the lysis of red blood cells and surrounding brain injury (anti-complement), and other nonselective treatments including antibiotics, have been shown to prevent progression of IVH to PHH in rodents following IVH [50–52]. These therapies are theorized to dampen the glial and immune response following the hemorrhagic event, thus preventing ventricular dilation and brain damage. Likewise, more targeted therapies such as nuclear factor (NF)-kB and mammalian target of rapamycin (mTOR) inhibitors may selectively mediate site-specific injuries to the white matter and ventricular zone and thus prevent hydrocephalus-associated brain damage [53, 54]. Several of these studies have utilized omics approaches to better understand the inflammatory response, validate existing biomarkers, and elucidate novel biomarkers associated with hydrocephalus. For example, hydrocephalus is associated with an elevation in CSF tumor necrosis factor alpha (TNFα) and other CSF proteome and metabolome changes [55]. Interestingly, Kajana et al. detected accumulations of extracellular signaling vesicles in the CSF thought to influence T cell function [56, 57]. The overlap of inflammatory signature dysfunction among hydrocephalus etiologies in patients and rodent models points toward inflammation as a key therapeutic target that warrants further investigation for ventriculomegaly-associated brain damage.

Therapies to repair the brain and improve long-term sequelae

A key neuroradiographic finding associated with hydrocephalus across etiologies and ages is termed "white matter injury" (WMI). WMI is a heterogeneous diagnosis, encompassing varying degrees of neuronal and glial dysfunction, that is associated with neurological comorbidities, such as spasticity, seizures, cognitive deficits, and socioemotional dysregulation. Paramount to the quality of life of people living with hydrocephalus is the development of therapies that prevent the development of and treat existing WMI (*Research Priority 2.3*, Table 1). WMI can be quantified using MRI techniques, such as diffusion tensor imaging (DTI), which has also been published and validated in rodent and large animal preclinical models. Several emerging therapies for the treatment of hydrocephalus have utilized DTI as an outcomes measure (Table 2).

In addition to WMI, ependymal damage and dysfunction, which have been implicated in congenital and acquired hydrocephalus, are often correlated with poor long-term neurological outcomes in patients (*Research Priority 2.3*, Table 1). The degree of periventricular brain damage is often correlated with neurocognitive outcomes, recently reviewed here [20, 21, 38, 58]. Previous therapies targeting neurorepair in PHH and post-traumatic hydrocephalus (PTH) models [59–63] have shown that repair of ependyma is critical for improvement to neurological sequelae associated with hydrocephalus. Regulatory factors, *Gemc1* and *Mcidas*, represent an emerging target shown to repair ependyma selectively at the site of injury via cellular reprogramming [64, 65].

In development, NPSCs receive their inputs from signaling and growth factors in CSF. Impaired signaling to these NPSCs, as well as mutations in NPSCs themselves, contribute to primary pediatric hydrocephalus [66]. In this regard, genetic manipulations prenatally or postnatally to improve neurodevelopment may represent a promising treatment approach for pediatric hydrocephalus and is the focus of ongoing studies in humans and rodents.

Importantly, regulation of the neurogenic niche has been shown to be dependent upon factors in the CSF that are secreted by the ChP and taken up by the ependymal cells and radial glia. Indeed, the ChP is the critical source for these CSF signals and therefore an attractive target for initiating brain repair following injury [67]. For this reason, identifying CSF signals that regulate the neurogenic niche and can be targeted to initiate and enhance repair is another research priority.

Theme 2: leverage big data to identify novel hydrocephalus treatment targets

Beyond developing treatment strategies that target known hydrocephalus pathways, researchers can mine hydrocephalus datasets to identify druggable pathways previously unrecognized to be involved in the condition (*Theme 2,* Table 1). Relying solely on published literature and preliminary studies on the cellular and molecular underpinnings of a disorder to develop treatments can result in an overrepresentation of particular pathways. Over the last few decades, omics data approaches have emerged as powerful tools for rapidly providing data to formulate novel hypotheses (Fig. 1). Omics approaches to interrogate RNA, DNA, protein and metabolites present an unbiased representation of data, enabling data-driven

