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ORIGINAL ARTICLE

Genetic etiologies associated with infantile hydrocephalus in a Chinese infantile cohort

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Abstract

Background Infantile hydrocephalus (IHC) is commonly related to other central nervous system diseases, which may have adverse effects on prognosis. The causes of IHC are heterogeneous, and the genetic etiologies are not fully understood. This study aimed to analyze the genetic etiologies of an IHC cohort.

Methods The data for 110 IHC patients who had received exome sequencing at the Clinical Genetic Center of the Children's Hospital of Fudan University between 2016 and 2019 were reviewed and analyzed retrospectively. An exome-wide association analysis (EWAS) was performed within this cohort using IHC as the study phenotype.

Results Of the 110 IHC patients, a pathogenic or likely pathogenic variant was identified in 16 (15%) patients, spanning 13 genes. The genes were mainly associated with metabolic disorders, brain abnormalities, and genetic syndromes. IHC patients who had unclear clinical etiology were more likely to possess a genetic etiology. Based on previous studies and on our EWAS results, *ZEB1*, *SBF2*, and *GNAI2* were over-represented among IHC patients and might affect the signaling pathways involved in IHC formation.

Conclusions Our study showed heterogeneous genetic etiologies in an IHC cohort. It is essential to perform genetic testing on IHC patients who have unclear clinical etiology, and genes associated with metabolic disorders, brain abnormalities, and genetic syndromes should be noted. In addition, when aiming to discover IHC susceptibility genes, genes that might influence the signaling pathways involved in IHC formation should be prioritized.

Keywords Etiology · Genetic · Hydrocephalus · Infantile

Introduction

Infantile hydrocephalus (IHC), a neurologic condition characterized by an abnormal accumulation of cerebrospinal fluid within the cerebral ventricular system that leads to an enlargement of the ventricles or subarachnoid space, is reported in approximately 1.1 per 1000 infants [1]. The immense impact of IHC on the family and society is clear, as it carries a high risk of neurodevelopmental damage and high mortality [2, 3]. The pathogenesis of IHC is heterogeneous, and genetic factors play an important part [4]. To date, the four most commonly documented hydrocephalus-related genes are *L1CAM*, *AP1S2*, *CCDC88C*, and *MPDZ* [5–8], whose disruption leads to diseases with hydrocephalus being the main or sole clinical characteristic. In most instances, hydrocephalus may present as a component of certain genetic syndromes with various genes involved [9]. Hydrocephalus can be recognized at different ages, which

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can be indicative of different underlying etiologies [4], and hydrocephalus caused by different means can be associated with different outcomes [10]. However, there are little data on the genetic causes among IHC patients with different onset ages and with different outcomes. In addition, most previous studies have focused on evaluating the genetic etiologies among hydrocephalus patients without a clear extrinsic cause, whereas few studies have investigated the genetic etiologies among IHC patients with clear clinical etiologies.

In this present study, we analyzed a cohort of IHC patients who had undergone genetic testing at the Clinical Genetic Center of the Children's Hospital of Fudan University between January 1, 2016, and December 31, 2019. In particular, we focused on the genetic findings, and made comparisons about the different genetic etiology rates among IHC patients with clear or unclear clinical etiologies, different gestational ages, different onset ages and different outcomes to obtain a better understanding of the genetic etiologies associated with IHC.

Methods

Participant recruitment and data collection

Patients were recruited from the Children's Hospital of Fudan University between January 1, 2016, and December 31, 2019, with the following inclusion criteria: (1) a diagnosis of hydrocephalus within the first year of life determined by cranial imaging (ultrasound, computed tomography or magnetic resonance imaging) and (2) genetic testing at the Clinical Genetic Center of the Children's Hospital of Fudan University. Patients who did not meet these criteria or declined to participate were excluded from this study. This study was approved by the Ethics Committee of the Children's Hospital of Fudan University, and informed consent was obtained from all the study participants.

For each patient, clinical information, laboratory results, and cranial imaging findings were obtained through the review of medical records. The evaluation of outcomes was made at the final follow-up visits, and developmental delays were assessed based on the Gesell Developmental Scale.

Statistical analysis consisted mainly of the Chi-square test for comparisons of categorical variables with a threshold of $P < 0.05$ considered to be statistically significant. For cells with < 5 observations, Fisher's exact test was applied. Statistical analysis was performed using SPSS 20.0 software.

Exome sequencing

Genomic DNA from peripheral blood was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The Agilent ClearSeq Inherited Disease Kit Illumina Cluster (Agilent Technologies, Santa Clara, CA, USA) and SBS Kits (Illumina Inc., San Diego, CA, USA) were used for clinical exome sequencing, whileereas the Agilent SureSelect Human All Exon Kit (Agilent Technologies, Santa Clara, CA, USA), Illumina TruSeq Rapid PE Cluster and SBS Kits (Illumina Inc., San Diego, CA, USA) were applied for whole-exome sequencing. Next-generation sequencing on an Illumina HiSeq 2000/2500 platform (Illumina Inc., San Diego, CA, USA) was utilized to perform the sequencing. Clean reads aligned to the reference human genome (UCSC hg19) were generated by discarding low-quality reads from raw data. Variant calling was performed using the Genome Analysis Tool Kit. ClinVar, online Mendelian inheritance in man and the human gene mutation database (professional version) for known pathogenic and likely pathogenic variants were applied. Numerous missense functional predictors, noncoding regulatory region annotations, and others were used. The allelic frequencies were annotated from the 1000 genomes, the Exome Sequencing Project (EVS6500), the Exome Aggregation Consortium (ExAC), and the Kyoto and Dutch allelic frequency databases to filter out variants of high frequency.

