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Original research

IFIH1 variants are associated with generalised epilepsy preceded by febrile seizures

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ABSTRACT

Background *IFIH1* variants have been reported to be associated with immune-related disorders with/without seizures. It is unknown whether *IFIH1* variants are associated with common epilepsy without acquired causes and the mechanism underlying phenotypic variation remains elusive.

Methods Trio-based whole-exome sequencing was performed on patients with febrile seizures or epilepsy with antecedent febrile seizures. Previously reported variants were systematically reviewed to investigate genotype-phenotype associations.

Results Two de novo heterozygous and three biallelic missense variants were identified in five patients with generalised epilepsy with antecedent febrile seizures. The variants were predicted to be damaging by in silico tools and were associated with hydrogen bonding changes to neighbouring amino acids or decreased protein stability. Patients exhibited an early onset age and became seizure-free with favourable outcome. Further analysis revealed that de novo missense variants located in the Hel region resulted in seizures with multiple neurological abnormalities, while those in the pincer domain or C-terminal domain led to seizures with normal neurodevelopment, suggesting a sub-molecular effect. Biallelic missense variants, which were inherited from unaffected parents and presented low allele frequencies in general populations, were associated with seizures without neurological abnormalities. Truncation variants were related to refractory epilepsy and severe developmental delay, suggesting a genotype-phenotype correlation. *IFIH1* is predominantly expressed in the neonatal stage and decreases dramatically in the adulthood, which is consistent with the early onset age and favourable outcome of the patients.

Conclusions *IFIH1* variants are potentially associated with generalised epilepsy with antecedent febrile seizures. The sub-molecular implication and genotype-phenotype association help explain phenotype variations of *IFIH1* variants.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The *IFIH1* variants were previously reported to be associated with autoimmune and autoinflammatory disorders with/without seizures.
⇒ The relationship between *IFIH1* variants and epilepsy remains elusive.

WHAT THIS STUDY ADDS

⇒ Two de novo heterozygous and three biallelic missense variants were identified in five patients with generalised epilepsy with antecedent febrile seizures.
⇒ Further analysis reveals that the severity of the phenotype associated with *IFIH1* variants correlates with specific genotypes and locations of the variants.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ *IFIH1* variants are associated with generalised epilepsy with antecedent febrile seizures with good prognosis.
⇒ The sub-molecular implication and genotype-phenotype association help explain phenotype variations of *IFIH1* variants.

INTRODUCTION

Epilepsy is a common neurological disease that is characterised by recurrent spontaneous seizures. The development of epilepsy can be attributed to a variety of factors, such as structural, genetic, infectious, metabolic and immune abnormalities.[1] Infection of the central nervous system is a major risk factor for epilepsy. Focal or systemic inflammatory processes may lead to aberrant neural connectivity and a hyperexcitable neuronal network, which mediate the onset of epilepsy.[2 3]

The interferon induced with helicase C domain 1 gene (*IFIH1*, MIM *606951), which is located on chromosome 2q24.2, is ubiquitously expressed, including in the brain.[4 5] It encodes melanoma differentiation-associated protein 5, which is a cytoplasmic sensor that recognises viral RNA and plays a crucial role in the innate immune response during viral infections.[6 7] Homozygous *IFIH1* knockout mice exhibited increased virus-associated morbidity and mortality, and a diminished cytokine response to various viral pathogens.[8 9]

In humans, variants in the *IFIH1* gene have been implicated in autoimmune and autoinflammatory disorders,[10] such as autosomal dominant Aicardi-Goutières syndrome 7 (AGS7, MIM #615846) and autosomal dominant Singleton-Merten syndrome 1 (SGMRT1, MIM #182250). AGS7 is a rare genetic disorder characterised by early onset encephalopathy, intracranial calcification and elevated levels

of type I interferons in the cerebrospinal fluid.[11 12] SGMRT1 is characterised by aberrations in blood vessels, dentition and osseous structures.[13] Seizures were occasionally observed in patients with *IFIH1* variants. However, it is unknown whether *IFIH1* variants are associated with common epilepsy without acquired causes, and the mechanism underlying phenotype variation remains elusive.

In this study, we performed trio-based whole-exome sequencing (WES) on a group of cases with febrile seizures or epilepsy with antecedent febrile seizures. Two de novo heterozygous and three biallelic *IFIH1* missense variants were identified in five unrelated cases without neurodevelopmental delay. We further reviewed all previously reported *IFIH1* variants associated with epilepsy, aiming to investigate the genetic and phenotypic characteristics of *IFIH1*-associated epilepsy and explore the mechanism underlying the variation in phenotype.

MATERIALS AND METHODS

Patients

A total of 314 cases with febrile seizures or epilepsy with antecedent febrile seizures were recruited between 2018 and 2021 at the Second Affiliated Hospital of Guangzhou Medical University, the Guangdong 999 Brain Hospital and the Second Hospital of Shandong University in China through the China Epilepsy Gene 1.0 Project platform.

The study gathered clinical data on the affected individuals, including their current age, gender, age at seizure onset, seizure progression, family history, developmental status and efficacy of antiepileptic drugs. MRI scans were conducted to identify abnormalities in the brain. Long-term video EEG examinations were performed, which involved tests such as the open-close eyes test, intermittent photic stimulation, hyperventilation and sleep recording. The findings were evaluated by two certified electroencephalographers. The identification of epileptic seizures and epilepsies adhered to the classification and terminology standards established by the Commission on Classification and Terminology of the International League Against Epilepsy.[14–18] Febrile seizure was diagnosed based on (1) seizures occurring in childhood between 1 month and 6 years of age that were accompanied by a fever, (2) febrile illness not caused by central nervous system infection and (3) exclusion of other acute symptomatic seizures. Cases with acquired causes, such as brain tumours, head trauma, cerebrovascular diseases and hypoxic-ischaemic encephalopathy, were excluded. The trios comprising patients and their biological parents underwent screening for genetic variants through WES.

