Title: Genomic approaches to improve the clinical diagnosis and management of congenital hydrocephalus patients

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Key Words: Congenital hydrocephalus, genomics, complex inheritance, neurodevelopmental disorders, whole-exome sequencing, genomic medicine

Abstract word count: 143

Text word count: 3947

Number of references: 73

Number of tables and/or figures: 3

Number of videos: 0

Abstract:

Congenital hydrocephalus (CH), characterized by incomplete clearance of cerebrospinal fluid and subsequent enlargement of brain ventricles, is the most common congenital brain disorder. The lack of curative strategies for CH reflects our poor understanding of the underlying pathogenesis. Here, we present an overview of recent findings in the pathogenesis of CH from human genetic studies and discuss the implications of these findings for treatment for CH. Findings from these -omics data has the potential to re-classify CH according to a molecular nomenclature which may increase precision for genetic counseling, outcome prognostication, and treatment stratification. Beyond immediate patient benefit, genomic data may also inform future clinical trials and catalyze the development of non-surgical, molecularly-targeted therapies. We therefore advocate further application of genomic sequencing in clinical practice by the neurosurgical community as a diagnostic adjunct in the evaluation and management of patients diagnosed with CH.

Introduction:

Hydrocephalus is defined as a pathological accumulation of cerebrospinal fluid (CSF) in the ventricular system of the brain. Given this broad definition, it has been proposed to more narrowly define this disorder as a “distension of the cerebral ventricular system due to inadequate passage of CSF from its point of production to its point of absorption” 1. Although secondary hydrocephalus is often found in the setting of obstructive brain tumors, leptomeningeal disease, infection and hemorrhage, these patients typically develop elevated intracranial pressure and demonstrate a clear obstructive pathology 2. Infantile hydrocephalus, however, is often present without a known antecedent or identifiable obstruction, and is therefore classified as primary (idiopathic) or congenital hydrocephalus (CH) 3. CH is the most common developmental neurological disease that affects 1/1,000 live births 4 and costs the US healthcare system in excess of $2 billion annually 4,5. CH can occur in the setting of complete/partial intraventricular obstruction (non-communicating hydrocephalus), most often due to aqueductal stenosis; however, commonly this infantile form of hydrocephalus is “communicating” and no physical obstruction to CSF flow is apparent.

Surgical CSF shunting or endoscopic third ventriculostomy (ETV) procedures aimed at reducing CSF accumulation are the current mainstay of treatment. While these procedures can be lifesaving, they carry high rates of morbidity, complication, and failure, often requiring repeat surgeries and life-long neurosurgical management 3. Even after surgical intervention, however, ventriculomegaly persists in many CH patients, and shunting often does not improve neurodevelopmental outcomes in this patient population 6-8. Paradoxically, some CH patients demonstrate normal or low intracranial pressure (ICP) at the time of shunt placement 3,9. Together, these observations highlight the fundamental challenge of managing such a heterogenous disease, and argues that some CH patients may not benefit from neurosurgical intervention. This therefore raises the question of whether ventriculomegaly in shunt-refractory hydrocephalus is a primary disorder of CSF homeostasis, or an associated epiphenomenon of a more critically impaired neurodevelopmental process. An improved understanding of the cellular and molecular mechanisms that drive the pathogenesis of CH in human patients could improve diagnostic testing, therapeutic options, and patient outcomes.

Perhaps the most efficient and unbiased approach to gain insight into CH pathogenesis is to identify novel disease-causing genetic variations. Genetic studies in animals 11 and in human syndromic CH 10-13 show that single inherited variants can exert large effects on CH risk 14,15. Nevertheless, despite reports of multiplex pedigrees that feature CH as the predominant phenotypic feature (i.e., “non-syndromic" CH), the number of genes identified and the proportion of CH patients explained by these genes small 16. Variants in these genes are predicted to account for less than 5% of primary CH cases 17, despite that greater than 40% of all CH cases have been predicted to have an underlying genetic etiology 18. Thus, most of CH cases remain genetically unexplained, emphasizing the need for continued gene discovery. The identification of novel human CH disease genes may guide generation of animal models that can help elucidate disease mechanisms, catalyze the discovery of medically-based treatments, and reduce neurosurgical refraction.