Exome-wide analysis study

An exome-wide analysis study (EWAS) was performed within this cohort. Informed consent for DNA analysis was obtained from the study participants in accordance with the time of collection. The details for the sequencing strategy, variant filtration, and genetic diagnosis have been published previously by our group [11].

Strategy to identify over-represented genes and variants in IHC patients

To screen for possible candidate genes or variants, we included another 10,000 patient samples from the Clinical Genetic Center of the Children's Hospital of Fudan University. The inclusion criteria were as follows: patients underwent genetic testing for hyperbilirubinemia or skin problems. Patients who underwent genetic testing for any of the following conditions and who had any positive genetic findings were excluded: hydrocephalus, brain malformations, brain tumor, arachnoid cyst, or encephalopathy. Because the control and case individuals were not matched for age, sex, ethnicity or other covariates, we applied a bootstrap strategy to avoid possible sample selection bias ($n = 1000$). For each run, we randomly selected 300 control samples for analysis. At the variant level, we performed Fisher's exact tests for each candidate to test whether the variant was over-represented in case samples. At the gene level, we calculated the pathogenicity score for each candidate by summarizing the

REVEL [12] score of the variant and adjusting it to 0–1. For genes containing nonsense variants, the score was defined as 1. Student's *t* test was then performed to test whether the pathogenicity score was higher in case samples. When summarizing the 1000 results, we followed the gene collapsing approach [13]. A threshold of $P < 0.05$ was considered statistically significant, and all tests were one-sided. All analyses were performed using R software (version 3.5.1, http://cran.r-project.org).

Results

Patient characteristics

A total of 110 infants diagnosed with IHC were enrolled in this study (Fig. 1). Overall, 86 (78%) patients had undergone clinical exome sequencing, and 24 (22%) patients had undergone whole-exome sequencing. The detailed demographics and certain clinical characteristics of the study population are summarized in Table 1. Among them, 61 (55%) patients were male. The average birth weight was 2239 g, and 40% (38/95) of the patients were born at term. Overall, 55% of the patients were recommended for surgery, among which 16 underwent a single surgery, including a ventriculoperitoneal shunt (VP) in eight patients, a third ventriculostomy in two patients, an external ventricular drainage (EVD) in two patients, an Ommaya reservoir implantation (OMMAYA) in one patient and unspecific surgery in three patients. Moreover, 18 patients underwent repeated surgeries, including EVD + VP (3 patients), OMMAYA + VP (4 patients), OMMAYA + EVD (4 patients), and OMMAYA + EVD + VP (7 patients). Another 16 patients refused the surgery recommendation.

Distribution of clinical etiologies

The recognizable clinical etiologies of hydrocephalus in this cohort are summarized in Fig. 2. A total of 48 (44%, 48/110) patients had intracranial hemorrhage (IH), including IH alone in 31 (28%, 31/110) patients, IH combined with intracranial infection (II) in 14 (13%, 14/110) patients and IH combined with brain malformations (BM) in 3 (3%, 3/110) patients. In addition, II alone occurred in 11 (10%, 11/110) patients, and BM alone occurred in 8 (7%, 8/110) patients. Arachnoid cyst occurred in 2 (2%, 2/110) patients, and a tumor occurred in 1 (1%, 1/110) patient. Another 40 (36%, 40/110) patients had no clear clinical cause. In this cohort, IH was the most common clinical etiology of hydrocephalus.

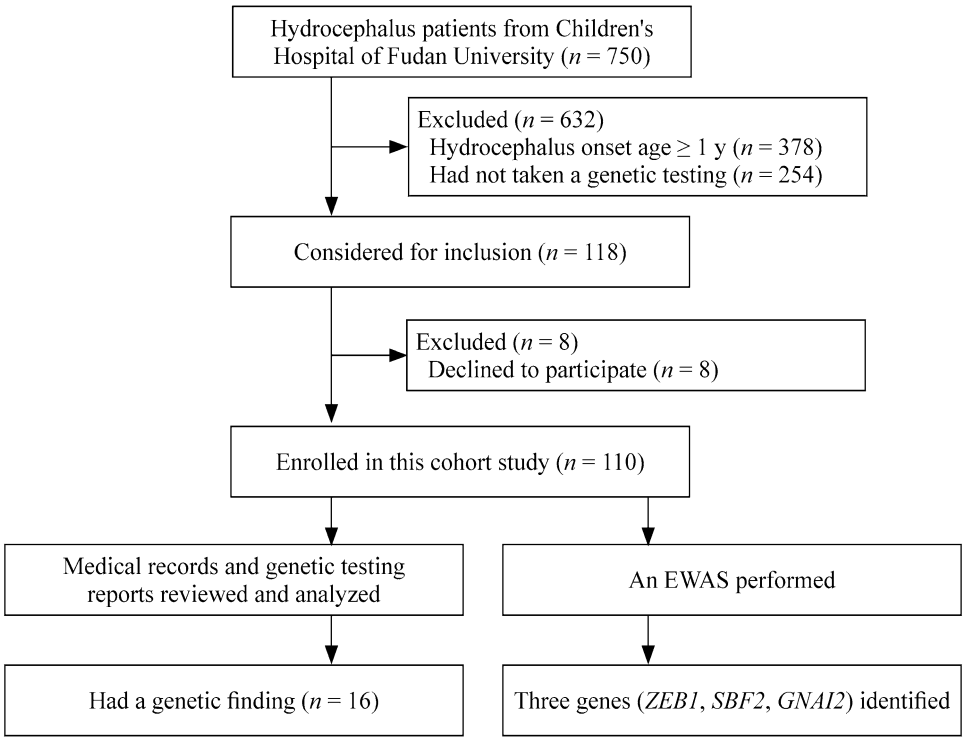

Fig. 1 Flow diagram of infantile hydrocephalus patient recruitment and analysis. *EWAS* exome-wide association analysis