Whole-exome sequencing and bioinformatic analysis

The collection of genomic DNA from blood samples of the patients and their parents (trios) was conducted for the purpose of segregation analysis. Subsequently, trio-based WES (patients and their biological parents) was performed with NextSeq2000 (Illumina, San Diego, California, USA). The sequencing data were obtained through the utilisation of massive parallel sequencing, with an average depth exceeding 125 times and coverage of the capture regions exceeding 98%. Sequence alignment and variant calling were executed following established protocols as previously documented.[19 20] The frequencies of the identified variants were retrieved from the Genome Aggregation Database (gnomAD), including East Asian and general populations.

The potential disease-causing variants were systematically examined and assessed individually using five models: (1) epilepsy-associated gene; (2) dominant/de novo; (3) autosomal recessive inheritance, including homozygous and compound heterozygous variants; (4) X linked and (5) co-segregation analysis if available. Genes with recurrent de novo variants, biallelic variants, hemizygous variants or variants with segregations were chosen for subsequent evaluation of gene-disease associations. The *IFIH1* gene was selected as one of the candidate genes with recurrent de novo variants and compound heterozygotes in the trio-based cohort. Sanger sequencing was employed to authenticate the positive findings and ascertain the source of the variant. All *IFIH1* variants were annotated with reference to transcript NM_022168.4 in this study.

Computational modelling and genetic analysis

To anticipate the consequences of *IFIH1* missense variants and assess the pathogenicity of potential variants, the protein structure was generated using the AlphaFold software.[21 22] The analysis and visualisation of three-dimensional protein structures were conducted using PyMOL V.2.4 software. The stability of mutant proteins was calculated using I-Mutant V.2.0 software. The prediction of free energy changes between native and variant proteins was based on Gibbs free energy values, while energy differences were calculated using free energy change value (DDG). The consequences of all missense variants were predicted by 23 in silico tools (http://www.genemed.tech/varcards/welcome/index), including PolyPhen2, MutationTaster, CADD, REVEL, Fathmm-MKL, GenoCanyon and GERP++, etc.

Systematic review on *IFIH1* variants and epilepsy

We systematically searched literature from the Human Genetic Mutation Database (HGMD, https://www.hgmd.cf.ac.uk/ac/index.php; Professional 2024.01 edition), OMIM (https://www.omim.org/) and the PubMed database (https://pubmed.ncbi.nlm.nih.gov/) until September 2023 to retrieve all previously reported *IFIH1* variants and related phenotypes. To further characterise the genetic and phenotypic features of *IFIH1*-associated epilepsy, the inheritance origin, allele frequency, variant type, variant location of the *IFIH1* variants and clinical characteristics of the patients were systematically reviewed.

IFIH1 temporal expression profile analysis

The general temporal expression profiles of *IFIH1* at different development stages, including embryoid body, blastocyst, fetus, neonate, infant, juvenile and adult stages, were retrieved from the UniGene database. Specifically, to analysis the temporal expression pattern in the brain, the *IFIH1* expression in multiple brain areas at different developmental stages (from 8 postconceptional weeks to 40 years) was obtained from the human RNA-sequencing data in the BrainSpan database (http://www.brainspan.org/). The expression levels were normalised to reads per kilobase per million mapped reads. The expression pattern is fitted by the third-order polynomial least squares by using GraphPad Prism V.9.

RESULTS

Identification of *IFIH1* variants

Two heterozygous missense variants (c.2906G>T/p.Gly969Val and c.2966T>A/p.Val989Glu) and three pairs of compound heterozygous missense variants (c.263G>A/p.Ser88Asn and c.1046A>G/p.Lys349Arg, c.1709T>G/p.Met570Arg and c.2366C>T/p.Thr789Ile and c.2069T>C/p.Leu690Pro and c.2232T>A/p.Phe744Leu) were identified in five unrelated patients with epilepsy. The two heterozygous variants were de