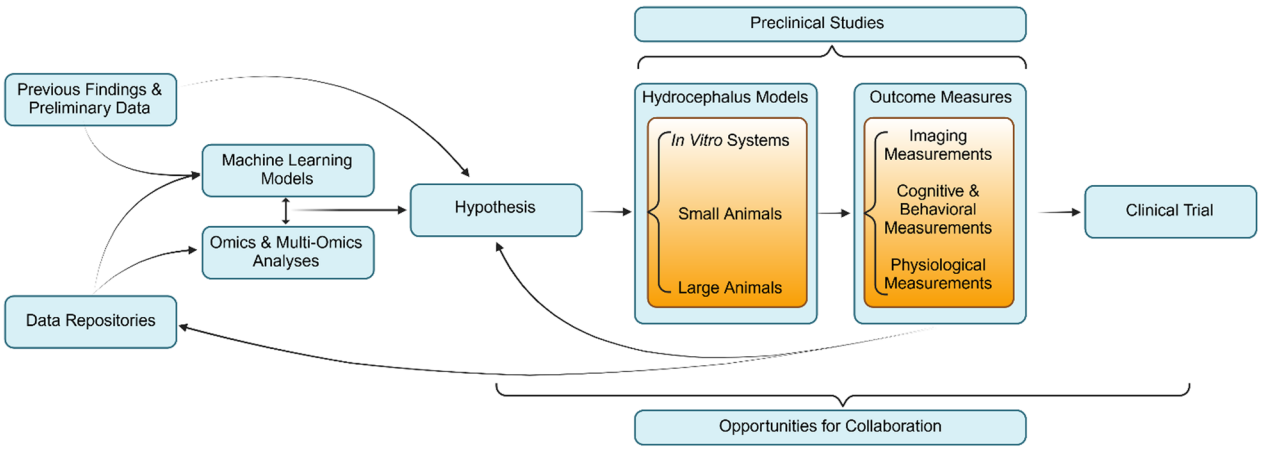

Fig. 1 Schematic outlining proposed pipeline for utilizing research priorities in hydrocephalus to take hypotheses and observations to clinical practice

hypothesis generation. Further, multi-omics analyses—which assess the interactions among multiple levels of a system—can provide a more precise elaboration of a biological system than an individual omics methodology allows. Thus, a research priority from the workshop discussions was to use omics and multi-omics approaches to identify key pathogenic changes associated with hydrocephalus and provide targets for potential therapeutic interventions (*Research Priority 3.1*, Table 1).

Omics and multi-omics techniques in hydrocephalus research

Omics and multi-omics methodologies can help disentangle the molecular intricacies of hydrocephalus [68]. Historical considerations for genetic causes of hydrocephalus, limited to family pedigrees, were scarce and primarily linked to congenital malformations and neurodevelopmental disorders, representing less than 5% of pediatric cases [69]. However, recent undertakings in genome and exome analysis have identified genetic underpinnings in the pathogenesis of hydrocephalus at a rate of up to 20% [66, 70, 71]. While genomics has traditionally been utilized for identifying DNA mutations associated with congenital hydrocephalus in family trios, the advancement of genomic techniques and plummeting costs have expanded our ability to interrogate the genome using omics and multi-omics approaches. For instance, integrating RNA-seq with genomic analyses (such as identifying genetic loci that account for differences in transcription) improves the identification of putative disease drivers, shedding light on the functional consequences of genetic mutations [72–74]. Beyond DNA and RNA, epigenomics is an invaluable tool for elucidating nongenetic heritable changes. These studies allow for a greater understanding of genetic predispositions to hydrocephalus and pave the way to defining the polygenic risk for hydrocephalus development. Drawing parallels with successful therapeutic developments in conditions like cancer [75] and cardiac ailments [76], epigenomic insights offer promising avenues for therapeutic exploration in hydrocephalus.

Omics technologies can power the creation of cellular and spatial maps or "atlases" of the brain, brain matters, and ventricular tissues that can be used to identify pathological changes and novel targets [77]. For example, a single-cell atlas of the ChP was leveraged to identify the differential expression of NKCC1 across development as mentioned above [78, 79]. Integrating single-cell and spatial transcriptomic techniques with other approaches in preclinical models of hydrocephalus, human tissue, or liquid biopsies could unveil cellular or spatially distinct molecular signatures within the CSF and ventricular system [80], enhance our understanding of hydrocephalus, and guide the development of precision interventions and personalized treatments tailored to specific tissue regions.