Table 1 Demographic and certain clinical characteristics of the study population

Characteristics	Cases ($n=110$)
Male gender	61/110 (56)
Birth weight (g), mean ± SD ($n=81$)	2239 ± 907
Proportion of term-born	38/95 (40)
Family medical history	
Hydrocephalus	2/88 (2)
Neurological disorders (except for hydrocephalus)	5/88 (6)
Non-neurological disorders	9/88 (10)
Maternal history of miscarriage due to fetal factors	10/88 (11)
Normal	62/88 (71)
Proportion of abnormal perinatal histories	65/89 (73)
Proportion of abnormal maternal pregnancy histories	48/84 (57)
Proportion of convulsion	22/110 (20)
Proportion of increased head circumference	17/90 (19)
Proportion of tight fontanel	24/90 (27)
Proportion of separation of cranial suture	11/90 (12)
Proportion of setting-sun sign	4/90 (4)
Muscular tension	
Hypertonia	22/110 (20)
Hypotonia	41/110 (37)
Normal	47/110 (43)
Surgical treatments	
Single surgery	16/91 (18)
Repeated surgeries	18/91 (20)
Surgery suggested but refused by the family	16/91 (18)
No surgery	41/91 (45)
Rate of surgery recommendation	50/91 (55)

Data are *n/N* (%). *SD* standard deviation

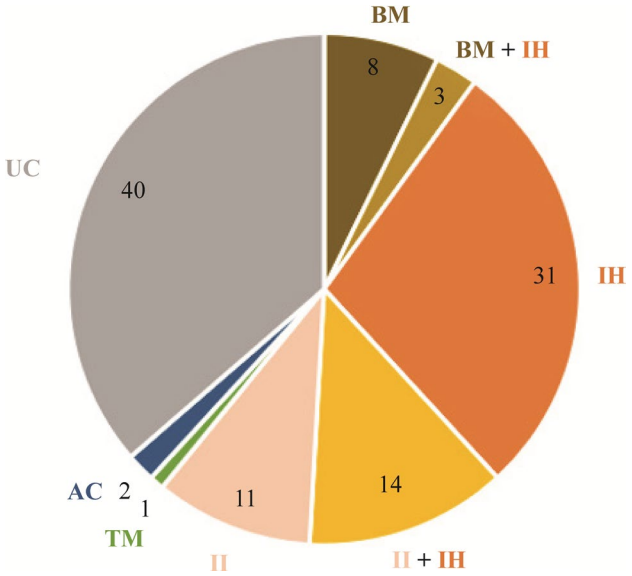

Fig. 2 Distribution of clinical etiologies in this cohort. *IH* intracranial hemorrhage, *II* intracranial infection, *BM* brain malformations, *AC* arachnoid cyst, *TM* tumor, *UC* unclear

Contribution of genetic disorders in infants with clear or unclear clinical etiologies

Overall, 22 pathogenic or likely pathogenic variants spanning 13 genes were identified in 16 (15%, 16/110) patients, including 12 variants identified by whole-exome sequencing in 8 (33.3%, 8/24) patients and 10 variants identified by clinical exome sequencing in 8 (9.3%, 8/86) patients. The genetic diagnosis rate of whole-exome sequencing was significantly higher than clinical exome sequencing ($P=0.007$). The distribution of genetic etiologies in this cohort was as follows: 7 (44%, 7/16) in metabolic disorders, 5 (31%, 5/16) in genetic syndromes, 3 (19%, 3/16) in brain malformations and 1 (6%, 1/16) in myopathy. The detailed information for the patients with a genetic etiology is listed in Table 2.

IHC infants without a clear clinical etiology accounted for 36% (40/110) of all patients included in this study, and 11 (28%, 11/40) patients were identified as having a genetic etiology. IHC infants with a clear clinical etiology accounted for 64% (70/110) of all cases enrolled in this cohort and 5 (7%, 5/70) patients had a genetic etiology. The genetic etiology rates between the patients with unclear clinical etiology and the patients with clear clinical etiologies were significantly different ($P=0.004$).

Distribution of etiologies among preterm patients and term patients

Of the patients in this study, 38 infants were born at term, and 57 infants were born preterm. For 15 patients, the exact gestational age could not be obtained. The detailed clinical and genetic etiology distributions among preterm patients and term patients are presented in Table 3. Among the patients born at term, approximately half (47%, 18/38) did not have a clear clinical etiology. Among the preterm patients, only 19% (11/57) did not have a clear clinical etiology; so the clear clinical etiology rate was 81% (46/57), with IH (occurring in a total of 35 patients; 61%, 35/57) being the most common clinical etiology. Among the patients who had unclear clinical etiology, there was no significant difference between the genetic etiology rates of the preterm patients (0%, 0/11) and the term patients (28%, 5/18) ($P=0.126$). In contrast, among the patients who had clear clinical etiologies, there was a significant difference between the genetic

Table 2 The genetic spectrum and clinical information among the patients with a genetic etiology in this cohort