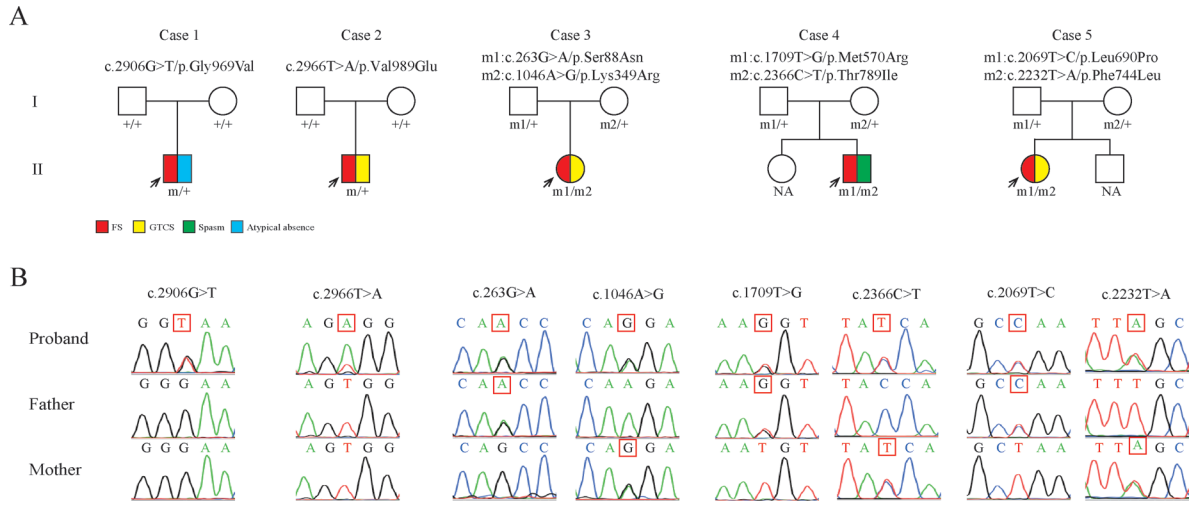

Figure 1 Genetic data of cases with *IFIH1* variants. (A) Pedigrees of the five cases with *IFIH1* missense variants and their corresponding phenotypes. FS, febrile seizure; GTCS, generalised tonic-clonic seizure. (B) DNA sequencing chromatogram of *IFIH1* variants. The red box indicate the positions of the variants.

novo, and the compound heterozygous variants were inherited from their asymptomatic parents (figure 1 and table 1).

The two heterozygous missense variants (c.2906G>T/p.Gly969Val and c.2966T>A/p.Val989Glu) arose de novo (PS2) and present none allele frequencies (gnomAD V.2.1.1 and V.2.1.1 controls) or extremely low allele frequencies (gnomAD V.4.1.0) in the gnomAD populations (PM2). They were highly conserved in various species and were predicted to be damaging by multiple in silico tools (PP3). According to the American College of Medical Genetics and Genomic (ACMG) guidelines,[23] the two variants were evaluated as 'likely pathogenic' (PS2+PM2+PP3; online supplemental table 1). The other six missense variants of the compound heterozygotes presented none or low frequencies (<0.005) in the gnomAD populations, predicted to be damaging by multiple in silico tools, and were assessed as 'uncertain significance' (online supplemental table 1). The heterozygous missense variant (c.2232T>A/p.Phe744Leu) in the compound heterozygotes was found to present one homozygote in the gnomAD V.4.1.0, but not present homozygotes in other gnomAD databases (V.2.1.1, V.2.1.1 controls and V.3.1.2 databases).

None of the patients had any other variants evaluated as pathogenic or likely pathogenic by ACMG guidelines in the genes that are recognised to be linked with epileptic phenotypes.[24]

Molecular locations and effects of *IFIH1* variants

The IFIH1 protein contains seven domains: caspase activation recruitment domain 1 (CARD1), caspase activation recruitment domain 2 (CARD2), helicase domain 1 (Hel1), helicase domain 2i (Hel2i), helicase domain 2 (Hel2), pincer domain (P) and C-terminal domain (CTD).[25] The N-terminal tandem CARD1 and CARD2 play a role together in activating mitochondrial antiviral-signalling protein; the centrally located Hel region, including Hel1, Hel2i and Hel2, is the core functional part of the IFIH1 protein, which facilitates the recognition of double-stranded (ds)RNA and the C-terminal CTD domain is involved in dsRNA binding.[26 27] The variant c.263G>A/p.Ser88Asn was located in the CARD1, variants c.1046A>G/p.Lys349Arg, c.1709T>G/p.Met570Arg, c.2069T>C/p.Leu690Pro, c.2232T>A/p.Phe744Leu and c.2366C>T/p.Thr789Ile were located in the Hel domain, while the remaining two variants (c.2906G>T/p.Gly969Val and c.2966T>A/p.Val989Glu) were located in the CTD (figure 2A,B).

The molecular effects of the missense variants were predicted by protein modelling using AlphaFold (figure 2C). Among the two de novo heterozygous variants, c.2906G>T/p.Gly969Val was predicted to have no change in hydrogen bonding with

Table 1 Clinical features of the patients with *IFIH1* variants

Case no.	*IFIH1* variants (NM_022168.4)	Origin	Sex	FS onset age	aFS onset age	Seizure course	EEG	Brain CT/MRI	ASM	DD	Seizure free duration	Diagnosis
1	c.2906G>T/p.Gly969Val	De novo	M	Infant	Infant	FS twice; atypical absence seizures 1–2 times per week	Diffuse discharges	Normal	LCM	Speech delay	1 year	EFS+
2	c.2966T>A/p.Val989Glu	De novo	M	Toddler	Preschooler	FS, 2–3 times/year; GTCS, once	Generalised SW	Normal	VPA, CNZ	Normal	4 years	EFS+
3	c.263G>A/p.Ser88Asn	Paternal	F	Infant	Infant	FS, once (after vaccination), GTCS, 3 times during 1 month	Normal	Normal	VPA	Normal	2.5 years	GE
	c.1046A>G/p.Lys349Arg	Maternal										
4	c.1709T>G/p.Met570Arg	Paternal	M	Toddler	Toddler	FS, once; spasm 1–2 times per day	Interictal: generalised SW/PSW; ictal: spasms	Normal	VPA	Normal	1 year	EFS+
	c.2366C>T/p.Thr789Ile	Maternal										
5	c.2069T>C/p.Leu690Pro	Paternal	F	Preschooler	Teenager	FS, once; GTCS twice during 13 years	generalised SW	Normal	LTG, PER	Normal	2 years	EFS+
	c.2232T>A/p.Phe744Leu	Maternal										

aFS, afebrile seizures; ASM, antiseizure medication; CNZ, clonazepam; DD, developmental disorder; EFS+, epilepsy with febrile seizures plus; F, female; FS, febrile seizures; GE, generalised epilepsy; GTCS, generalised tonic-clonic seizure; LCM, lacosamide; LTG, lamotrigine; M, male; PER, perampanel; PSW, poly spike-and-slow waves; SW, spike-and-slow waves; VPA, valproate.

