Supplementary Material

The genetic spectrum of febrile infection-related epilepsy syndrome (FIRES) and refractory status epilepticus

Overview of Supplementary Material

Supplementary Table 1. Individuals excluded from FIRES subgroup (n=34)

Diagnoses for individuals excluded from the FIRES cohort	**Individuals**	**Reason for exclusion**
Acute necrotizing encephalitis of unknown etiology	1	No fever prodrome, history of seizure 2 weeks prior
GEFS+	1	History of prior seizures
Angelman's syndrome	1	History of prior seizures
SCN2A	1	History of neonatal seizures, epilepsy
Acute necrotizing encephalitis with HHV6 virus, *RANBP2*	1	Did not have seizures
SCN1A	1	History of prior seizures
PCDH19	1	pre-existing history of seizures, prior to status presentation
Idiopathic epilepsy	4	History of prior seizures, did not have RSE
Pyruvate Carboxylase deficiency	1	Metabolic disorder
Hypoxic Ischemic Encephalopathy	2	Acquired acute symptomatic seizures
Epilepsia partialis continua	1	History of prior seizures
LIG3 mitochondrial encephalopathy	1	Did not have RSE
MOG encephalitis	1	Did not have seizures
Febrile status epilepticus	1	Fevers at onset of status epilepticus
MELAS	1	Long standing illness, prior seizures
CACNA1A	1	Febrile with seizure onset
FIRES	8	Remote history in another country lacking documentation *One patient with +GAD antibodies, another with history of HSV encephalitis*
NORSE*	6	Remote history in another country/lacking documentation

* See Supplementary Table 2 for individuals excluded with NORSE.

Supplementary Table 2. Individuals excluded from FIRES subgroup with NORSE (n=6)

Individual with NORSE	Findings	Sufficient documentation for chart review
1	*PCDH19*, recent vaccination for flu ; NORSE	No; consultation without outside records
2	NORSE	No; remote history in outside country without documentation
3	NORSE	No; Telephone consult at CHOP without outside records
4	NORSE/MOG encephalitis	Yes
5	NORSE	Yes
6	NORSE	Yes

There were 3 individuals with NORSE with sufficient clinical information, therefore further analyses and clinical data on NORSE were excluded from this study.

Supplementary Table 3. Genetic testing in our cohort of 25 individuals with FIRES

Participant	Year diagnosed with FIRES (age, years)	Single Gene	Karyotype	SNP Microarray	Epilepsy Gene Panel	Exome	Mitochondrial	Other
1	2010 (7.35 years)							
2	2012 (5.06 years)							
3	2012 (7.81 years)			Normal result				
4	2013 (11.9 years)	*SCN1A* sequencing report N/A for review			• *GAMT,* c.581T>C p.(V194A), heterozygous, inheritance unknown, VOUS			
5	2013 (9.21 years)			Normal result	• *NRXN1,* c.2627C>T p.(A876V), heterozygous, inheritance unknown, VOUS • *ZEB2,* c.808-4A>G p.? heterozygous, inheritance unknown, VOUS			
6	2013 (18.7 years)		Normal result	Normal result				
7	2013 (10.8 years)	*TWIST* sequencing: Negative	Normal result		Negative			
8	2014 (0.63 years)					• *SCN5A*, c.5477G>A p.(R1826H), heterozygous, inheritance unknown, pathogenic	• *HSPD1*, c.425G>A p.(A142L), heterozygous, VOUS • *DARS2,* c.228-20dupT, IVS2-20dupT, heterozygous, VOUS	Maturity Onset Diabetes of the Young (MODY) Panel: • *BLK,* c.26C>T p.(P9L), heterozygous, inheritance unknown, VOUS WES reanalysis: no additional variants
9	2014 (6.52 years)	*SCN1A* sequencing: Negative		Normal result		• *ITPR1,* c.187A>G p.(M63V), heterozygous, de novo, VOUS • *BTD*, c.1330G>C	Negative	

						p.(D444H), heterozygous, maternally inherited pathogenic • *CFTR*, c.1521_1523delCTT p.(F508del), heterozygous, maternally inherited, pathogenic • *COL18A1*, c.2824_2840delGGCCCCCCAGGCCCCCC, heterozygous, inheritance unknown, pathogenic • *POLRIC*, c.796delG heterozygous, paternally inherited, pathogenic		
10	2014 (3.39 years)			Normal result	• *PNKP*, c.1123G>T p.(G375W), heterozygous, inheritance unknown, likely pathogenic	Duo reanalysis • *CPA6*, c.919G>A p.(A307T), heterozygous, inheritance unknown, VOUS • *PNKP*, c.1123G>T p.(G375W), heterozygous, inheritance unknown, pathogenic	Negative	
11	2014 (8.47 years)	*POLG* sequencing: Negative	Normal result	Normal result	N/A for review			
12	2016 (4.96 years)				• *CNTN2*, c.2735C>T p.(P912L), heterozygous, inheritance unknown, VOUS			Humoral Dysfunction Panel: • *LRBA*, c.6695T>C p.(I2232T), heterozygous, inheritance unknown, VOUS
13	2016 (12.38 years)				Negative	N/A for review		