Case number	Gene	Variant classification	Variant	Zygosity	WES/CES	Genetic etiology	Clinical etiology	Clinical phenotypes
005[a]	*POMGNT1*	LP	NM_017739:exon16:c.1325G > A(p.R442H)	Het	WES	Genetic syndrome	Unclear	Cerebellar hypoplasia, agenesis of the corpus callosum, white matter lesions, mental retardation, developmental delay, nystagmus, hypotonia, IHC
005[a]	*POMGNT1*	LP	NM_017739:exon21:c.1873G > A(p.G625R)	Het				
007	*MTHFR*	LP	NM_005957:exon8:c.1267dupG	Hom	WES	Metabolic disorder	II + IH	Poor response, poor feeding, hypotonia, purulent meningitis, encephalopathy, homocysteinemia, IHC
009[a]	*MTHFR*	LP	NM_005957:exon8:c.1316 T > C(p.L439P)	Het	WES	Metabolic disorder	Unclear	Seizure, development delay, homocysteinemia, IHC
009[a]	*MTHFR*	LP	NM_005957:exon3:c.323C > T(p.S108F)	Het				
027[a]	*MNX1*	LP	NM_005515:exon2:c.778_783del TTCAAG	Het	WES	Genetic syndrome	II	Anterior sacral meningocele, tethered cord, anorectal stenosis and IHC
030	*MMACHC*	P	NM_015506:exon4:c.609G > A(p.W203X)	Het	WES	Metabolic disorder	Unclear	Decreased visual acuity, anemia, atelencephalia, decreased serum methionine, IHC
030	*MMACHC*	P	NM_015506:exon4:c.658_660del AAG	Het				
036	*TUBA1A*	P	NM_006009:exon4:c.1226 T > C(p.V409A)	Het	CES	Brain malformation	BM	Seizure, lissencephaly, thin corpus callosum, hypoplasia of the brainstem, IHC

Table 2 (continued)

Case number	Gene	Variant classification	Variant	Zygosity	WES/CES	Genetic etiology	Clinical etiology	Clinical phenotypes
043	*FGFR3*	LP	NM_000142:exon9:c.1138G > A(p.G380R)	Het	CES	Brain malformation	BM	Macrocephaly, skull deformity, incapable of walking and IHC
060	*PDHA1*	P	NM_000284:exon10:c.926_928dupAAG	Het	WES	Metabolic disorder	Unclear	Hypotonia, development delay, agenesis of the corpus callosum, hyperlactacidemia, increased urine pyruvic acid, IHC
064	*MTM1*	P	NM_000252:exon9:c.721C > T(p.R241C)	Hemi	CES	Myopathy	Unclear	Neonatal respiratory distress, hypotonia, poor response, uncoordinated swallowing, IHC
081	*SOX2*	P	NM_003106:exon1:c.384del(p.G129AfsTer25)	Het	CES	Genetic syndrome	AC	Hydramnios, arachnoid cyst, IHC
083[a]	*FLVCR2*	LP	NM_017791:exon4:c.997C > T(p.R333C)	Het	Panel	Genetic syndrome	Unclear	Microcephaly, seizures, calcifications in pons and bilateral frontal lobe, thin corpus callosum, IHC
083[a]	*FLVCR2*	LP	NM_017791:exon4:c.986C > T(p.T329I)	Het				
086	*PDHA1*	P	NM_000284:exon11:c.1132C > T(p.R378C)	Hemi	WES	Metabolic disorder	Unclear	Development delay, episodic abnormal eye movements, hyperlactacidemia and IHC

Table 2 (continued)

Case number	Gene	Variant classification	Variant	Zygosity	WES/CES	Genetic etiology	Clinical etiology	Clinical phenotypes
104[a]	*MTHFR*	P	NM_005957:exon12:c.1915A > C(p.T639P)	Het	WES	Metabolic disorder	Unclear	Poor response, lethargy, hypertonia, seizures, encephalopathy, homocysteinemia, IHC
104[a]	*MTHFR*	P	NM_005957:exon4:c.568 T > C(p.F190L)	Het				
105	*L1CAM*	P	NM_000425:exon22:c.2920G > T(p.E974X)	Hemi	CES	Brain malformation	Unclear	Agenesis of the corpus callosum and IHC
108	*NSD1*	P	NM_022455:exon5:c.2488delA	Het	CES	Genetic syndrome	Unclear	Pointed chin, development delay, mental retardation, agenesis of corpus callosum, IHC
109	*LIAS*	LP	NM_006859:exon6:c.587C > A(p.T196N)	Het	CES	Metabolic disorder	Unclear	Poor response, poor feeding, cerebral atrophy, cerebromalacia, increased glycine and IHC
109	*LIAS*	LP	NM_006859:exon10:c.1063G > C(p.A355P)	Het				

P pathogenic, *LP* likely pathogenic, *Het* heterozygous, *Hemi* hemizygous, *Hom* homozygous, *CES* clinical exome sequencing, *WES* whole-exome sequencing, *IH* intracranial hemorrhage, *II* intracranial infection, *BM* brain malformations, *AC* arachnoid cyst, *IHC* infantile hydrocephalus. [a]This patient had a correlative family history

etiology rates of the preterm patients (2%, 1/46) and the term patients (20%, 4/20) ($P = 0.027$).

Distribution of etiologies among the patients with different diagnosed ages

The distribution of age at diagnosis in this study was 22 (21%, 22/103) during pregnancy, 44 (43%, 44/103) before 1 month of age, 31 (30%, 31/103) from 1 month (including 1 month) to 6 months and 6 (6%, 6/103) from 6 months (including 6 months) to 1 year. Information about age at diagnosis was not available for the remaining seven patients. The detailed clinical and genetic etiology distributions among the patients with different diagnosed ages are given in Table 3. The genetic etiology rates among the patients with unclear clinical etiology were 25% (2/8) in the "during pregnancy" group, 50% (2/4) in the "< 1 month" group, 31% (5/16) in the "1–6 months" group and 17% (1/6) in the "6 months–1 year" group. The genetic etiology rates among the patients with clear clinical etiologies were 14% (2/14) in the "during pregnancy" group, 3% (1/40) in the "< 1 month" group, 13% (2/15) in the "1–6 months" group. There were no statistically significant differences in the genetic etiology rates among the four groups with unclear clinical etiology nor among the three groups with clear clinical etiologies ($P = 0.713$; $P = 0.196$).

