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CWH43 Variants Are Associated With Disease Risk and Clinical Phenotypic Measures in Patients With Normal Pressure Hydrocephalus

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Abstract

Background and Objectives

Variants in the *CWH43* gene have been associated with normal pressure hydrocephalus (NPH). We aimed to replicate these findings, identify additional *CWH43* variants, and further define the clinical phenotype associated with *CWH43* variants.

Methods

We determined the prevalence of *CWH43* variants by whole-genome sequencing (WGS) in 94 patients with NPH. The odds of having *CWH43* variant carriers develop NPH were determined through comparison with 532 Mayo Clinic Biobank volunteers without a history of NPH. For patients with NPH, we documented the head circumference, prevalence of disproportionate enlargement of subarachnoid hydrocephalus (DESH), microvascular changes on MRI quantified by the Fazekas scale, and ambulatory response to ventriculoperitoneal shunting.

Results

We identified rare (MAF <0.05) coding *CWH43* variants in 15 patients with NPH. Ten patients (Leu533Terfs, n = 8; Lys696Asnfs, n = 2) harbored previously reported predicted loss-of-function variants, and combined burden analysis confirmed risk association with NPH (OR 2.60, 95% CI 1.12–6.03, $p = 0.027$). Additional missense variations observed included Ile292Thr (n = 2), Ala469Ser (n = 2), and Ala626Val (n = 1). Though not quite statistically significant, in single variable analysis, the odds of having a head circumference above the 75th percentile of normal controls was more than 5 times higher for *CWH43* variant carriers compared with that for noncarriers (unadjusted OR 5.67, 95% CI 0.96–108.55, $p = 0.057$), and this was consistent after adjusting for sex and height (OR 5.42, 95% CI 0.87–106.37, $p = 0.073$). DESH was present in 56.7% of noncarriers and only 21.4% of carriers ($p = 0.016$), while sulcal trapping was also more prevalent among noncarriers (67.2% vs 35.7%, $p = 0.030$). All 8 of the 15 variant carriers who underwent ventriculoperitoneal shunting at our institution experienced ambulatory improvements.

Discussion

CWH43 variants are frequent in patients with NPH. Predicted loss-of-function mutations were the most common; we identified missense mutations that require further study. Our findings suggest that congenital factors, rather than malabsorption or vascular dysfunction, are primary contributors to the *CWH43*-related NPH clinical syndrome.

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Glossary

CI = confidence interval; **DESH** = disproportionate enlargement of subarachnoid hydrocephalus; **LOF** = loss of function; **MMSE** = Mini-Mental Status Examination; **NPH** = normal pressure hydrocephalus; **OR** = odds ratio; **STMS** = Short Test of Mental Status; **VQSR** = Variant Quality Score Recalibration; **WGS** = whole-genome sequencing.

Introduction

Clinical features of normal pressure hydrocephalus (NPH) include progressive gait impairment, urinary dysfunction, and cognitive decline.[1] Characteristic radiographic features include ventriculomegaly and disproportionately enlarged subarachnoid space hydrocephalus (DESH)[2] suggesting abnormal CSF absorption.[3] The classic NPH phenotype may also result from vascular[4,5] and congenital factors.[6,7] While clinically similar, each of these etiologies has distinctive radiographic features on brain MRI and variable responses to ventriculoperitoneal shunting.

There is growing evidence that variants in specific genes may contribute to NPH. In a Finnish study of 375 shunted patients with NPH, 4.8% of patients had a family member who was also shunted for NPH and 11% of patients had relatives with ≥2 NPH clinical features.[8] Variants in several genes have been associated with the NPH phenotype including *SFMBT1*,[9] *DNAH14*,[10] *CFAP43*,[11] and *CWH43*.[12] A recent investigation reported 2 heterozygous predicted loss-of-function (LOF) variants (Leu533Terfs and Lys696Asnfs) within the *CWH43* gene in 15% of 53 unrelated patients with idiopathic NPH.[12] To validate the clinical and genetic findings, they created a *CWH43* knock-out mouse model that displayed an NPH-like phenotype (hydrocephalus with impaired gait and balance), reduced ependymal ciliary populations, and decreased locomotion of glycosylphosphatidylinositol-anchored proteins on the surfaces of choroid plexus and ependymal cells. This work suggests a genetic relationship to CSF dynamics resulting in NPH; however, the human clinical and radiographic corollary of *CWH43* variants remains unclear.