Locus and phenotypic heterogeneity, incomplete penetrance, and pleiotropy have hindered CH gene discovery efforts using traditional family-based linkage approaches 16. CH and other clinically heterogenous neurodevelopmental disorders (NDD) also show strong signs of negative selection, suggesting that the genetic architecture of these diseases consists of rare variants with large deleterious effects. These observations, along with the sporadic nature of CH, suggest that a significant proportion of variants that strongly contribute to disease pathogenesis in CH are likely to have arisen *de novo* in the population. To that end, whole exome sequencing (WES) of case-parent trios (affected patient and their parents) is an efficient and cost-effective method of disease-causing gene discovery that enables selective sequencing of all protein coding genomic regions 19. This makes WES a suitable technique for the detection of rare deleterious variants, especially when used to sequence a large cohort of patients and their unaffected family members. WES has recently demonstrated the importance of rare damaging *de novo* mutations (DNMs) and transmitted (inherited) mutations with variable expressivity and incomplete penetrance as important mechanisms of disease in several different genetically heterogeneous NDDs, including autism, epilepsy, and intellectual disability 20,21. Genetic studies using other omics data followed by functional experiments also discover several novel loci associated with CH 22-25. In this article, we review recent gene discovery efforts for CH and discuss the clinical and research utility of WES in understanding the molecular pathogenesis of this disorder. We suggest WES and other next-generation sequencing-based technologies will become mainstay diagnostic and prognostic adjuncts for patients clinically diagnosed with CH.

Main text:

**Applying genomic sequencing to the discovery of CH genes and pathways**

Only eighteen years ago, the human genome project was completed after thirteen years of work at a total cost of $2.7 billion 26. Over the last two decades, technological advancement and innovative multiparallel sequencing approaches have driven the time and cost investment of genomic sequencing far lower than would be predicted by the exponential growth of computational power, allowing next-generation sequencing (NGS) technologies to be widely applicable in both research and clinical environments (**Figure 1**). With particular relevance to recent CH gene discovery, WES applies targeted capture of the exome – the protein coding regions of the genome – along with flanking intronic regions followed by massively parallel sequencing to recognize variation across the exome relative to a reference sequence 27. Comprising only 1.2% of the human genome, the exome is disproportionately represented in genetic diseases, harboring an estimated 85.5% of all deleterious variants causative of human diseases (**Figure 2**) 28. Where proband-maternal-paternal trio data is available, WES can also identify coding region and splice site *de novo* mutations (DNMs), which have been highly associated to CH pathogenesis 29-31. Thus, WES presents itself as a more time- and cost-effective methodology for unbiased CH gene discovery and diagnosis for patients with idiopathic hydrocephalus and has repeatedly been proven successful in gene discovery for CH as well as other congenital or neurological conditions 20,23,26,30,32-36. Additionally, genomic studies can direct future functional studies into CH pathogenesis, identifying underlying disease processes and potentially catalyzing the development of future therapeutic options 22.

It is also important to delineate that many genes have been reported as associated with CH, however for most of these, defects or dysfunction in the genetic code do not necessarily translate into clinical manifestations of hydrocephalus in patients 37. For the purposes of this review, we will be discussing only the currently-known high-confidence CH gene candidates. These high-confidence genes are those for which strong evidence exists that primary CH results directly from their alteration, including multiple damaging DNMs, an exome-wide significant mutational burden, and/or multiple independent transmitted mutations co-segregating with CH in independent pedigrees (**Table 1**).

Although the rate of gene discovery is increasing exponentially, relatively few high-confidence CH genes have been described, and those discovered comprise a small portion of the expected number of CH-associated genes (**Figure 1**). Thus, while a genetic panel may be less expensive and able to identify the most common variants in known CH genes, WES has the advantage of superior resolution to identify gene dysfunction, which can translate to enhanced discovery of novel CH genes, as well as novel single nucleotide (SNVs), copy number (CNVs), and other structural variants on known CH genes 38. Importantly, WES analysis of CH patients can also contribute to phenotypic expansion of currently-described syndromes for which a CH phenotype is not currently documented, further aiding diagnostic yield.