Table 3 Distribution of etiologies in this cohort

Variables	Patients born preterm or term[a]		Patients of different diagnosed ages[b]				Patients of different outcomes[c]			
	Preterm ($n=57$)	Term ($n=38$)	During pregnancy ($n=22$)	<1 mon ($n=44$)	1 mon–6 mon ($n=31$)	6 mon–1 y ($n=6$)	Developmental delay ($n=42$)	Neurological sequelae ($n=11$)	Deceased ($n=7$)	Improved ($n=9$)
Clinical etiology distribution, *n*										
BM	4	4	4	0	3	0	5	0	1	0
BM + IH	2	1	1	2	0	0	1	0	0	0
IH	22	6	7	22	2	0	5	6	1	3
II	6	5	0	7	4	0	0	2	2	4
II + IH	11	3	0	9	5	0	4	0	0	2
TM	0	0	0	0	1	0	0	1	0	0
AC	1	1	2	0	0	0	0	0	0	0
UC	11	18	8	4	16	6	27	2	3	0
Genetic etiology distribution, *n*										
MD	0	5	0	1	5	1	5	0	1	0
GS	1	2	2	1	1	0	2	1	0	1
BM	0	2	2	0	1	0	1	1	0	0
MP	0	0	0	1	0	0	0	0	0	0
NEG	56	29	18	41	24	5	34	9	6	8
Genetic etiology rates among the patients with unclear clinical etiology, % (*n*/*N*)										
	0 (0/11)	28 (5/18)	25 (2/8)	50 (2/4)	31 (5/16)	17 (1/6)	22 (6/27)	50 (1/2)	33 (1/3)	/
Genetic etiology rates among the patients with clear clinical etiologies, % (*n*/*N*)										
	2 (1/46)	20 (4/20)	14 (2/14)	3 (1/40)	13 (2/15)	/	13 (2/15)	11 (1/9)	0 (0/4)	11 (1/9)

BM brain malformations, *IH* intracranial hemorrhage, *II* intracranial infection, *TM* tumor, *AC* arachnoid cyst, *UC* unclear, *MD* metabolic diseases, *GS* genetic syndromes, *MP* myopathy, *NEG* negative, *1 mon–6 mon* including 1 mon, *6 mon–1 y* including 6 mon, *developmental delay* assessed by the Gesell Developmental Scale, *neurological sequelae* persistent ventriculomegaly, brain dysplasia or epilepsy, *improved* hydrocephalus and ventriculomegaly improved without any complications. [a]The exact gestational age of 15 patients could not be obtained; [b]Information about age at diagnosis was not available for 7 patients; [c]The outcomes of 41 patients could not be determined. "/" none

Distribution of etiologies among patients with different outcomes

We evaluated the outcomes of 69 patients. The outcomes of 41 patients could not be determined owing to, limited medical record availability in 29 cases and to patients rejecting treatment and leaving the hospital when informed of the possible poor outcomes in 12 cases. Among the 69 patients with outcomes were available, 42 (61%, 42/69) showed developmental delays and 14 had undergone surgeries; 11 (16%, 11/69) patients developed other neurological sequelae including seven patients who had undergone surgeries; and 7 (10%, 7/69) died including one patient who had undergone surgery. Only 9 (13%, 9/69) patients improved without any sequelae during follow-up, and six of these patients had undergone surgeries. The detailed clinical and genetic etiology distributions among the patients with different outcomes are shown in Table 3. The genetic etiology distribution was as follows: among the patients with unclear clinical etiology, there were 6 (22%, 6/27) patients in the "developmental delay" group, 1 (50%, 1/2) patients in the "neurological sequelae" group, and 1 (33%, 1/3) patient in the "deceased" group among the patients with clear clinical etiologies, there were 2 (13%, 2/15) patients in the "developmental delay" group, 1 (11%, 1/9) patient in the "neurological sequelae" group, 1 (11%, 1/9) patient in the "improved" group and zero (0%, 0/4) patients in the "deceased" group. Comparative analyses in this section were not practicable owing to the large number of patients lost to follow-up.

Exome-wide association study

We detected 18 genes and 31 variants that were over-represented in the 110 IHC patients described above (Methods section and Supplementary Tables 1 and 2). Of these 47 genes, six (*NPHP1*, *FANCL*, *ERCC4*, *NME8*, *ERCC3* and *DMPK*) were annotated as hydrocephalus-related genes recorded in DisGeNET [14]. In addition, another three genes (*ZEB1*, *SBF2*, and *GNAI2*) also may be related to IHC because they are involved in the pathways of hydrocephalus formation (elaborated in the Discussion section). The position and encoded proteins of the variants detected in *ZEB1*, *SBF2*, and *GNAI2* are listed in Table 4.

Discussion

As a neurological disorder with high morbidity, IHC requires early and sometimes multiple neurosurgical interventions [15, 16] and can result in marked neurological presentations, neurodevelopmental delays or even death [10]. Although surgical treatments can alleviate the ventriculomegaly and can relieve the increased intraventricular pressure, which may cause a crescendo of neurovascular damage further compromising the brain development [15, 17], severe prognoses are common among those who have undergone surgeries owing to shunt infection or shunt failure [3, 16]. Among the patients who had undergone surgical treatments in our study population, only six patients improved without any sequelae, whereas 22 patients received severe prognoses.