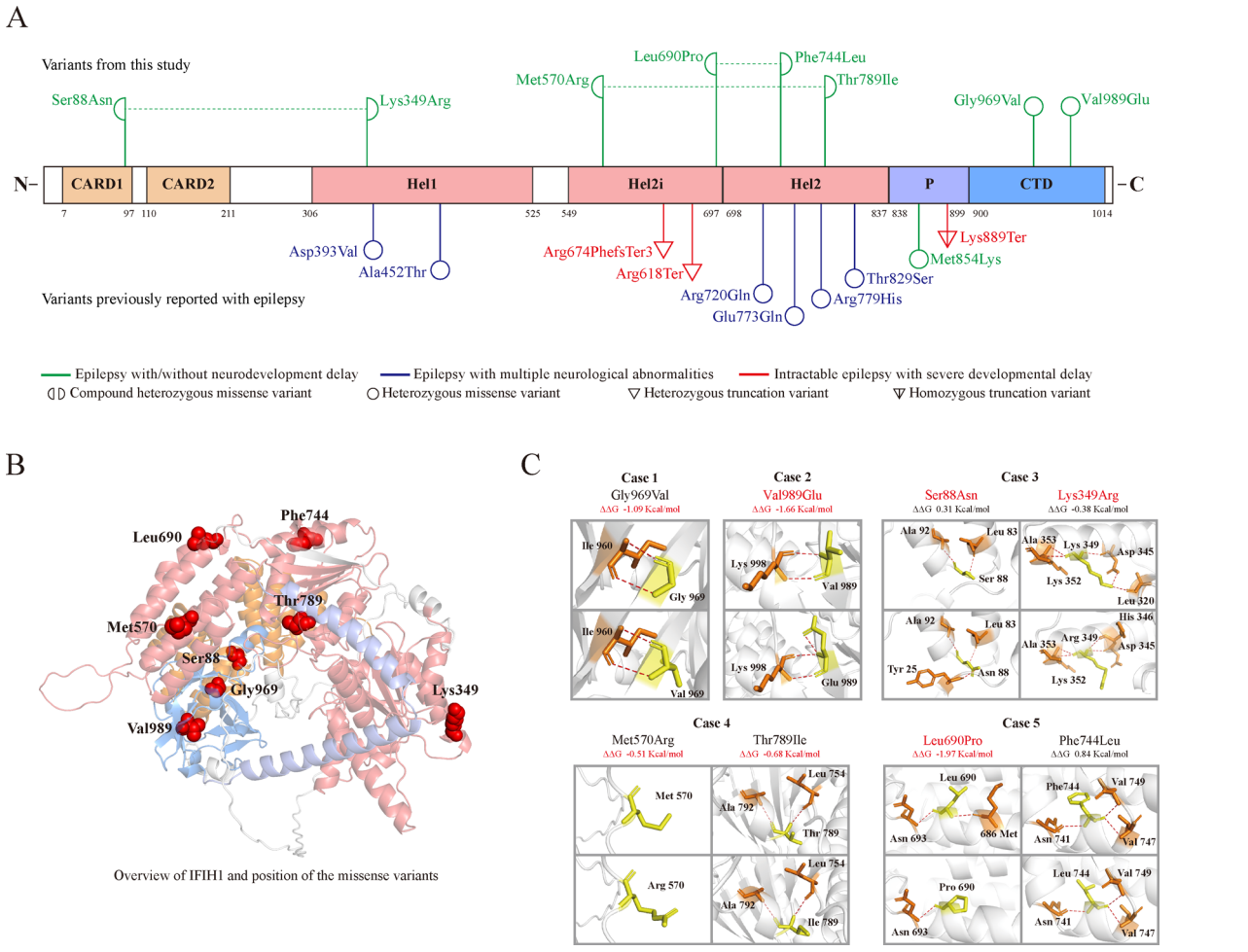

Figure 2 Schematic presentation of IFIH1 structure and molecular effect of the missense variants on IFIH1 protein. (A) Schematic diagram of IFIH1 structure and the location of *IFIH1* variants identified in this study (top) and previously reported *IFIH1* variants associated with epilepsy (bottom). (B) Schematic illustration of the location of missense variants in the three-dimensional structure of IFIH1. (C) Hydrogen bond changes and free energy stability changes of the variants identified in this study. Variants with marked changes are highlighted in red. CARD1, caspase activation recruitment domain 1; CARD2, caspase activation recruitment domain 2; CTD, C-terminal domain; Hel1, helicase domain 1; Hel2, helicase domain 2; Hel2i, helicase domain 2i; P, pincer domain.

neighbouring residues but was predicted to decrease protein stability, and c.2966T>A/p.Val989Glu was predicted to change both hydrogen bonding and protein stability. One pair of compound heterozygous variants (c.263G>A/p.Ser88Asn and c.1046A>G/p.Lys349Arg) were predicted to change hydrogen bonding in both alleles. Another pair (c.2069T>C/p.Leu690Pro and c.2232T>A/p.Phe744Leu) were predicted to change hydrogen bonding in one of the paired variants. A third pair (c.1709T>G/p.Met570Arg and c.2366C>T/p.Thr789Ile) was predicted to have no hydrogen bond changes but decrease the protein stability in both variants.