Another utility of omics approaches is the interrogation of CSF. With CSF drainage and collection as common practice in the care of hydrocephalic and non-hydrocephalic control subjects, the lack of large omics datasets on CSF composition was highlighted during the workshops as a missed opportunity in the field. Delving into the proteomic and metabolomic landscape of CSF could provide insights into the dynamic interplay of molecular and metabolic mechanisms underlying hydrocephalus pathogenesis. For example, precise measures of protein levels indicative of neuroinflammation or altered metabolic profiles could serve as actionable biomarkers, guiding diagnostics and the development of targeted therapeutic interventions that could improve patient care. Requisites for these

analyses are advancements in methods for the isolation and purification of samples, as well as analytical methods to distinguish true biological signals from background and contaminant signals. In that vein, Petrova et al. have developed analytical approaches to apply untargeted metabolomics for the generation of a mouse embryonic CSF metabolite library [81]. They have used this reference library for comparisons with the metabolome of embryonic CSF from a mouse model of autism; however, similar investigations comparing CSF from hydrocephalus models may provide novel treatment targets or biomarkers of disease state. Thus, a research priority for the field is to use omics and multi-omics technologies to guide hydrocephalus diagnostics and therapeutics (*Research Priority 3.2*, Table 1).

The need for large datasets

Omics and multi-omics studies require large datasets, posing a challenge in hydrocephalus research, which typically investigates small, clinically heterogeneous, and geographically limited patient populations. Thus, data sharing and standardization among hydrocephalus research groups will be critical for those using these technologies (*Research Priority 3.3*, Table 1). Importantly, pathogenic changes and genetic predispositions likely vary by hydrocephalus etiology. Although developing therapeutic interventions for every gene and molecular target is impractical, exploring datasets across various etiologies of hydrocephalus to identify common molecular pathways is likely to expedite drug repurposing and refine precision medicine tailored to nuanced variations. This cross-etiology perspective has been proposed in rare disease research to move towards categorizing diseases on a molecular basis rather than the traditional symptom-based definitions to streamline drug development [82]. Currently, there are 48 publicly available datasets related to hydrocephalus research in the National Center for Biotechnology Information (NCBI) and the European Nucleotide Archive (ENA) (Supplemental File 4 Table 2). Although technical variation between datasets limits their interpretability, these resources can be used to formulate a hypothesis to be tested in a new study. Multidisciplinary and multi-site collaborations that encompass various etiologies are also likely to be crucial in providing sufficient datasets for groundbreaking discoveries. Existing hydrocephalus consortiums and research networks, such as the Adult Hydrocephalus Clinical Research Network (AHCRN), the Hydrocephalus Clinical Research Network (HCRN), and the HA Network for Discovery Science (HANDS), should prioritize the creation of more hydrocephalus datasets.

Applying machine learning for data analysis

Key advancements in bioinformatics, including the analysis of large datasets generated by omics and multi-omics technologies, have come from machine learning (ML). ML algorithms, built upon a foundation of statistics, compute models that capture complex realities and predict outcomes of systematic interactions. In hydrocephalus, ML has been used to analyze morphometric parameters and radiological features to support accurate diagnosis [83]. For other conditions, such as cancer, ML models have been used in combination with omics approaches to predict treatment response [84]. Because ML models identify complex patterns in high-dimensional and heterogenous data, the inner logic of the models is often not explanatory [85]. The use of ML models for high-stakes decisions has been criticized because of the inherent lack of transparency [86] and suggests that ML models may not be well-suited for understanding the underlying mechanisms of a disease. However, ML can be used to combine large amounts of data, including omics datasets, imaging, clinical features and outcomes, to identify potential pathways and druggable targets for disease intervention. These models can then be used to formulate hypotheses explaining underlying mechanisms and testing novel therapeutic strategies (Fig. 1). Thus, a priority for hydrocephalus research is to use ML to analyze new and existing hydrocephalus datasets (*Research Priority 3.4*, Table 1). Advancements in ML are happening at tremendous rates, making its use in research intimidating for many biomedical scientists. Tools such as Automated Machine Learning streamline the traditionally ambiguous model development process, thus reducing the threshold for needed expertise. However, collaborations between hydrocephalus researchers and data scientists are likely needed for the field to take full advantage of ML opportunities [87].