14	2016 (8.07 years)		Normal result		Negative	Negative	• MT-TK m.8342 G>A p.? approximately 3% heteroplasmy, likely pathogenic variant (extracted from brain tissue, approximately 4% heteroplasmy in the blood)	
15	2017 (2.39 years)	*CPT2* sequencing: Negative		Normal result	Negative	• *CPA6*, c.932G>A p.(R311Q), heterozygous, maternally inherited, VOUS	Negative	
16	2018 (5.95 years)		Normal result			Negative	• *MT-ND5* m.13154T>C p.(I273T), approximately 16%, maternally inherited, VOUS	
17	2018 (2.79 years)				Negative	Negative	• *ADCK4*, c.1336G>C p.(G446R), heterozygous, inheritance unknown, VOUS • *MRPS22*, c.766C>T p.(R256C), heterozygous, inheritance unknown, VOUS • *TYMP*, c.1207G>A p.(G403L), heterozygous, inheritance unknown, VOUS	WES Reanalysis: negative
18	2018 (6.61 years)	*SCN1A* sequencing: N/A for review *POLG* - sequencing:			• *PNPO*, c.527C>G p.(S176C), heterozygous, VOUS			

		N/A for review						
19	2019 (7.25 years)	*HLA-B*: Negative	Normal result	• 1.49Mb interstitial duplication within chromosome Xq23, inheritance unknown, VOUS		• *GRIN2D*, c.250G>C p.(V84L), heterozygous, maternally inherited, VOUS • *SPTBN5*, c.2366G>A p.(R789G), heterozygous, maternally inherited, VOUS • *SPTBN5*, c.749G>T p.(G250V), heterozygous, paternally inherited, VOUS		Fragile X: Negative
20	2019 (2.59 years)			Normal result	• *JMJD1C* c.3933T>C (silent mutation), het, VOUS			
21	2019 (9.84 years)					• ATP1A3, c. 719T>C p.(V240A), heterozygous, paternally inherited, VOUS	Negative	
22	2020 (17.0 years)				N/A for review	• *ADAM17*, c.1894G>A p.(V632I), heterozygous, inheritance unknown, VOUS • *ADAM17*, c.178C>A p.(L60I), heterozygous, inheritance unknown, VOUS • *CR2*, c.2518C>G p.(R840G), heterozygous, inheritance unknown, VOUS • *NTRK1*, c.1643G>A p.(R548Q), heterozygous,	Negative	

						inheritance unknown, VOUS		
23	2020 (6.88 years)		N/A for review		Negative	• *LPA*, c.2462C>T p.(P821L), heterozygous, paternally inherited, VOUS	Negative	
24	2020 (6.34 years)				• *DIAPH1*, c.2158C>T p.(L720F), heterozygous, paternally inherited, VOUS • *RANBP2*, c.1320A>T p.(L450F) heterozygous, maternally inherited, VOUS	• *FLG*, c.8911A>T p.(R2971*), heterozygous, paternally inherited, Pathogenic	Negative	
25	2021 (16.7 years)	*HBB* sequencing: • *HBB*, c.92+5G>C, Intronic, heterozygous, inheritance unknown, VOUS *HBA1:* Negative *HBA2:* Negative	Normal result	Normal result	• *CACNA2D2*, c.227G>A p.(R76H), heterozygous, inheritance unknown, VOUS • *CNTNAP2*, c.14C>T p.(P5L), heterozygous, inheritance unknown, VOUS • *GTPBP3*, c.1255G>A p.(G419S), heterozygous, inheritance unknown, VOUS • *PACS2*, c.2597T>C p.(V866A), heterozygous, inheritance unknown, VOUS • *PEX10*, c.845T>G p. (L282W), heterozygous, inheritance unknown, VOUS • *RELN*, c.576A>G (Silent), heterozygous, inheritance unknown, VOUS	• *DUOX2* c.2895_2898del p.(F966Sfs*29), heterozygous, inheritance unknown, Likely Pathogenic • *SLC9A7*, c.1985T>C p.(L662P), hemizygous, maternal inheritance, VOUS		

Supplementary Table 4. Individuals with RSE identified from CHOP cohort

Case	Age (Sex)	Clinical presentation	Gene and variant	Outcome
1	23m (F)	Refractory focal status epilepticus in the setting of metapneumovirus	*CACNA1A* p.Leu618Ser	Well-controlled epilepsy and mild developmental delay
2	4m (M)	Focal status epilepticus that was initially responsive to one dose of lorazepam, however within a couple hours his seizures recurred, requiring escalating doses of medication	*CACNA1A* p.Val1396Met	Refractory epilepsy, moderate global developmental delay, autism, ataxia, and hemiplegic migraine
3	11m (F)	Twins born from consanguinous parents. Both presented with refractory status epilepticus in the setting of a febrile illness. Both had acute brain injury with restricted diffusion and signal abnormalities in the brainstem and deep gray regions in the brain consistent with acute necrotizing encephalitis	*RANBP2* p.Asp2631Ala	Unknown
4	7m (F)		*RANBP2* p.Asp2631Ala	Developmental delay
5	4m (F)	Refractory clinical SE. Received several doses of anti-seizure medications, then was found to be in subclinical status epilepticus upon initiating EEG	*KCNA2* p.Pro405Leu	Neurotypical at last follow-up at 7m
6	6m (F)	RSE in the setting of fever after vaccine administration	*PCDH19* p.Thr404Ile	Unknown