Whole-genome sequencing (WGS) shares many of the advantages of WES over traditional genetic testing but includes information on variants in the noncoding region including in sites which can lead to disease phenotypes if altered such as promoter, enhancer, and branch splice site regions. Additionally, WGS boasts increased uniformity of coverage depth across the exome to inform variant calling in regions of the genome not amenable to capture 35. Despite this, WGS is currently several times more expensive with only moderately increased diagnostic yield over WES. For that reason, it is common practice to use the more expensive WGS as a secondary approach only in cases where genetic etiology is expected, but WES analysis was not able to resolve a genetic basis of disease.

With increasing accessibility and affordability of next-generation sequencing (NGS) technologies, and especially WES, there has been an explosion in gene and pathway discovery for many diseases, including CH (**Figure 1**). The discovery of new genes and structural variants associated with CH is improving our understanding of the biological pathways involved in this elusive pathology. Further, the identification of these genetic mutations is shedding light on the relative contribution of the aggregate disease profile that must be considered when applying genetic analysis such as WES to the diagnosis of CH. Therefore, we advocate a tier-sequencing approach (i.e., WES followed by WGS in patients whose finding was negative from WES) for routine medical practice. Studies of clinical utility and cost-effectiveness have showed that genomic sequencing in complex pediatric patients saves significant costs and dramatically improves the diagnostic yield of traditional cytogenic or targeted panel sequencing approaches 39-41.

**Relative Contribution of Chromosomal Aneuploidy and Copy Number Variants**

While strong evidence of ubiquitous, primary CH has yet to be described as a result of chromosomal aneuploidy or copy number variation, there are a number of structural variants where the extent of association to CH is unknown.

Historically, down syndrome 42, or trisomy 21, has been linked to CH in large cohort studies featuring 11,000 cases and 7.8 million controls 43. Down syndrome is the most common autosomal trisomy 3 and is often characterized by delays in physical growth and varying intellectual disability. Independent studies have shown that Down syndrome patients have a greater activation of the mTORC1 pathway in brain tissue than those patients without Down syndrome. Hydrocephalus is one of many neuroanatomical phenotypes linked with an increase in activation of mTORC1 44. Compoundingly, a recent study has reported significant association of CNV deletion events in trisomy 21 patients with fetal ventriculomegaly 45. Furthermore, others have identified a number of copy number variations as potential causes of fetal ventriculomegaly and thus potential agents of CH pathogenesis 46.

After Down syndrome, trisomy 18 47 is the second most common autosomal trisomy 43. It is one of many cytogenetic abnormalities associated with hydrocephalus 3. However, this association is very rare. It is unusual to see CH present alongside trisomy 18 in the absence of other brain abnormalities. Further research is necessary to determine if this presentation of CH is coincidental or a high-confidence association 47.

Lastly, Furey et al. report five verified *de novo* CNVs after applying XHMM algorithms to call CNVs from a cohort of 125 WES-sequenced trios, with two duplication events occurring at the SHH locus. The protein coded by SHH is the canonical ligand for PTCH1, a high-confidence CH gene that regulates neurogenesis by conveying spatial information to ventral neural progenitor cells in the neural tube 30. Notably, *de novo* single nucleotide variants in SHH were also disproportionally enriched compared to the expected number in a later study of 232 CH trios discussed later in this review 36. Further studies will be needed to fully elucidate the involvement of SHH in the pathogenesis of CH.

**Relative contribution of single nucleotide variants and small insertion/deletion events**

Contrastingly, single nucleotide variants (SNVs) and small (less than100 base pair) insertion/deletion events (indels) comprise the majority of novel CH gene and pathway discoveries through genomic approaches. Current WES capture and analysis is known to be accurate in identifying the vast majority of SNV and indel events, and novel technologies are developed daily to increase the coverage and calling of WES while decreasing cost.