Table 4 Variants detected in *ZEB1*, *SBF2*, and *GNAI2*

Gene	Variant	Zygosity	SIFT	Polyphen2	MutationTaster	HGMD
SBF2	NM_030962:exon12:c.1171G > A(p.A391T)	Het	0.032	0.984	1.000	–
SBF2	NM_030962:exon29:c.3877A > G(p.K1293E)	Het	0.434	0.097	1.000	–
SBF2	NM_030962:exon28:c.3754A > T(p.S1252C)	Het	0.009	0.541	0.843	–
SBF2	NM_030962:exon24:c.3056A > T(p.Q1019L)	Het	0.807	0.013	1.000	–
SBF2	NM_030962:exon36:c.5037C > T(p.R1679R)	Het	–	–	–	–
SBF2	NM_030962:exon11:c.1066C > T(p.R356X)	Het	–	–	1.000	DM
SBF2	NM_030962:exon11:c.1067G > T(p.R356L)	Het	0.000	0.934	1.000	–
SBF2	NM_030962:exon20:c.2390A > G(p.Y797C)	Het	0.173	0.713	0.999	–
SBF2	NM_030962:exon23:c.2813A > G(p.E938G)	Het	0.000	1.000	1.000	–
SBF2	NM_030962:exon6:c.527 T > G(p.L176W)	Het	0.001	0.997	1.000	–
SBF2	NM_030962:exon32:c.4328A > C(p.E1443A)	Het	0.002	0.983	1.000	–
ZEB1	NM_030751:exon4:c.444_461delinsG(p.G150Wfs*3)	Het	–	–	–	DM
ZEB1	NM_030751:exon4:c.479_480delinsA(p.N160Kfs*26)	Het	–	–	–	–
ZEB1	NM_030751:exon9:c.2995G > C(p.E999Q)	Het	0.046	0.877	0.999	–
GNAI2	NM_002070:exon5:c.465-8A > C	Het	–	–	–	–

SIFT sorting intolerant from tolerant, *Polyphen2* polymorphism phenotyping v2, *HGMD* Human Gene Mutation Database, *DM* disease-causing mutations, *Het* heterozygous. "–" none

Because IHC may be caused by various underlying etiologies [18], including genetic causes that remain incompletely understood, we performed a retrospective study focused on the genetic landscape of IHC and discovered a total of 16 (15%, 16/110) IHC patients with a genetic etiology. The genetic factors associated with IHC are heterogeneous [19]. Among the 16 patients with a genetic finding in this study, variations in genes associated with metabolic disorders were most common. In 2018, a study reported ten patients with metabolic disorders who were not diagnosed until hydrocephalus had developed [20]. If those patients had received an earlier diagnosis through genetic testing and had been provided with early, precise intervention, the hydrocephalus may not have developed. The second most common class of mutated genes found in our study was genetic syndrome-related genes. In a large proportion of patients, IHC was merely a symptom of various syndromes. Paying attention to the concomitant abnormalities and actively adopting genetic testing will facilitate identification of the underlying cause of hydrocephalus. The third most common identified gene class included genes responsible for causing brain malformations. A combination of brain imaging and genetic testing will make it possible to reveal the brain abnormalities and the underlying genetic causes of IHC in a timely manner. In addition to the genes associated with the three types of diseases discussed above, patient 064 had a variant in *MTM1*, which is the gene responsible for X-linked centronuclear myopathy. Joseph et al. [21] proposed that the symptoms of greater length at birth, large head circumference (with or without hydrocephalus), elongated face and slender digits, may suggest an early clinical diagnosis of this disease, which can usually be confirmed by muscle biopsy. With the development of genetic testing, these earlier invasive methods may be substituted.

In addition, the genetic etiology rate of the patients with unclear clinical etiology was significantly higher than the genetic etiology rate of the patients with clear clinical etiologies. Consistent with previous literature reports, most hydrocephalus cases are present at birth or shortly after birth [2]. In this study, 66 (64%, 66/103) patients were diagnosed before 1 month of age. Most of the patients with a genetic etiology were distributed in the "1–6 months" group, but there were no significant differences of the genetic etiology rates among the different diagnosed age groups conditional on the clinical etiology being neither clear nor unclear. When investigating the genetic etiology distribution among the patients with different outcomes, comparative analyses were not practicable owing to a large number of patients lost to follow-up. When comparing the genetic etiology rates in patients born at term and before term, the rate was significantly lower in the preterm group than in the term group among the patients with clear clinical etiologies; however, the genetic etiology rates were not significantly different among the patients with unclear clinical etiology. IH was the most common clinical etiology of hydrocephalus in this cohort, and a total of 48 patients presented with IH, including 35 were preterm patients. Preterm hydrocephalus might be more commonly caused by IH owing to the intrinsic fragility of the germinal matrix vasculature and the fluctuation of cerebral blood flow [22–24], which may help to explain the low genetic etiology rate among the preterm patients with clear clinical etiologies. Thus, we inferred that a genetic etiology was more common to be found among IHC patients who had unclear clinical etiology. Although most of the patients with a genetic etiology were patients with unclear clinical etiology, it is worth mentioning that five patients with a clear clinical cause in this study also had a genetic etiology, and their genetic etiology rate was significantly higher in the term group than the preterm group. Those findings suggested that even among IHC patients (especially among term IHC patients) with a clear clinical etiology, the possibility of a genetic underpinning cannot be ruled out.