In this study, we performed whole-genome sequencing (WGS) in 94 unrelated patients diagnosed with NPH. We aimed to confirm the previously reported disease risk associated with *CWH43* LOF variants, characterize *CWH43* variants, and define the genotype-phenotype relationship by leveraging detailed clinical and radiographic information.

Methods

Participant Selection and Clinical Characterization

Ninety-four patients, representing multiple ethnicities, were diagnosed with the clinical syndrome of NPH,[1] had radiologic evidence of hydrocephalus (Evans ratio >0.31), and no evidence for secondary hydrocephalus (e.g., obstruction or inflammatory process). Patients presented to the Department of Neurology at the Mayo Clinic Florida (Jacksonville, FL) and were assessed from 2000 to 2021 (Table 1).

We reviewed patient medical records and extracted demographic, clinical, and radiographic features of NPH. Extracted clinical features included the presence of cognitive impairment; performance on bedside screening tests of cognition, including the Folstein Mini-Mental Status Examination (MMSE)[13] or Kokmen Short Test of Mental Status (STMS)[14]; and the presence of gait impairment, urinary dysfunction, and head circumference. The 98th percentile of head circumference for healthy men and women is 59 cm and 57.5 cm, respectively.[15,16] When relevant, the response to ventriculoperitoneal shunting was recorded, based on the patient/care partner subjective reporting. Radiographic features were determined by consensus between 2 behavioral neurologists (P.W.T., N.G.R.) and included the presence of ventriculomegaly, Evan index, presence of aqueductal stenosis, Fazekas score,[17] small vessel disease score,[18] and the presence of DESH (combination of ventriculomegaly, enlarged Sylvian fissures, high tight cortical sulci, and sulcal trapping). MRI T1-weighted and T2/FLAIR sequences were used to assess morphological changes and white matter lesions, respectively. Surrogate markers for vascular disease included quantification of white matter disease with the Fazekas score (median and binarized into low vs high scores) and the small vessel disease score, which incorporates Fazekas score with the presence of microhemorrhages and lacunar infarcts.[17,18]

All patients underwent a video recording of their walking before and within 1 hour after a high-volume lumbar puncture (HVLP) resulting in removal of approximately 30 mL of CSF. A "positive" result was based on the clinical judgment of a behavioral neurologist (N.G.R., G.S.D., N.E.T., P.W.T.) and/or movement disorders specialist (P.W.T.). Patients with a positive result were

Table 1 Cohort Racial/Ethnic Composition

	All		*CWH43+*		*CWH43−*	
	n	%	N	%	n	%
Race						
Black or African American	9	10.1	0	0.0	9	12.0
White	85	89.90	15	100.0	70	88.0
Ethnicity						
Not Hispanic or Latino	93	98.9	15	100.0	78	98.7
Hispanic	1	1.1	0	0	1	2.3

referred for shunting based on previously reported expectations of temporary CSF removal corresponding to a probability of improvement with shunting.[19]

A well-characterized clinical control series of 532 individuals without a diagnosis of NPH from the Mayo Clinic Biobank with WGS data were included.[20] Controls were non-Hispanic White and unrelated. Most of them were men (n = 296; 55.6%), and the average age during blood draw was 66 years (range: 55–96) compared with 78 years (range: 48–92) for patients with NPH.

Standard Protocol Approvals, Registrations, and Patient Consents

Patients with NPH and controls signed informed consent permitting review of available clinical records and neuroimaging data and processing and storage of available biofluids (including blood and DNA) within the Mayo Clinic Biobank. Research protocols were approved by the Mayo Clinic Institutional Review Board. This work involved no human experimentation, and thus, no approval by an ethical standards committee or institutional/licensing committee was necessary. This work involved no disclosure of nonanonymous patient information.