Clinical characteristics of the cases with *IFIH1* variants

The detailed clinical features of the five cases with *IFIH1* variants are summarised in table 1. All patients exhibited generalised epilepsys with preceding febrile seizures. Four of them (cases 1, 2, 4 and 5) experienced antecedent febrile seizures, which ranged from 1 to 4 years, with a median age of 1.6 years. Subsequently, they developed afebrile seizures and were diagnosed with epilepsy with febrile seizures plus. Case 3 had a febrile seizure 5 hours later after vaccination at the age of 1 year. She then experienced unprovoked generalised tonic-clonic seizures three times within 1 month and was diagnosed with generalised epilepsy.

All of the five patients had generalised seizures, including atypical absence seizures (case 1), spasm (case 4) and generalised tonic-clonic seizures (cases 2, 3 and 5). Four patients presented diffuse or generalised discharges on the EEG recording (figure 3), while one patient showed a normal interictal EEG (case 3). The brain MRIs of all the cases were normal, and none of them had basal ganglia calcification or abnormalities of blood vessels in the brain. All the patients were seizure-free after administration of one or two antiseizure medications. Case 1 showed mild speech delay, and the other four cases had normal development.

In summary, patients with *IFIH1* variants had generalised seizures with antecedent febrile seizures, generalised or diffused discharges on EEGs and achieved seizure-free outcomes with a good prognosis.

Genetic and phenotypic characteristics of *IFIH1*-related epilepsy

Until September 2023, a total of 54 variants, including 42 missense variants and 12 truncation variants, had been reported. Among these variants, 24 were reported in patients with AGS7, 5

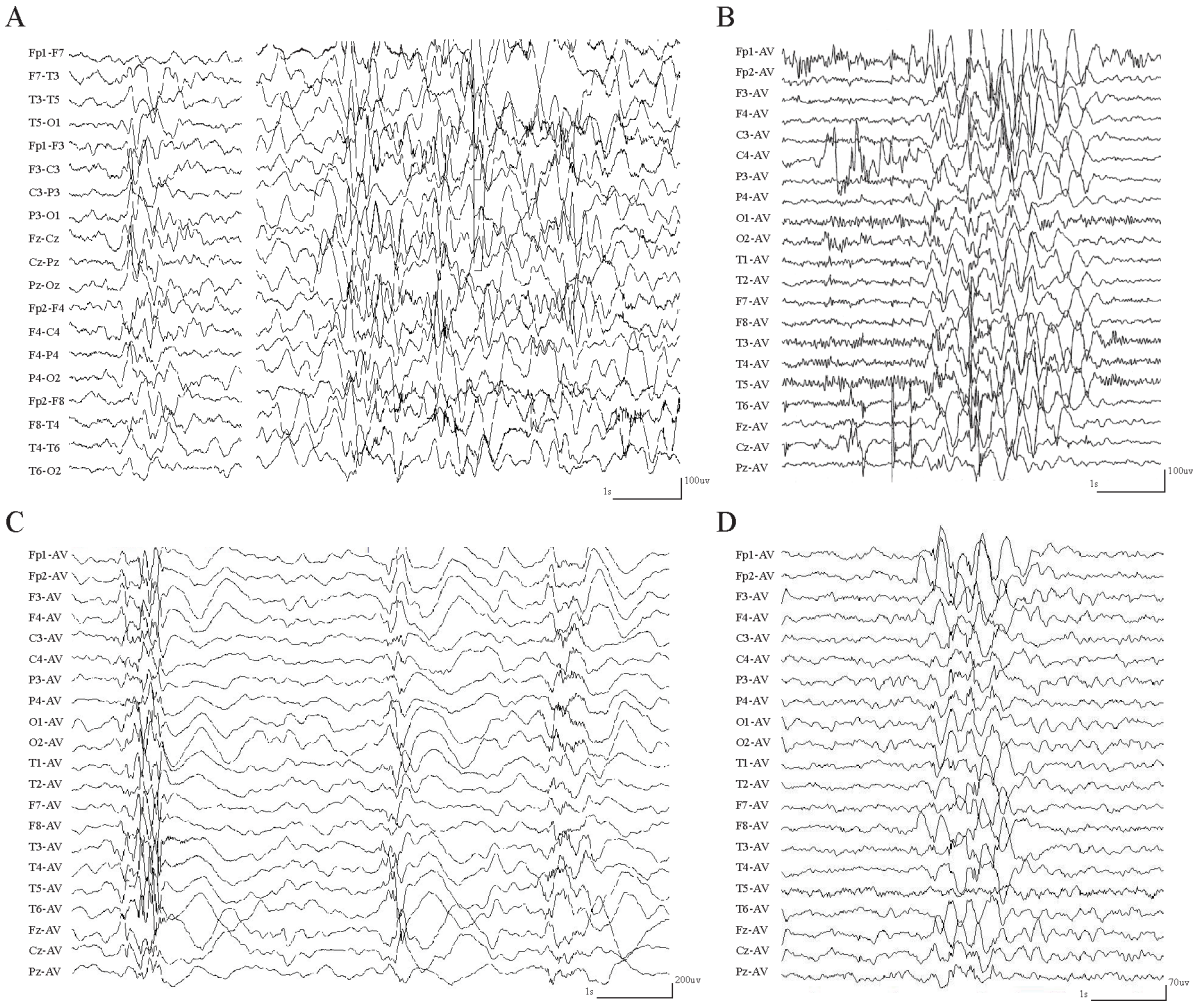

Figure 3 Representative EEG recordings of the patients with *IFIH1* variants. (A) Interictal EEG of case 1 showed diffuse discharges in both cerebral hemispheres during sleep (obtained at the age of 3 years). (B) Interictal EEG of case 2 showed irregular generalised spike-and-slow waves awake (obtained at the age of 4 years). (C) Interictal EEG of case 4 showed generalised spike-and-slow waves and poly spike-and-slow waves during sleep (obtained at the age of 1 year). (D) Interictal EEG of case 5 showed irregular generalised spike-and-slow waves during sleep (obtained at the age of 13 years). The low-frequency filter: 1.6 Hz; the high-frequency filter: 70 Hz.