With advancing methodologies for omics, imaging, and ML, we stand at the cusp of a new era in hydrocephalus research characterized by precise molecular insights, complex system analysis, and improved targeted therapeutic strategies. Integrating diverse omics datasets with other data modalities, including imaging and neurocognitive behavior outcomes, will unravel the complex tapestry of hydrocephalus pathology, paving the way for non-invasive management strategies.

Theme 3: Design preclinical studies with a focus toward clinical translation

To generate better clinical therapies for those with hydrocephalus [11], workshop participants recognized the need to improve animal modeling, implement consistent endpoint measurements in preclinical and clinical trials,

and collaborate across basic and translational scientists and patient care teams (*Theme 3,* Table 1).

Improve animal modeling

Preclinical testing aims to demonstrate efficacy with an acceptable side effect profile to warrant a trial for safety in humans (*primum non nocere*). A key aspiration noted in the workshops was to identify or design optimal preclinical models to reflect clinical trials (*Research Priority 4*, Table 1). Workshop participants acknowledged the diversity of existing models—cell-based, organotypic, and various animal models—each offering unique strengths and weaknesses [15, 88]. However, no single model can fully replicate the complexities and heterogeneity of human hydrocephalus, necessitating distinct models for different etiologies, such as obstructive versus communicating hydrocephalus. Similarly, hydrocephalus is a lifelong condition with myriad comorbid neurological conditions, thus necessitating studies designed to monitor the long-term impact on quality of life.

Understanding hydrocephalus at a granular level requires cell and tissue-based analyses across preclinical models and human tissues. In this regard, current models in small and large animals that utilize injectable agents (blood products, inflammatory molecules, inorganic metals or polymers) into the ventricles or cisterna magna subarachnoid space have been influential in the field and recapitulate two main features of acquired hydrocephalus: 1) ventriculomegaly (modeling a mismatch between CSF production and reabsorption) and 2) parenchymal injury (modeling injury to neural progenitors and white matter tracts, which correlate with cerebral palsy and cognitive deficits). However, these models have limitations; importantly, as some models of acquired hydrocephalus generate asymptomatic ventriculomegaly that do not necessitate shunt surgery. By contrast, any animal model that generates more severe symptoms will have direct clinical relevance, as the options for patients with severe symptoms are usually limited to surgery. Though clinically relevant and preferable for studies, truly symptomatic animal models are generally not possible, especially in larger animals, due to the need to euthanize the animals for ethical reasons.

Small animals (frogs, mice, rats, zebrafish) allow for the use of transgenic modification and well-established viral gain- and loss-of-function approaches that expand the toolkit for investigating mechanisms of disease. Indeed, many spontaneous or induced mutations in rodents result in hydrocephalus, recently reviewed in [21], and the addition of induced and genetic mouse models of NPH will undoubtedly aid in understanding mechanisms of disease and serve as platforms for therapy testing [89, 90]. Large animals (rabbits, ferrets, pigs, sheep), often cited for their benefit in easing the transition to FDA trials based on their more relevant size and physiology, represent more than a scaling conversion toward human-sized brains. Brain physiology associated with gyrated cortices enables insights into neurophysiology, neurodevelopment, neuroimmunology, and relevant surgical modeling [76–78]. Moreover, larger animals better replicate human ventricular shape, volume-to-surface area ratio, and hydrodynamics [91–94]. Certain forms of ventriculomegaly have only been demonstrated in large animals, such as sheep [95, 96]. Therefore, these animals represent CSF volumes and hydrodynamic profiles that cannot be modeled in smaller animals. A few groups are leading with new models in large animals, including dogs and cats, but limitations to genetic manipulation restrict these models to acquired (post-hemorrhagic, post-infectious) rather than congenital forms of hydrocephalus [97, 98]. Ultimately, selecting the appropriate preclinical animal model is crucial for accurately capturing the manifestations of hydrocephalus and assessing treatment effects. Workshop attendees emphasized the trade-offs between small and large animal models, balancing experimental cost, speed, and access to molecular tools. As advancements continue in shunting techniques and behavioral testing in small animals, there is potential for more effective preclinical therapy testing within these models, while still recognizing the critical nuances specific to hydrocephalus that influence model selection.