Genetic Assessment

WGS was performed using genomic DNA extracted from whole blood at the Mayo Clinic Genome Analysis Core (Rochester, MN). Sequencing analysis was performed by Mayo Clinic Bioinformatics core using the Genome GPS v4.0 pipeline to generate a joint variant call file (gVCF). Functional annotations of variants were performed using ANNOVAR (version 2016Feb01). Genotype calls with genotype quality (GQ) <10 and/or read depth (DP) <10 were set to missing. For all analyses, only variants that passed Variant Quality Score Recalibration (VQSR) and with a call rate > 95% were considered, unless otherwise specified. The transition/transversion ratio for this final variant call set is 2.04. The gVCF was then imported into Golden Helix SNP and Variation Suite (SVS; v8.8.3 Golden Helix Inc., MT) for further variant calling annotations. Using SVS, variants within *CWH43* were extracted and annotation tracks: NCBI RefSeq Genes 109 Interim v2, NCBI dbSNP 149, and BROAD gnomAD Genomes Variant Frequencies 2.1.1 were performed. Data for all exons were captured with an average coverage of the coding exons of CWH43 in the NPH series of 125X (minimum coverage of 42X) and Mayo Clinic Biobank controls with an average 88X (minimum coverage of 8X). Genetic alterations were confirmed using PCR and Sanger sequencing. Primers were designed for exons 7, 11, 12, 15, and 16, the location of Ile292Thr, Ala469Ser, Leu533Ter, Ala626Val, His689Asn, and Lys696AsnfsTer23, for validation with Sanger sequencing. Bidirectional Sanger sequencing was performed in all 94 clinically diagnosed cases with NPH (primers and conditions available on request). Sequences were aligned and annotated using Seqscape software (v3.0) (Thermo Fisher, MA). Call rates were >98% for all variants, and there was no evidence of a departure from Hardy-Weinberg equilibrium (all $p > 0.05$).

Statistical Methods

Case-Control Genetic Association Analysis

Associations of individual Leu533Terfs and Lys696Asnfs CWH43 variants, and the presence of the minor allele for either of these 2 CWH43 variants, with odds of NPH were examined using multivariable logistic regression models that were adjusted for age at blood draw and sex. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated. No adjustment for multiple testing was made, and p values <0.05 were considered statistically significant. All tests were 2-sided. Statistical analyses were performed using SAS (version 9.4; SAS Institute, Inc., Cary, NC).

Clinical Measures

Statistical analyses of demographic data and clinical comparisons of CWH43 variant carriers and noncarriers among patients with NPH was performed using IBM SPSS Statistics version 28.0.1.0. Continuous data were descriptively summarized with the sample median (minimum, 25th percentile; maximum, 75th percentile). Categorical data were descriptively summarized with the frequency and percentage of patients. The Fisher exact test and Wilcoxon rank sum test were used for categorical and continuous variables, respectively. p values <0.05 were considered as statistically significant. Likelihood ratios (LRs) were reported where appropriate. Due to the small number of African American and Hispanic individuals (n = 10), these individuals were excluded from statistical analysis. Data for non-White and Hispanic individuals are listed in eTable 1 (links.lww.com/NXG/A618).

We were unable to directly extract the 75th percentile from the study conducted by Krefft et al.[6] because only the sex-based median and range of head circumferences were reported; therefore, we used the method described by Hozo et al.[21] to estimate the mean and SD assuming the mean is equal to the median and assuming the SD is equal to the maximum value minus the minimum value and then divided by 6. Using the qnorm function from the R Stats package, the 75th percentile for head circumference in normal controls was estimated to be 58.9 cm for male individuals and 55.5 cm for female individuals. CIs for proportions and differences in proportions were based on the scoring method. Single variable and multivariable logistic regression models were used to examine associations of CWH43 mutations with head circumference above the 75th percentile for normal controls; ORs and 95% CIs were reported.