To further elucidate the genetic causes of IHC, we conducted an EWAS of IHC within this cohort. Based on previous reports and on our results, we speculated that *ZEB1, SBF2*, and *GNAI2* might be associated with the formation of IHC. The literature suggests that *ZEB1* mediates the activation of nuclear factor-κB (NF-κB) [25] and that *ZEB1* inhibition promotes the apoptosis of osteosarcoma cells via a NF-κB-related signaling pathway [26]. This evidence indicates that *ZEB1* may mediate the activation of NF-κB, which is known to mediate neuroinflammation and impair ependymal ciliogenesis during early life after birth, thus leading to earlier hydrocephalus formation [27]. Moreover, *ZEB1* has been reported to be a downstream signaling molecule in the sonic hedgehog pathway [28], a signaling pathway involved in hydrocephalus formation [29]. Similar to *ZEB1*, *SBF2*, and *GNAI2* might be associated with molecular signaling pathways involved in hydrocephalus formation, including the transforming growth factor-β (TGF-β)/Smad and phosphatidylinositol 3-hydroxy kinase (PI3K)/protein kinase B (AKT) pathways, respectively [30, 31]. A previous study has reported that silencing *SBF2* significantly promotes pancreatic cancer cell apoptosis via the TGF-β/Smad signaling pathway, which is also involved in hydrocephalus formation [30, 32]. *GNAI2* (G protein, alpha-inhibiting 2B) might be related to the PI3K/AKT signaling pathway because G-alpha inhibitory proteins are clearly involved in extracellular signal-regulated kinase (ERK) activation [33], and a slight increase in ERK activity leads to a distinct enhancement of PI3K/AKT phosphorylation [34]. Thus, we speculate mutations within those three genes may have an impact on the

signaling pathways related to hydrocephalus development and may be involved in the formation of IHC. Further studies are needed to confirm our findings.

Our study had several limitations. Owing to the retrospective nature of our data collection, not all data could be obtained, which might lead to underestimates of certain results. In addition, this was a single-center study that included a period of 4 years, which represents a small sample. Owing to the small number of patients in the study, we could not make a more complete comparison of the genetic and clinical etiologies of IHC patients.

In conclusion, the large proportion of early-onset and agnogenic IHC cases indicates that genetic components might play an important role in the etiologies. The utility of genetic testing can help to reveal the genetic etiologies in IHC patients, especially in those who had unclear clinical etiology. Genes associated with metabolic disorders, brain abnormalities and genetic syndromes should be considered in a specific panel for detecting the genetic etiology of IHC patients when whole-exome sequencing or whole-genome sequencing are not available. We also identified genes that might have an impact on the signaling pathways involved in IHC formation, and these genes should be evaluated as potential IHC susceptibility genes.

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Author contributions MHF designed the study, collected data, drafted the initial manuscript, reviewed the manuscript, and revised the manuscript. DXR, CHY, LYL, WBB, and WHJ designed the data collection instruments, performed the initial analyses, and were involved in writing the manuscript. CGQ, WLS, and CY reviewed all cases and were involved with the study design, data analysis, and writing of the manuscript. YL and ZWH were involved with the study design, supervised data collection, and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

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Compliance with ethical standards

Ethical approval This study was approved by the Ethics Committee of the Children's Hospital of Fudan University, and informed consent was obtained from all the study participants.

Conflict of interest The authors have no conflict of interest relevant to this article to disclose.

Data availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Munch TN, Rostgaard K, Rasmussen MLH, Wohlfahrt J, Juhler M, Melbye M. Familial aggregation of congenital hydrocephalus in a nationwide cohort. Brain. 2012;135:2409–15.
2. Wright Z, Larrew TW, Eskandari R. Pediatric hydrocephalus: current state of diagnosis and treatment. Pediatr Rev. 2016;37:478–90.
3. Gmeiner M, Wagner H, Zacherl C, Polanski P, Auer C, van Ouwerkerk WJR, et al. Long-term mortality rates in pediatric hydrocephalus-a retrospective single-center study. Childs Nerv Syst. 2017;33:101–9.
4. Kousi M, Katsanis N. The genetic basis of hydrocephalus. Annu Rev Neurosci. 2016;39:409–35.
5. Adle-Biassette H, Saugier-Veber P, Fallet-Bianco C, Delezoide AL, Razavi F, Drouot N, et al. Neuropathological review of 138 cases genetically tested for X-linked hydrocephalus: evidence for closely related clinical entities of unknown molecular bases. Acta Neuropathol. 2013;126:427–42.
6. Drielsma A, Jalas C, Simonis N, Désir J, Simanovsky N, Pirson I, et al. Two novel CCDC88C mutations confirm the role of DAPLE in autosomal recessive congenital hydrocephalus. J Med Genet. 2012;49:708–12.
7. Ekici AB, Hilfinger D, Jatzwauk M, Thiel CT, Wenzel D, Lorenz I, et al. Disturbed Wnt signalling due to a mutation in CCDC88C causes an autosomal recessive non-syndromic hydrocephalus with medial diverticulum. Mol Syndromol. 2010;1:99–112.
8. Saillour Y, Zanni G, Des Portes V, Heron D, Guibaud L, Iba-Zizen MT, et al. Mutations in the *AP1S2* gene encoding the sigma 2 subunit of the adaptor protein 1 complex are associated with syndromic X-linked mental retardation with hydrocephalus and calcifications in basal ganglia. J Med Genet. 2007;44:739–44.
9. Verhagen JMA, Schrander-Stumpel CTRM, Krapels IPC, de Die-Smulders CEM, van Lint FHM, Willekes C, et al. Congenital hydrocephalus in clinical practice: a genetic diagnostic approach. Eur J Med Genet. 2011;54:e542–7.
10. Tully HM, Ishak GE, Rue TC, Dempsey JC, Browd SR, Millen KJ, et al. Two hundred thirty-six children with developmental hydrocephalus: causes and clinical consequences. J Child Neurol. 2016;31:309–20.
11. Dong XR, Liu B, Yang L, Wang HJ, Wu BB, Liu RC, et al. Clinical exome sequencing as the first-tier test for diagnosing developmental disorders covering both CNV and SNV: a Chinese cohort. J Med Genet. 2020;57:558–66.
12. Ioannidis NM, Rothstein JH, Pejaver V, Middha S, McDonnell SK, Baheti S, et al. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. Am J Hum Genet. 2016;99:877–85.
13. Povysil G, Petrovski S, Hostyk J, Aggarwal V, Allen AS, Goldstein DB. Rare-variant collapsing analyses for complex traits: guidelines and applications. Nat Rev Genet. 2019;20:747–59.
14. Pinero J, Ramirez-Anguita JM, Sauch-Pitarch J, Ronzano F, Centeno E, Sanz F, et al. The DisGeNET knowledge platform for disease genomics: 2019 update. Nucleic Acids Res. 2020;48:D845–55.
15. Harris CA, McAllister JP 2nd. What we should know about the cellular and tissue response causing catheter obstruction in the