in patients with SGMRT1 and 4 in patients with AGS7 combined with SGMRT1. The other variants were associated with disorders such as cardiovascular disease, inflammatory bowel disease and spastic paraplegia (online supplemental table 2).

Regarding epilepsy, a total of 10 *IFIH1* variants, including 7 missense and 3 truncations with detailed phenotypes, were previously reported. Their genetic and clinical characteristics are summarised in table 2, and the locations of the variants are demonstrated in figure 2A.

Six missense variants were located at the Hel region, among which five were de novo heterozygotes. These variants were associated with seizures with multiple neurological abnormalities, such as severe developmental delay, microcephaly, rapid neurological decline, spastic quadriplegia and intracranial calcification.[12 25 28–31]

One de novo missense variant, which was located in the pincer region, was identified in a patient with febrile seizures plus. Except for moderate motor and mental retardation, no other neurological abnormalities were observed.

In contrast, in the present study, de novo missense variants located within the C-terminal domain were associated with generalised epilepsy with normal psychomotor development and without brain abnormalities. It suggested a sub-molecular mechanism underlying phenotype heterogeneity, that is, de novo heterozygous missense variants in different domains potentially played distinct functional roles and thus were associated with different clinical phenotypes.

The compound heterozygous missense variants, of each with low allele frequencies in the general population and inherited from unaffected parents, were associated with generalised seizures without neurological abnormalities.

Three truncation variants, including one de novo heterozygous nonsense, one heterozygous frameshift and one pair of homozygous nonsense, were previously reported in three cases with intractable seizures and severe developmental delay.[28 30 32] Among them, two were diagnosed with epileptic encephalopathy and one had been deceased. Brain structural abnormalities were observed in all the three patients with truncation variants, suggesting a genotype-phenotype correlation.

The temporal expression profile of *IFIH1*

In the present study, patients showed an early onset age and achieved seizure freedom after treatment. Our recent study showed that the age of onset and disease outcome correlated with the stage of genetic expression (dependent stage).[33 34] We thus

Table 2 Genetic and clinical features of *IFIH1* variants previously reported in epilepsy

Variants		gnomAD V.4.1.0	gnomAD V.2.1.1	Sex	Inheritance	Location	Diagnosis	Seizure	Other manifestations	Brain imaging	Reference
Missense variants	c.1178A>T/p.Asp393Val	–	–	M	De novo	Hel1	Neuroregression	Seizures	Fever and rash, severe spastic tetraparesis, axial hypotonia and a loss of speech, and a rapid loss of motor and intellectual skills	Basal ganglia calcification	Rice *et al*[25]
	c.1354G>A/p.Ala452Thr	–	–	M	De novo	Hel1	AGS7	EFS+	Hypertonia, spastic quadriplegia and microcephaly	Brain atrophy, bilateral in the basal ganglia and white matter calcification	Oda *et al*[12]
	c.2159G>A/p.Arg720Gln	6.841×10^{-7}	–	M	De novo	Hel2	AGS7	Intractable epilepsy (died of pneumonia at the age of 2 years)	Irritable and fed poorly, spastic-dystonic tetraparesis, intrauterine growth retardation and microcephaly	Cerebral atrophy with calcifications and white matter disease	Rice *et al* [25]
	c.2317G>C/p.Arg773Gln	–	–	M	De novo	Hel2	Cerebral palsy	EE	Spastic diplegia, intelligence disturbance and neurodevelopmental delay	Calcification	Rosello *et al*[31]
	c.2336G>A/p.Arg779His	1.374×10^{-6}	4.011×10^{-6}	F	De novo	Hel2	AGS7	EFS+	Dystonia, hypotonia, spastic quadriplegia and progressive microcephaly	Brain atrophy, bilateral basal ganglia and subcortical calcification	Oda *et al*[12]
	c.2486C>G/p.Thr829Ser	–	–	M	NA	Hel2	AGS7	Seizures	Spastic diplegia and rapid neurological decline	Abnormal white matter and calcification	Rice *et al*[29]
	c.2561T>A/p.Met854Lys	–	–	F	De novo	Pincer	AGS7 and SGMRT1	EFS+ (febrile seizures every year)	Moderate motor and mental retardation	Bilateral calcification of the deep frontal lobes and the globus pallida	Takeichi *et al*[42]
Truncation variants	c.1852C>T/p.Arg618Ter	1.426×10^{-5}	1.992×10^{-5}	F	De novo	Hel2i	AGS7	Seizures (deceased)	Microcephaly, muscular hypotonia and global developmental delay	NA	Liu *et al*[7]
	c.2020_c.2023delAGAT/ p.Arg674PhefsTer3	–	–	NA	NA	Hel2i	Early onset epileptic encephalopathy	Frequent focal seizures (severely)	Severe developmental delay	A wide range of symmetrical abnormalities in the white matter	Ma *et al*[28]
	c.2665A>T/p.Lys889Ter	1.369×10^{-6}	3.989×10^{-6}	F	Homozygous (paternal and maternal)	Pincer	3-phosphoglycerate dehydrogenase deficiency	EE (occurring once or twice per day with mild myoclonia)	Fever and respiratory infections, microcephaly, severe psychomotor retardation	Simplified gyral pattern, hypoplastic corpus callosum	Zaki *et al*[32]