Data Availability

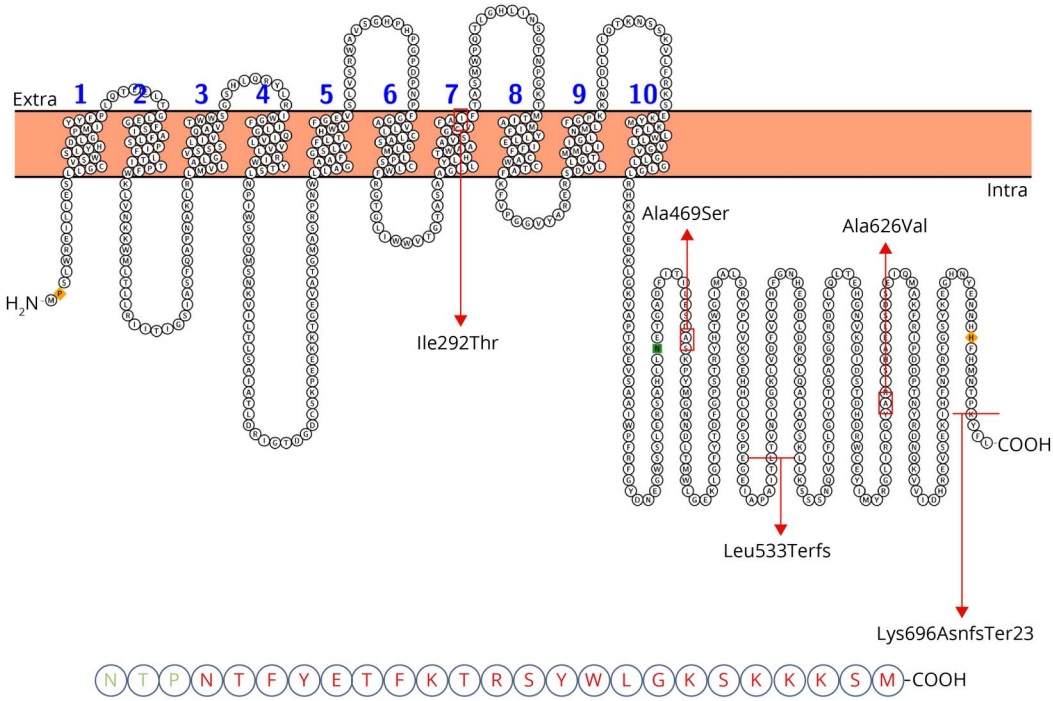
Anonymized study data will be shared pending review of a request to the corresponding author from qualified individuals.

Results

Genetic Characterization of *CWH43* Variants in Normal Pressure Hydrocephalus

WGS in 94 patients with NPH identified 7 variants altering the protein sequencing of *CWH43* (eTable 2, links.lww.com/NXG/A618). Fifteen of 94 patients had *CWH43* variants that could

Figure 1 CWH43 Protein Structure With Possible Function Changing Variants



Protein figure created with Protter (wlab.ethz.ch/protter)4. This figure shows the 3 point mutations (Ile292Thr, Ala469Ser, and p.Ala469Ser), 1 nonsense mutation (Leu533Terfs), which results in premature termination of protein, and 1 deletion (Lys696AsnfsTer23) that leads to a frameshift mutation that adds 22 new amino acids to protein and causes alternation of the carboxy terminus of protein.

affect the protein function with 10 patients harboring the LOF mutations[12]; rare missense point mutations were identified in 5 other patients with NPH (Ile292Thr, Ala469Ser, and Ala626Val) (Figure 1). Associations between *CWH43* variants and odds of NPH were assessed in non-Hispanic White participants as summarized in Table 2. There was a significant association between the previously reported NPH-related variant Leu533Terfs (rs147750792; Figure 2) and odds of NPH (OR = 2.91, 95% CI 1.14–7.39, p = 0.025; Table 2). However, no single variant associated with NPH for Lys696Asnfs (rs538616012) despite an increased frequency in patients with NPH (OR = 1.57, 95% CI = 0.27–9.19, p = 0.61). The presence of the minor allele for either Leu533Terfs or Lys696Asnfs was associated with an elevated risk of NPH (OR = 2.60, 95% CI = 1.12–6.03, p = 0.027).

Phenotyping

Table 3 details a comparison of patient demographic, clinical, and radiologic findings for *CWH43* variant carriers (n = 15) vs noncarriers. Among the 94 patients who underwent WGS, 64% were female, 90% White, 10% African American, 99% not Hispanic/Latino, and 1% Hispanic. There were no statistically significant differences between variant carriers and noncarriers regarding sex, race, or ethnicity.