treatment of hydrocephalus. Neurosurgery. 2012;70:1589–601 (discussion 601–2).
16. Laeke T, Tirsit A, Biluts H, Murali D, Wester K. Pediatric hydrocephalus in Ethiopia: treatment failures and infections: a hospital-based, retrospective study. World Neurosurg. 2017;100:30–7.
17. McAllister JP 2nd. Pathophysiology of congenital and neonatal hydrocephalus. Semin Fetal Neonatal Med. 2012;17:285–94.
18. Ma ML, Wu MX, Li YB, Wu D, Zhang BK. Shunt surgery for early-onset severe hydrocephalus in methylmalonic acidemia: report on two cases and review of the literature. Childs Nerv Syst. 2018;34:1417–21.
19. Furey CG, Zeng X, Dong W, Jin SC, Choi J, Timberlake AT, et al. Human genetics and molecular mechanisms of congenital hydrocephalus. World Neurosurg. 2018;119:441–3.
20. Zhang KH, Gao M, Wang GY, Shi YY, Li XY, Lv YQ, et al. Hydrocephalus in cblC type methylmalonic acidemia. Metab Brain Dis. 2019;34:451–8.
21. Joseph M, Pai GS, Holden KR, Herman G. X-linked myotubular myopathy: clinical observations in ten additional cases. Am J Med Genet. 1995;59:168–73.
22. Kulkarni AV, Riva-Cambrin J, Butler J, Browd SR, Drake JM, Holubkov R, et al. Outcomes of CSF shunting in children: comparison of Hydrocephalus Clinical Research Network cohort with historical controls: clinical article. J Neurosurg Pediatr. 2013;12:334–8.
23. Ballabh P. Pathogenesis and prevention of intraventricular hemorrhage. Clin Perinatol. 2014;41:47–67.
24. de Bijl-Marcus KA, Brouwer AJ, de Vries LS, van Wezel-Meijler G. The effect of head positioning and head tilting on the incidence of intraventricular hemorrhage in very preterm infants: a systematic review. Neonatology. 2017;111:267–79.
25. Wang HQ, Zhao SL, Chen B, Fu CH, Dang YW, Fang PH, et al. Repression of the expression of PPP3CC by ZEB1 confers activation of NF-kappaB and contributes to invasion and growth in glioma cells. Jpn J Clin Oncol. 2018;48:175–83.
26. Xu XM, Liu W, Cao ZH, Liu MX. Effects of ZEB1 on regulating osteosarcoma cells via NF-kappaB/iNOS. Eur Rev Med Pharmacol Sci. 2017;21:1184–90.
27. Lattke M, Magnutzki A, Walther P, Wirth T, Baumann B. Nuclear factor kappaB activation impairs ependymal ciliogenesis and links neuroinflammation to hydrocephalus formation. J Neurosci. 2012;32:11511–23.
28. Singh S, Howell D, Trivedi N, Kessler K, Ong T, Rosmaninho P, et al. Zeb1 controls neuron differentiation and germinal zone exit by a mesenchymal-epithelial-like transition. Elife. 2016;5:e12717.
29. Gavino C, Richard S. Patched1 haploinsufficiency impairs ependymal cilia function of the quaking viable mice, leading to fatal hydrocephalus. Mol Cell Neurosci. 2011;47:100–7.
30. Liu CG, Li GR, Wang PP, Wang Y, Pan J. Characterization of spontaneous hydrocephalus development in the young atherosclerosis-prone mice. Neuroreport. 2017;28:1108–14.
31. Mirzaa G, Parry DA, Fry AE, Giamanco KA, Schwartzentruber J, Vanstone M, et al. De novo *CCND2* mutations leading to stabilization of cyclin D2 cause megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome. Nat Genet. 2014;46:510–5.
32. Long J, Liu Z, Wu XD, Xu YH, Ge CL. siRNA-mediated *SBF2* silencing may inhibit pancreatic cancer cells via attenuation of the TGF-beta signaling pathway. Technol Cancer Res Treat. 2016;15:308–13.
33. Werry TD, Christopoulos A, Sexton PM. Mechanisms of ERK1/2 regulation by seven-transmembrane-domain receptors. Curr Pharm Des. 2006;12:1683–702.
34. Huang X, Fu Y, Charbeneau RA, Neubig RR. GNAI2 and regulators of G protein signaling as a potential Noonan syndrome mechanism. Med Hypotheses. 2009;73:56–9.