AGS7, Aicardi-Goutières syndrome 7; EE, epileptic encephalopathy; EFS+, epilepsy with febrile seizures plus; F, female; gnomAD, Genome Aggregation Database; GTCS, generalised tonic-clonic seizure; Hel, helicase domain; M, male; NA, not available; SGMRT1, Singleton-Merten syndrome 1.

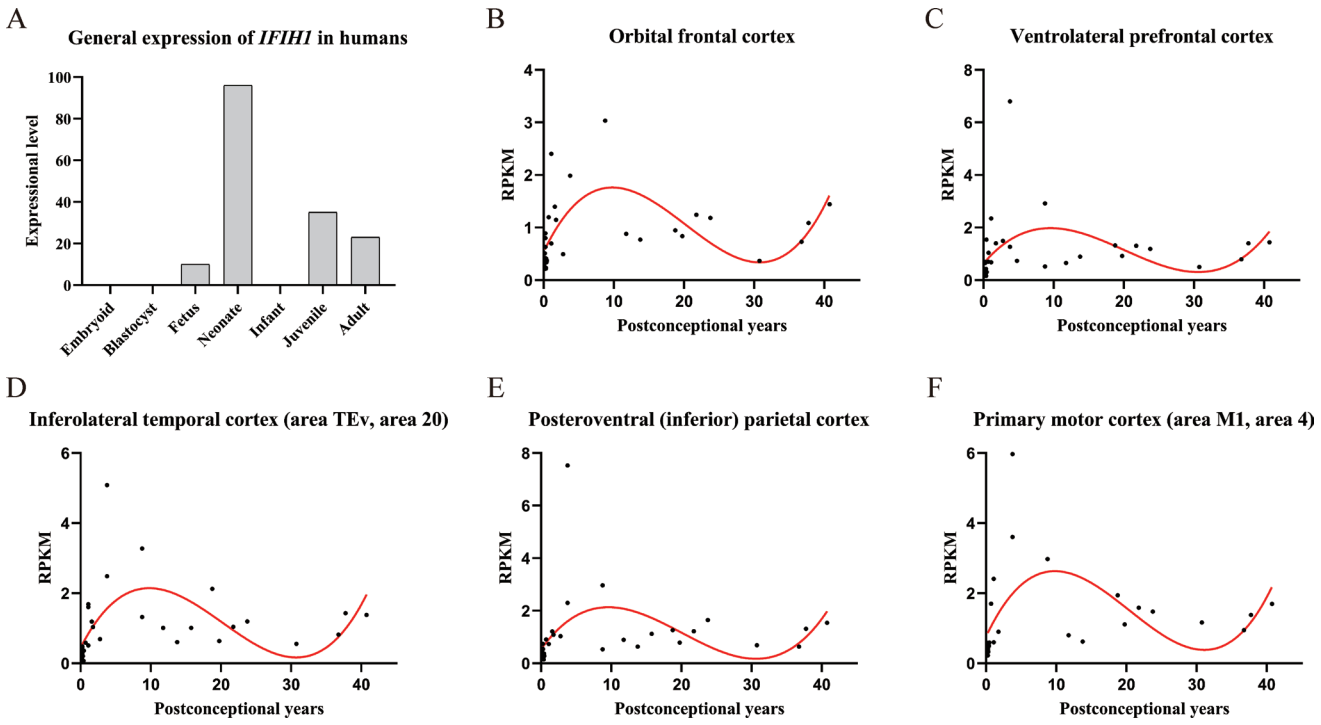

Figure 4 The temporal expression profile of *IFIH1* in humans. (A) Temporal expression pattern of *IFIH1* in humans retrieved from the UniGene database. (B–F) Temporal expression patterns of *IFIH1* in different cortices of the human brain retrieved from the BrainSpan database. RPKM, reads per kilobase per million mapped read.

explored the temporal expression of *IFIH1*. The data retrieved from the UniGene database showed that *IFIH1* was predominately expressed in the neonate stage, decreased dramatically in the juvenile and adult stages (figure 4A). In the brain, including the frontal, temporal, parietal and motor cortex, *IFIH1* expression was detected during fetal development, increased after birth with a peak before 10 years, decreased significantly in adulthood with a nadir at approximately 30 years of age and showed a slight increase later around 40 years of age (figure 4B–F).

DISCUSSION

In this study, we identified novel *IFIH1* variants, including two de novo heterozygous and three biallelic missense variants, in five unrelated cases with generalised epilepsy with antecedent febrile seizures and without neurodevelopmental delay. The two de novo missense variants were assessed as 'likely pathogenic' according to the ACMG guidelines. The variants of the compound heterozygotes presented low allele frequencies and were predicted to be damaging by multiple in silico tools and change hydrogen bonding with neighbouring residues or decrease protein stability. All of the patients achieved seizure freedom with a good prognosis. Further analysis revealed that the de novo heterozygous missense variants located in the Hel region were associated with seizures with multiple severe neurological abnormalities, while those located in the C-terminal domain were associated with febrile seizures with normal neurodevelopment, suggesting a sub-molecular effect underlying phenotype heterogeneity, that is, different domains potentially played distinct functional roles and thus were associated with different clinical phenotypes. Biallelic missense variants, which were inherited from unaffected parents and presented low allele frequencies in the general population, were associated with seizures without neurological abnormalities. Additionally, heterozygous and homozygous truncation variants were related to intractable seizures with severe developmental delay, suggesting a genotype-phenotype association.