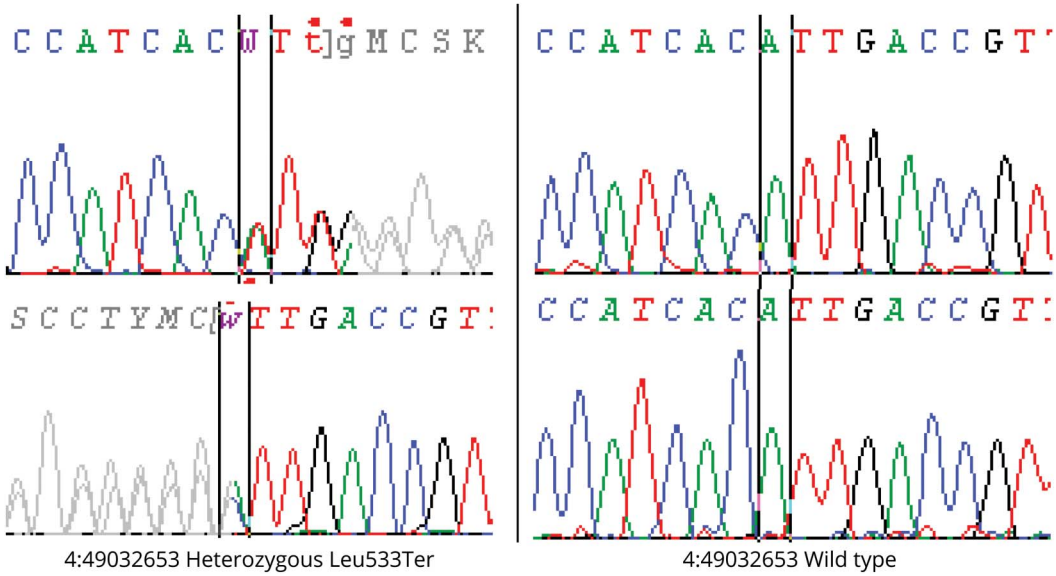
Cognition impairment (73%), urinary dysfunction (73%), and gait difficulties (94%) were present in most of the patients at presentation, with 56% of patients having all 3 features of NPH. No clinical differences reached statistical significance. Of the 84 patients, 49 underwent ventriculoperitoneal shunting (58.3%).

Table 2 Associations Between *CWH43* Variants and Risk of NPH

		No. (%) of individuals with a copy of the minor allele for the given variant		Association with NPH	
Variant	**Amino acid**	**Patients with NPH (N = 84)**	**Controls (N = 532)**	**OR (95% CI)**	***p* Value**
rs147750792	p.Leu533Terfs	8 (9.5%)	22 (4.1%)	2.91 (1.14–7.39)	0.025
rs538616012	p.Lys696Asnfs	2 (2.4%)	7 (1.3%)	1.57 (0.27–9.19)	0.61
rs147750792 or rs538616012	p.Leu533Terfs or p.Lys696Asnfs	10 (11.9%)	29 (5.5%)	2.60 (1.12–6.03)	0.027

Abbreviations: CI = confidence interval; OR = odds ratio.
ORs, 95% CIs, and *p* values result from logistic regression models that were adjusted for age at blood draw and sex. ORs correspond to presence of the minor allele for the given variant or presence of the minor allele for either variant for the "rs147750792 or rs538616012" variable.

Figure 2 Leu533Ter Variant



Sanger DNA sequencing data show us early determination of the protein according to wild type.

Of the 49 shunted patients, 48 underwent a gait assessment before and after high volume cerebrospinal removal. Of these 48 patients, 2 patients did not experience gait improvement but proceeded to shunting based on high clinical suspicion of symptomatic hydrocephalus based on imaging findings and clinical symptoms. Two patients did not undergo a lumbar puncture because of safety concerns from aqueductal stenosis but proceeded to shunting. Subjective ambulatory improvement was noted by the patient and/or care partner in 100% of shunted individuals for whom follow-up data were available (44/49). This was corroborated by the treating physician who compared walking with video-archived pre-HVLP walking.

Eleven CWH43 variant carriers (3/4 female, 6/7 male) had a head circumference >98th percentile.[15] The median head circumference for female and male individuals was 57.0 cm (range: 53–59) and 59.0 cm (range: 56.0–62.5), respectively. We also compared both groups with previously reported normal controls.[6] In our cohort of patients with NPH, 69% (40/58, 95% CI 56–79) had a head circumference >75th percentile for normal controls; 91% (10/11, 95% CI 62–98) among CWH43 variant carriers and 64% (30/47, 95% CI 50–76) among noncarriers (Table 4). When comparing patients with NPH carrying a CWH43 variant with noncarriers, the estimated difference in the proportion of patients with a head circumference larger than the 75th percentile of normal controls was 27.1% (95% CI −0.04 to 43.2). Though not quite statistically significant, in single variable analysis, the odds of having a head circumference above the 75th percentile of normal controls was more than 5 times higher for CWH43 variant carriers compared with noncarriers (unadjusted OR 5.67, 95% CI 0.96–108.55, $p = 0.057$), and this was consistent after adjusting for sex and height (OR 5.42, 95% CI 0.87–106.37, $p = 0.073$).