Autosomal-dominant inheritance and *de novo* variants

Recently, analysis of WES data from CH trios (proband, mother, father) for DNMs and rare, autosomal dominant variants has yielded rich results for the discovery of novel CH genes and expansion of CH pathway involvements 36,48,49. Recent studies have applied WES of CH patients and found significant evidence tying variants in FOXJ1 into CH pathogenesis in both de novo and transmitted modalities of inheritance. These have implicated impaired cilia formation and function to the development of CH symptoms, further suggesting a currently-unknown underlying disease mechanism for CH 24,25 Most recently, analysis of 381 neurosurgically-treated CH patients (232 trios) found that damaging DNMs account for >17% of all CH cases with five genes (*TRIM71*, *SMARCC1*, *PTEN*, *PIK3CA* and *FOXJ1*) identified as having significant de novo mutation burden 36. From this report and application of similar methods, CH pathway homologies may be elucidated. For example, the finding that PI3K signaling genes *PIK3CA*, *PTEN* and *MTOR* are frequently mutated in sporadic CH implicates the PI3K pathway in hydrocephalus molecular pathology 50. Further analysis of recurrent DNMs has also identified *FOXJ1*, *FMN2*, and *PTCH1*, which all harbored multiple DNMs and damaging inherited variants, as likely CH genes 36. Additionally, *TRIM71*, *PTCH1*, and *SMARCC1*, all high-confidence or probable CH genes, were found to have significant burden of *de novo* and transmitted variants in an exome sequencing study of 125 trios with 52 additional probands 48. Additionally, *de novo* variants in *SHH*, which encodes PTCH1 ligand, were also disproportionately elevated in the CH cohort. Notably, all four genes (*TRIM71, PTCH1, SMARCC1,* SHH) are required for neural tube development and regulate prenatal neural stem cell progression, implicating impaired neurogenesis in the pathology of a subset of CH cases 31,48,51,52. Another report examined a cohort of exome-sequenced, L1CAM-negative CH patients applying XHMM algorithms to predict *de novo* copy number variants in addition to traditional analyses, and identified *SLC12A6* and *SLC12A7*, closely-related K-Cl cotransporters, as novel CH-associated genes 49. Thus, WES can be applied to the detection of a wide number of autosomal-dominant variants, with particular focus being given to DNMs in instances where trio data is available.

Autosomal-recessive inheritance

WES of CH patients has also identified rare autosomal recessive variants, further elucidating the genetic profile of CH. Prior to the widespread application of WES, however, gene discovery through rare variants was much slower. For example, Al-Dosari et al. describe a case study of two consanguineous families which underwent linkage analysis and autozygosity mapping followed by direct sequencing of a 6.9Mb interval, revealing *MPDZ* as a novel CH disease gene with a constellation of associated additional clinical findings including neurodevelopmental delay, seizures, coloboma, ocular disorders, and craniofacial malformations as well as various systemic defects beyond the nervous system 53. Ekici et al. describe the second autosomal-recessive CH gene identified without WES. Through positional cloning and targeted sequencing of a consanguineous family, they identified a novel splice site mutation in *CCDC88C* resulting in a consistent complex hydrocephalic brain malformation with associated craniofacial abnormalities 54,55. WES-mediated studies, by comparison, are faster and able to identify single CH disease genes from complex multi-syndromic or pleiotropic phenotypes. Slavotinek et al. report exome sequencing of five probands from three nonconsanguineous families with cerebral ventriculomegaly, echogenic kidneys and histopathological findings of congenital nephrosis to identify novel deleterious autosomal-recessive variants in *CRMB2* in all sequenced probands 56. In another case study, WES identified compound-heterozygous variants in *ATP1A3* in a single patient inherited from unaffected parents. These mutations were subsequently confirmed as causal of CH by immohistochemical studies of ATP1A3 activity in mouse embryonic brain tissue 57. Shaheen et al. increased the study cohort size, performing a WES and Axiom SNP-chip array study on a moderate size CH cohort of 27 families, each with two or more children diagnosed with CH. The group was able to identify likely-causal mutations in 21 of 27 families (78%) and described two novel CH genes, *EML1* and *WDR81*, highlighting ciliopathies as a major contributing factor to CH pathology 38. WES, therefore, demonstrates robust performance in ascertaining causal autosomal-recessive CH variants, with particular power in consanguineous families or in those where pedigree data supports an autosomal-recessive modality of inheritance.