IFIH1, which serves as a cytoplasmic sensor for viral nucleic acids, belongs to the retinoic acid inducible gene-I receptor family.[35] It plays a crucial role in detecting viral infections and initiating a series of antiviral responses, such as the induction of type I interferons and pro-inflammatory cytokines (PICs).[29] PICs are crucial inflammatory mediators and play a pivotal role in neuroinflammation and epileptogenesis.[36 37] Moreover, PICs have also been found to be related to epileptogenic mediators such as glutamate, which may result in brain hyperexcitation and seizures.[38] Clinically, *IFIH1* variants have been found to be associated with a spectrum of immune phenotypes, such as AGS7, SGMRT1 and immunodeficiency.[26 39–41] Seizures were occasionally observed in patients with *IFIH1* variants. The present study identified *IFIH1* variants in five unrelated cases with generalised epilepsy with antecedent febrile seizures and without neurological abnormalities, indicating that *IFIH1* variants are potentially associated with generalised epilepsy with antecedent febrile seizures, expanding the phenotype spectrum of *IFIH1*.

The IFIH1 protein comprises several crucial domains (CARD, Hel, P and CTD). Previously reported de novo heterozygous missense variants associated with epilepsy clustered within the Hel domains. In addition to epilepsy, these variants also result in multiple neurological abnormalities. In contrast, de novo heterozygous missense variants outside the Hel domain, including one variant in the P domain reported in the literature[42] and two variants in the CTD domain in the present study, were associated with epilepsy with/without neurodevelopmental delay. These findings suggested a regional sub-molecular effect of *IFIH1* variants, that is, de novo heterozygous missense variants in different domains potentially resulted in distinct functional damage and thus were associated with different clinical phenotypes. The Hel region is the core functional part of the IFIH1 protein, which recognises

dsRNA and hydrolyses ATP. Previous studies showed that variants in the Hel region resulted in 4-fold to 10-fold higher levels of basal signalling activity, even in the absence of exogenous ligand,[25] potentially explained the associated phenotypes of seizures and multiple neurological abnormalities.

The minor allele frequency (MAF) in general populations is critical in the pathogenicity evaluation of genetic variants. Generally, de novo heterozygous variants that are associated with dominant genetic disorders are absent in general populations; in contrast, a single heterozygous variant in the paired compound heterozygotes or homozygotes usually present a low MAF, suggesting a mild damage of the single variant. In the present study, among *IFIH1* variants in the same Hel region, biallelic variants inherited from unaffected parents and presenting low MAF in the general population were associated with a relatively mild phenotype and good prognosis. In contrast, de novo heterozygous variants with no MAF were associated with seizures and multiple neurological abnormalities, potentially attributed to the severity of genetic damage caused by these variants.

The three previously reported cases with epilepsy with *IFIH1* truncation variants presented with severe developmental delay. It was noted that in patients with *IFIH1* truncation variants without epilepsy, their clinical manifestations varied significantly, ranging from late-onset dystonia parkinsonism[43] to infant-onset paediatric rheumatology,[44] and to very early onset inflammatory bowel disease.[26] Particularly, the patient with a homozygous truncation variant exhibited neonatal-onset life-threatening enteropathy and multiple viral and bacterial infections during the first year of life. However, after the age of 1, she did not encounter any further episodes of infectious diseases, and her development was normal.[26] One of the explanations might be that the damage to innate immunity was alleviated by the development of adaptive antigen-specific lymphocyte responses over time.[45–47] The present study showed that *IFIH1* expression was predominantly observed in the neonatal stage, decreased dramatically during the juvenile and adult stages, consistent with the transitionary process and remission of *IFIH1*-associated immune diseases.

Our recent study showed that the natural course and outcome of diseases are correlated with the stage of genetic expression (dependent stage).[33 34] In the present study, the patients with generalised epilepsy showed an early onset age between 1 and 4 years and achieved seizure freedom with a favourable outcome. Studies on the temporal expression profile in the brain showed that *IFIH1* was highly expressed before 10 years and decreased dramatically in adulthood, which is potentially one of the explanations for the favourable outcomes. Considering that *IFIH1* expression in the brain was slightly increased later around 40 years of age, attention should be paid to patients with late-onset epilepsy and immune disorders.

This study has several limitations. The functional effects of the identified variants were not examined. There is evidence suggesting that the pathogenic mechanism may involve loss-of-function (LOF). First, the three truncation variants are expected to terminate the IFIH1 protein, leading to LOF.[12 25] Second, compound heterozygous variants are highly likely to result in LOF. Further studies are needed to verify the functional alteration of the variants. Lastly, the sample size of mutated patients was limited, underscoring the need for large cohorts to validate the pathological role of *IFIH1* variants in epilepsy.

CONCLUSION

IFIH1 variants are potentially associated with generalised epilepsy with antecedent febrile seizures and good prognosis. Disclosing the sub-molecular effects and validation of *IFIH1*-epilepsy association is urgent needed in further studies and large cohorts, which help to understand the underlying mechanisms of phenotypic variation and help the early genetic diagnosis of patients with *IFIH1* variants.

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