Radiographic assessments were intended to identify features with etiologic significance. The Fazekas score was lower among variant carriers, but this was not statistically significant. Aqueductal stenosis was exclusively present in noncarriers (4/62, 6.5%) and none of the carriers, but this was not statistically significant. DESH was present in 41/81 patients and more prevalent among variant noncarriers (56.7% vs 21.4%; LR = 6.060, $p = 0.014$). Sulcal trapping was more prevalent among noncarriers (67.2% vs 35.7%, LR 4.719, $p = 0.030$).

Discussion

NPH is often described as *idiopathic*; however, its evolving genetic landscape has strengthened the growing body of evidence supporting 3 primary mechanisms contributing to the clinical syndrome of NPH: impaired CSF absorption, vascular factors, and congenital factors.[22] These features may occur independently of each other but often coexist in the aging population. Understanding the relative contributions of these mechanisms to NPH-related pathophysiology will facilitate improvements in the diagnosis and treatment of affected individuals. Advances in genetic technologies have facilitated the identification of genetic variation and genes that influence disease state and provide specific targets for biomarker development and preclinical studies. Recent studies in NPH have nominated 4 genes that may influence individual susceptibility to NPH; however, replication is crucial to identifying bona fide genetic determinants.[9-12] Predicted LOF variation in the *CWH43* gene has been associated with the risk of NPH.[12] In this study, we replicate the observed association and demonstrate that carriers have specific phenotypic features. Moreover, the presence of either of the previously

Table 3 Clinical and Radiographic Comparison of White Non-Hispanic Patients With NPH Based On *CWH43* Variant Carrier Status

		All		*CWH43+*		*CWH43−*		
	n	n/median	%/Range	n/median	%/Range	n/median	%/Range	*p* Value
Female (n, %)	84	28	33.3	6	40.0	22	31.9	0.374
Family history								
Cognitive impairment	84	37	44.0	7	46.7	30	43.5	0.521
Hydrocephalus	84	6	7.1	1	6.7	5	7.2	0.710
Age at clinical evaluation	84	76.0	48.4–89.2	75.99	48.4–85.3	76	56.8–89.2	0.959
Cognition								
Impairment	84	61	72.6	9	60.0	52	75.4	0.185
MMSE	62	26.0	11–30	27.00	20–30	26	11–30	0.569
STMS	12	32.5	28–36	29.50	28–35	33	29–36	0.545
Urinary								
Impairment[a]	84	61	72.6	10	66.7	51	73.9	0.390
Incontinence	79	57	72.2	10	66.7	47	73.4	0.407
Gait								
Impairment	84	79	94.0	13	86.7	66	95.7	0.216
Head circumference (cm)								
Female	17	57.0	53.0–59.0	57.8	56.0–59.0	56.0	53.0–59.0	1.000
Male	41	59.0	56.0–62.5	59.5	57.0–60.0	59.0	56.0–62.5	0.761
Head circ >98th percentile	58	36	62.1	9	81.8	27	57.4	0.123
Height (cm)								
Female	26	158.5	152–174	161.00	152–174	158	152–173	1.000
Male	55	175.0	162–191	177.00	165–184	175	162–191	0.536
Aqueductal stenosis	74	5	6.8	0	0.0	4	6.5	0.458
Evans index	81	0.4	0.29–0.50	0.36	0.29–0.46	0	0.29–0.50	0.968
Fazekas score	75	2.0	0–3	1.00	0–3	2	0–3	0.954
Fazekas (low)[b]	79	28	35.4	7	46.7	21	32.8	0.236
DESH[c]	81	41	50.6	3	21.4	38	56.7	**0.016**
Ventriculomegaly	82	82	100.0	14	100.0	68	100.0	—
Enlarged sylvian fissure	81	50	61.7	6	42.9	44	65.7	0.099
High tight sulci	81	57	70.4	7	50.0	50	74.6	0.068
Sulcal trapping	81	50	61.7	5	35.7	45	67.2	**0.030**
Small vessel disease score								
Simple	84	1.0	0–1	1.00	0–1	1	0–1	—
Amended	84	2.0	0–5	1.00	0–4	2	0–5	0.841
Shunted	84	49	58.3	9	60.0	40	58.0	0.561
Shunt improvement	48	48	100.0	8[d]	9.5	40	100.0	—