Implement consistent outcome measures in preclinical and clinical trials

Clinical studies in hydrocephalus management have traditionally centered on surgical outcomes, such as shunt failure and infection rates. However, patient engagement, especially during the patient perspective panels of the workshops, highlighted that cognitive function, pain management, and overall neurological outcomes are of paramount importance. Incorporating these patient-centered variables into clinical trial designs is crucial for truly understanding the impact of interventions (*Research Priority 5.1*, Table 1). Notably, even though treatment in the Drainage, Irrigation and Fibrinolytic Therapy (DRIFT) trial did not reduce the number of shunt placements, it reduced severe cognitive impairment after 2 years [99]. This highlights the need for trials to measure both immediate surgical success and long-term patient well-being.

To better assess long-term patient well-being, a broader patient care team, including neuropsychologists in collaboration with neurosurgeons, should be implemented in hydrocephalus research (*Research Priority 5.2*, Table 1). Understanding the neuropsychological phenotypes associated with hydrocephalus is essential for predicting developmental trajectories and requires longitudinal

studies [100]. For example, in the Management of Myelomeningocele (MOMS) trial, analysis of 30-month outcomes revealed improved mental development and motor function in children following interventions like fetal myelomeningocele repair [101]. The 2022 research workshop by the Hydrocephalus Association and Rudi Schulte Research Institute, titled, "Improving Cognitive and Psychological Outcomes in Hydrocephalus", highlighted key research priorities, including neuropsychological phenotypes, treatment-focused approaches, and translational tools, aimed at advancing cognitive and functional outcomes for individuals with hydrocephalus [100]. By integrating cognitive assessments and physiological measures, researchers can develop more targeted interventions and ultimately improve the quality of life for individuals affected by hydrocephalus. Additionally, sophisticated motor analyses, such as gait assessments in adult patients with iNPH, can provide valuable insights into recovery and rehabilitation strategies. Developing imaging correlates that guide intervention thresholds will also enhance decision-making and improve patient care.

In addition to the patient perspective, workshop participants were asked to submit ideal outcome measures for preclinical and clinical studies that are important for discerning therapy efficacy (*Research Priority 4*, Table 1). Participant responses reflected three major types of outcome measures including imaging metrics, cognitive and behavioral outcomes, and physiological measurements (Table 2). From a research perspective, understanding the pathophysiology of hydrocephalus requires rigorous study of various biomarkers and imaging techniques. Imaging metrics, particularly ventricular volume, are essential in evaluating patient outcomes related to hydrocephalus. While ventricular size can indicate the severity of certain conditions and is often associated with clinical symptoms, its utility in diagnosing shunt efficacy and failure is limited. Similarly, clinical experience in congenital hydrocephalus has shown that using ventriculomegaly in isolation as an indication for intervention may increase the risk of complications associated with over drainage, such as the development of subdural collections and slit ventricle syndrome, increasing the risk of shunt malfunction and complications. Ultimately, while large ventricles may be tolerated in some patients, the relationship between ventricular size and clinical outcomes necessitates further exploration. Moreover, innovative imaging modalities that assess CSF flow may provide deeper insights into treatment efficacy [102, 103].

Physiological measures are crucial for hydrocephalus management. Elevated ICP is a primary reason for surgical intervention, as normal pressure is essential for maintaining brain perfusion, a normal trajectory of head growth in infants, and clinical improvement. Monitoring ICP not only informs decisions regarding surgical treatment but also helps predict treatment failure, particularly when ventricle volumes do not change. In an effort to improve surveillance of shunt function in patients, recent advances in telemetric ICP monitors in Europe are notable [104, 105]. The progress in these technologies might be incorporated into similar diagnostic testing in preclinical models.

White matter integrity and neuro-connectivity, although not routinely assessed in clinical practice, hold significant promise for understanding the prognosis of hydrocephalus. Advanced imaging techniques, such as DTI, have shown that changes in white matter can provide insights into neurological outcomes, especially in conditions like cerebral palsy. Research suggests that pressure on specific white matter tracts, such as the internal capsule, can impair motor function, but these effects may be reversible following appropriate interventions like shunting [106]. More comprehensive studies that include these measurements could enhance our understanding of the long-term impacts of hydrocephalus and guide early interventions.