X-linked Inheritance

X-linked hydrocephalus (HSAS), which is characterized by intellectual disability and enlarged brain ventricles, was the first form of CH to be mapped to a defect in a specific gene. Through analysis of recombination events, Rosenthal et al. mapped the gene HSAS to an interval of two Mb in Xq28. Further analysis of patient-derived cDNA of the same chromosomal subregion revealed a novel mRNA species of L1, a highly-conserved glycoprotein involved in the migration and adhesion of neurons 14. Consequently, Camp et al. conducted a mutation analysis of *L1CAM*, an L1 gene found within the identified Xq28 subregion, in 25 HSAS families and subsequently confirmed *L1CAM* as the primary HSAS gene and the first identified CH gene 58. Sheen et al. later reported three familial cases of periventricular hypertropia (PH) with hydrocephalus and suggestive linkage to the Xq28 subregion. Specifically focusing on *FLNA* which had been previously reported as associated with PH, the group found significant phenotypic heterogeneity in FLNA variants, suggesting a more complex inheritance pattern 20,59,60. Lastly, Saillour et al. report variants in AP1S2 as causative in an X-linked syndrome characterized by hydrocephalus and intellectual disability 61.

**Value of a Diagnosis**

Together, CH afflicts millions of children globally. Although clinically the disease appears similar across patients, each CH gene variant is, to a degree, rare and can constitute a unique CH pathogenesis specific to each patient. An understanding of the underlying genetic pathway for each patient is important, not only for development of targeted therapy, but also for accurate diagnosis. Often, patients presenting with a genetic syndrome wait months-to-years before receiving a specific diagnosis, a challenging and time-consuming process termed “the diagnostic odyssey.” In a 2013 study of 613 rare disease patients, the average elapsed time from first clinical presentation to the correct diagnosis was 7.6 years in the United States. Moreover, each patient visited an average of eight separate physicians and received three misdiagnoses 62. Further, a recent analysis comparing Canadian rare disease patients who received a diagnosis to those who did not, found multifold benefits for the diagnosed patients.

Beyond the primary positive medical impact of increased access to disease-specific therapies, diagnosis provided positive social impacts, such as the ability for patients to join disease-specific support groups, and positive personal impacts including a higher reported sense of patient empowerment and self-confidence as well as the ability to conduct life-planning and reproductive decision-making. These factors all significantly contribute to positive outcomes and satisfaction as a result of diagnosis, and improve overall quality of life for patients 63. Although there has been no report of the personalized treatment for CH patients with known disease-causing mutations, personalized treatments have been initiated for several rare diseases, including ethosuximide for GNB encephalopathy 64, levodopa for CTNNB1/β-catenin deficient dystonia 65, and 5-aminoimidazole-4-carboxamide riboside for AMPD2- related Rationale for dopa-responsive 66, which suggests targeted gene therapies could soon come to fruition for CH patients who have undergone genomic sequencing. Furthermore, genetically diagnosing CH opens the possibility of stratifying surgical outcomes by gene variant; currently, there is no such data on this. Such analysis could ultimately predict responsiveness to shunting and/or aid in prognostication for patients and families.

**Future Directions**

Although WES has the potential to enhance the care and management of patients with CH, there are limitations in its diagnostic capabilities. Specifically in cases (1) lacking true genetic etiology; (2) with genetic etiology in regions of the genome not captured by WES such as promoter, enhancer, and branch-splice sites 67; with lowered detection threshold for chromosomal abnormalities; and (4) with genetic etiology in areas of the exome not amenable to capture. There are also areas upon which further study, novel technologies, and consensus guidelines may improve the utility of WES for the diagnosis of CH.

Functional Analysis for Variants of UnknownSignificance (VUS)

As genomic medicine becomes more commonplace, an increasing number of gene variants are being identified. Many of these alterations are in previously undescribed genes or are in areas of otherwise unknown clinical significance (VUS). Traditional genetic approaches for interpreting the importance of genetic variants, such as case-control or co-segregation studies, require a large sample size, which limits the discovery of rare, novel, or unique disease-causing entities. Computational predictions of pathogenicity described previously are useful in variant filtration, however, they lack the high confidence level required for clinical translation and direct application to patient care 68-70. Additionally, continued community annotation with current ACMG pathogenicity classifiers and data sharing via databases such as ClinVar can provide continually-increasing knowledge for future diagnostic efforts 70-72.