Abbreviations: MMSE = Mini-Mental Status Examination; NPH = normal pressure hydrocephalus; STMS = Short Test of Mental Status.
[a] Impairment = report of incontinence or urgency.
[b] Fazekas high = 0 or 1, low = 2 or 3.
[c] DESH = Ventriculomegaly + enlarged Sylvian fissures + High tight sulci.
[d] Follow-up data only available on 8 patients.
Bolded values correspond to a p value < 0.05.

Table 4 Fraction (%) of Patients With NPH With a Head Circumference Larger Than the Sex-Based 75th Percentile Head Circumference of Normal Controls

	Male	Female	All
NPH with *CWH43* mutation	6/7 (86%)	4/4 (100%)	10/11 (91%)
NPH without *CWH43* mutation	21/34 (62%)	9/13 (69%)	30/47 (64%)
All NPH	27/41 (66%)	13/17 (76%)	40/58 (69%)

Abbreviation: NPH = normal pressure hydrocephalus.

NPH-associated variants Leu533Terfs or Lys696Asnfs conferred a more than 2-fold increased risk of this condition.

Hydrocephalus caused by impaired CSF absorption is often seen in the setting of arachnoiditis; however, this is usually accompanied by increased CSF pressure. It is thought that insidious CSF absorption impairment may influence ventricular compliance leading to hydrocephalus without increased pressure, i.e., NPH. This has been demonstrated by measuring CSF outflow resistance (R_{CSF}) whereby R_{CSF} is increased in NPH. Boon et al. demonstrated that higher R_{CSF} values predicted shunt responsiveness.[3] Patients with impaired CSF absorption have more CSF in the intraventricular and extraventricular spaces. This is represented radiographically by DESH, which includes ventriculomegaly with high tight cortical sulci and enlarged sylvian fissures.[2] Impaired CSF absorption is likely the most common cause of NPH. DESH was present in 51% of our total NPH cohort but only 21% among *CWH43* variant carriers. This suggests that *CWH43* mutations are not a driver of a primary malabsorption NPH phenotype.

The relationship of hydrocephalus to the vascular system was initially identified by demonstrating that occlusion of the vein of Galen caused hydrocephalus.[23] A subsequent study caused hydrocephalus in dogs by blocking the major venous drainage of the head and the major anastomotic channels to the spinal venous system.[24] In addition to increased venous backpressure, vascular-related hydrocephalus may result from perturbation of the arterial system. The percussion wave (P1) on intracranial pressure monitoring demonstrates a direct effect of arterial pulsation on the CSF system. Petterossi et al. caused unilateral ventricular enlargement in sheep by using an intraventricular balloon to increase the CSF pulse pressure (without altering the mean intraventricular pressure).[25] Thus, it is not surprising that several clinical studies have identified an association between hypertension and hydrocephalus. Assessment of 1,112 participants in the Atherosclerosis Risk in Communities study demonstrated that both systolic blood pressure and pulse pressure increased one's odds of developing ventriculomegaly.[26] The SPRINT study showed that pulse pressure was related to larger ventricle size, more white matter damage, and cognitive decline. Moreover, the cognitive decline in those with increased pulse pressure was mediated by white matter damage.[27] More recently, a population-based study of 1,235 older individuals found that fulfilling the diagnostic criteria for probable NPH was related to the presence of moderate-to-severe white matter disease while hydrocephalic ventriculomegaly was related to hypertension, moderate-to-severe white matter disease, and diabetes mellitus.[4] A meta-analysis estimated that 25% of NPH is related to vascular disease.[28] Tisell et al. showed that patients with NPH and extensive white matter disease still benefited from shunting,[5] but perhaps more importantly, these findings also highlight manageable risk factors. Subcortical hyperintensities on T2-weighted MRI sequences support a hypothesis of vascular contributions to a patient's hydrocephalus and are most often quantified with the Fazekas scale, which is incorporated into the more recently developed small vessel disease score.[18] Within our NPH cohort, 35.4% of individuals had low Fazekas score. While *CWH43* variant carriers tended to have lower Fazekas scores, this did not reach statistical significance. Furthermore, having *CWH43* mutations provide a permissive milieu for NPH that does not require white matter disease for its pathogenesis.