Biomarkers and histopathology play a crucial role in understanding the pathophysiology of hydrocephalus and guiding treatment decisions. While CSF biomarkers, such as neurofilament light (NFL) and myelin basic protein (MBP), have emerged as general indicators of neurodegeneration, there remains a significant need to identify novel biomarkers specific to hydrocephalus to improve diagnostic accuracy and treatment outcomes. These emerging biomarkers may help differentiate between neurocognitive disorders, like iNPH and Alzheimer's disease, and provide insight into shunt effectiveness and patient outcomes. Histopathological analysis, particularly in post-mortem samples and preclinical models, offers valuable information about the underlying mechanisms of injury related to hydrocephalus. By combining insights from both biomarkers and histopathology, researchers can better understand the progression of the disease, identify targets for intervention, and ultimately improve therapeutic strategies for affected individuals.

Collaborate across basic and translational scientists and patient care teams

Designing clinical trials for hydrocephalus presents a monumental challenge that necessitates collaboration across multiple disciplines, including basic science, neurosurgery, and other patient care teams (*Research Priority 5.3*, Table 1). While the goal of achieving shunt freedom is aspirational, shunt dependency may be an acceptable trade-off if developmental outcomes and symptom management are optimized. Future trials must prioritize patient-driven multidisciplinary objectives and foster

collaboration among specialists to assess neurodevelopmental outcomes effectively.

An alternative approach to hydrocephalus treatment could involve treating pre-hydrocephalic conditions, particularly in high-risk populations such as premature neonates. Trials aimed at preventing the progression to temporizing devices or reducing conversions to permanent ventriculoperitoneal shunts could significantly impact patient care. Similarly, addressing the root cause of hydrocephalus, such as evolving brain injury that precedes post-traumatic hydrocephalus, may also be a critical area for investigation. Fostering a collaborative, multidisciplinary approach in clinical trial design will be essential to advancing effective treatments for hydrocephalus and improving outcomes for vulnerable patient populations.

Synthesis

Research workshops provide an opportunity for a field to reflect on progress and set new goal posts to focus research efforts. Prior research priorities proposed by the Hydrocephalus Association have seen progress in different arenas [107, 108]. Yet, the prolonged ramifications of hydrocephalus highlight the continued need for non-invasive hydrocephalus therapies. The discussions from these two workshops organized by the Hydrocephalus Association culminated in the identification of research priorities summarized by three overarching themes: 1) Expanding our understanding of CSF physiology, the precipitating mechanisms of CSF dysfunction, and the damage caused by hydrocephalus to develop intervention strategies; 2) Leveraging big data to identify novel hydrocephalus treatment targets; and 3) Selecting strategic outcome measures, endpoints, and preclinical models of hydrocephalus to assess the efficacy of interventions and facilitate translation into clinical trials. Advances in any of these themes will likely benefit efforts in others: for example, omics analyses could uncover novel hydrocephalus-associated pathways that could provide mechanistic insight into CSF dysfunction and inform the development of preclinical models.

Inherent in the pursuit of these priorities is the need for collaborative efforts among clinicians, healthcare specialists, research scientists, data scientists, and patients as well as funders to support large pre-clinical and clinical studies. Because hydrocephalus is a heterogenous condition encompassing numerous causes, patient populations, and associated sequelae, there is unlikely to be a single non-invasive treatment applicable to all hydrocephalus cases. However, collaborations comparing the physiology across different hydrocephalus etiologies to identify commonalities have the potential to identify treatment strategies with a broad impact. Additionally, recent advances in ML can only be used to their full effect when paired with vast datasets, which necessitate collaborations among research institutions and open-access databases and publications. Further, the journey from identifying a novel treatment target to developing a non-surgical intervention likely requires the use of multiple different disease models and can be expedited by purposefully creating research collaborations among experts and fostering multidisciplinary discourse in the field. Finally, to understand the consequences of novel treatment strategies on the outcomes important to patients, clinical trials must be designed for long-term follow up to facilitate longitudinal analysis, thus requiring expanded funding support. The priorities outlined here and the call for greater collaborative efforts among researchers and research institutions should serve as guidelines for the hydrocephalus community and funding agencies.

Supplementary Information

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Supplementary file 1 - Attendees.

Supplementary file 2 - 2023 Program PDF.

Supplementary file 3 - 2024 Program PDF.

Supplementary file 4 - Database list.

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Declarations

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