Continuous Review

As the discovery of new CH-related genes accelerates, and known gene phenotypes are further defined, a clearer picture of the full spectrum of CH is coming into focus. These new insights are unveiling the importance of previously unknown and under-appreciated gene mutations and alterations as disease-causing agents, and as such, is actively redefining our definitions of many pathologies such as CH. As our knowledge continues to grow, it is likely that variants of previously unknown significance will become diagnostic parameters, and phenotypes thought to be constrained to the pediatric population may be further defined in adults. Furthermore, periodically reviewing previous collected samples in undiagnosed patients is also important. Multiple studies have reported an increased diagnostic yield ranging from +5.9% – +22.0% from reanalysis of data several years after initial analysis 73,74. Surprisingly, however, despite this potential for improvement in patient care, there are no official guidelines or regulations regarding the reanalysis of samples and thus it is rarely performed. A 2017 study reported that only 1 out of the 21 surveyed labs continuously reviewed undiagnosed cases 75. As such, there remains an unmet clinical and scientific need for universal policies and regulations governing the periodic review of undiagnosed cases. Making this a universal practice will not only improve clinical care of individual patients but will increase the cohort of patients with unique mutations available to study and has the potential to meaningfully impact our understanding of genetic disease.

Multiomic Analysis:

With increasing availability of data and the continual development of new analytical techniques, integration of multiple modalities of patient data including genomics, transcriptomics, epigenomics, and phenomics, may present a new methodology for discovery of CH genes, phenotypic clusters, and pathway involvements. A few recent studies in CH and other congenital neurological diseases have applied integrative multiomic approaches 23,36, but widespread adoption could revolutionize the field of CH genomics, driving new discoveries and informing future functional investigation in a poorly-understood and deeply heterogeneous disease.

Conclusions:

Taken together, these studies present WES as an efficient and inexpensive methodology for both the discovery of novel CH genes and the clinical diagnosis of idiopathic hydrocephalus. Genomic investigation of CH has elucidated many novel CH genes and pathways and presents itself as a potent tool for the diagnosis of both known, and in conjunction with functional variant analysis, unknown forms of idiopathic hydrocephalus. Here we describe the process of discovery for all CH genes along with relative contribution towards aggregate disease profile. We also review the current knowledge in the field of CH through the lens of WES and identify shortcomings that prevent optimal utility of WES in the diagnosis of CH while also offering suggestions of future directions to ameliorate these shortcomings. Finally, we discuss the multifold positive implications of a specific genetic diagnosis through genomic medicine including more targeted therapy, greater patient sovereignty in healthcare and reproductive decisions, and the ability to join disease-, syndrome-, or gene-specific affinity groups. With WES and whole-genome sequencing becoming increasingly more affordable and accessible, we predict genomic medicine will soon become commonly applied to the clinical diagnosis of a multitude of idiopathic conditions, including, but not limited to, CH. We, therefore, encourage the routine use of WES and other in-depth genetic analysis for all patients with CH. This developing genetic medicine approach will allow a transition from the general CSF shunting approach currently taken for most CH patients, to a patient-centered approach focused on therapeutic decisions based on pathological mechanisms that have the ability to treat the underlying disease and improve patient outcomes.

Disclosures:

This work was supported by the Gruber Science Fellowship (G.A.), by the NIH Medical Scientist Training Program Training Grant T32GM136651 (P.Q.D), and by R01 NS111029-01A1, R01 NS109358, K12 228168 and the Rudi Schulte Research Institute (K.T.K.). S.C.J is supported by a K99/R00 Pathway to Independence Award (K99HL143036 and R00HL143036-02).

Describe any perceived Conflict(s) of Interest:

The authors declare that this review was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments:

We thank the many children and adults with CH and their families who have contributed to our studies. Without their commitment to improving the state of the science in CH, this work would not have been possible.

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