Congenital hydrocephalus may be suspected in individuals with stenosis or webbing of the cerebral aqueduct of Sylvius. While this may manifest with symptoms in early life, a subset of patients will experience an onset of the NPH syndrome in adulthood. Aqueductal stenosis was rare (7%) in our NPH cohort and absent among *CWH43* variant carriers. Independent of aqueductal stenosis, more than 20% of patients with NPH have a head circumference greater than the 90th percentile further supporting a role for other congenital factors.[6,7] We found that the odds of having a head circumference >75th percentile were more than 5 times greater for *CWH43* variant carriers compared with that for noncarriers. Other radiographic features of congenital hydrocephalus include ventriculomegaly without other components of DESH or features suggestive of *ex vacuo* ventriculomegaly (significant cortical atrophy). Taken together with the lack of DESH and WMD, our findings suggest that *CWH43* mutations lead to a congenital form of NPH phenotype without aqueductal stenosis.

Long-standing perturbations of intraventricular CSF flow dynamics may contribute to the NPH syndrome. In the brain, cilia are on the ependymal lining, and animal models lacking motile cilia develop hydrocephalus.[29,30] Olstad et al.[31] showed that the lack of motile cilia eliminated the directionality of intraventricular CSF flow and disrupted ventricular development in zebrafish. Thus, it is of great interest that variants in 3 different genes (*CWH43*, *CFAP43*, and *DNAH14*) seem to affect cilial movement and result in congenital hydrocephalus, which may become symptomatic in adulthood.[10-12]

The clinical phenotype of our patients harboring a *CWH43* variant indicates long-standing hydrocephalus, which manifests as symptomatic NPH in adulthood. Variant carriers tended not to have radiologic features of CSF malabsorption (DESH). However, when shunted, these patients improve. Our findings support the notion that NPH is becoming "less idiopathic," and adult-onset symptomatic hydrocephalus may be a syndrome with distinct causes.[22]

Limitations of this study include those inherent to a retrospective approach. The small number of non-White and Hispanic patients necessitated their removal from analysis of the comparison between variant carriers and noncarriers to avoid population stratification. This highlights a major need for increased minority recruitment in genetic studies. This will require coordination of multiple institutions to adequately power such studies. In addition, the sample size is relatively small, and therefore, the possibility of a type II error (i.e., false-negative finding) is important to consider; we cannot conclude that a true association of difference does not exist simply due to the occurrence of a nonsignificant *p* value in our study. Owing to this relatively small sample size, we did not make any adjustment for multiple testing, and therefore, it will be important to validate our statistically significant findings in an independent series. Finally, we cannot rule out the possibility that unmeasured confounding variables could have influenced the results of our genetic association analysis. While this is a relatively small series, our study replicates the findings of Yang and colleagues.[12] Our work also adds to the current body of knowledge by finding 3 additional point mutations that likely alter CWH43 protein function and may also cause the NPH syndrome. It also details the clinical phenotype of a genetic form of NPH. Other studies suggest that there may be other causative mutations for adult-onset hydrocephalus, which also require replication and phenotypic characterization.

Our study identified novel likely LOF *CWH43* variants and characterized the phenotypic features in variant carriers. Our observation that carriers have more than a 2-fold increased risk of NPH suggests the genetic component to disease may be larger than previously thought. Several other genes have also been recently nominated, and ongoing studies are characterizing these genes in our series. However, large-scale efforts are needed to perform unbiased genome-wide association studies to assess the influence of common variants on disease risk and WGS efforts for rare variants and more complex copy number or structural variation. Defining the genetic architecture of NPH will advance basic research and lead to better-designed therapeutic intervention studies and interpretation of clinical trial outcomes.

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Disclosure

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Appendix *(continued)*

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