Supplementary Text e-1: clinical case reports

Patient 1 (P1):

P1 was born to first cousins from Israel. She developed herpes simplex encephalitis (HSE) first at the age of eight months and then a second episode at 35 years. She recovered from the first episode but has since suffered from mental retardation and hemiparesis of the left side of the body. No CT or MRI scan was carried out during this episode. However, this infection probably affected the right hemisphere, as subsequent CT scans showed diffuse encephelomalacia in the right hemisphere, and this patient has suffered from left hemiplegia ever since. At the age of 35 years, she was hospitalized again due to HSE. PCR for HSV-1 in the cerebrospinal fluid (CSF) was positive. MRI showed severe brain damage, affecting the left temporal-parietal area and extending to the brainstem. The patient was treated intravenously with Zovriax (acyclovir), leading to recovery.

The patient is now 44 years old, and remains otherwise healthy. Her father and a sister and two brothers, who are 68, 46, 43, and 40 years old respectively, are heterozygous carriers of the same mutations found in the patient. No remarkable infectious disease courses have been noted from these family members.

Patient 2 (P2):

Patient 2 (P2) is a Turkish girl born to consanguineous parents. She developed HSE at the age of one year. She is now nine years old. She has remained healthy since her episode of HSE. No HSE or other remarkable infectious disease courses have been noted from other members of the family.

Patient 3 (P3):

Patient 3 (P3) was born to a non consanguineous French family. She developed HSE at the age of three years. Primary HSV-1 infection was confirmed by the appearance and augmentation of anti-HSV-1 IgG and IgM antibodies in the serum. The patient recovered with a sequela of left homonymous hemianopia, and modest epileptic syndrome with short loss of consciousness regularly happening during the years since her episode of HSE. At the ages of 31, she developed right eye retina necrosis with HSV-1 PCR positive, following an operation of retina complex detachment. She is now 43 years old. No other remarkable infectious disease courses have been noted.

Patient 4 (P4):

Patient 4 (P4) was born to a non-consanguineous Turkish family. She developed HSE at the age of one year. When she was nine-months old, herpetic lesions appeared on her mouth and one month later on her back (as herpetic whitlow). When she was twelve-months old, she was admitted into a regional general pediatrics clinic and diagnosed with herpes encephalitis with clinical and cerebrospinal fluid findings. HSV-1 PCR was positive in her CSF. She was treated with acyclovir, and was discharged in a good clinical status. However one month later, she was admitted again into hospital due to severe encephalitis syndrome including unconsciousness and convulsions. HSV-1 PCR was again positive for HSV-1. Anti-HSV-1 IgG and IgM were positive in the serum. Brain diffusion MRI and EEG showed bilateral temporal lesions. The patient was treat with phenobarbital, diphenylhidantoin, carbamazepin, lamotrigine, and foscavir 40 mg/kg/day for every 8 hours and weekly IVIG. On the 5 day of this therapy, clinical improvement was observed. The patient is now 6 years old, and has not developed other episodes of HSE, nor other severe infectious conditions.

Patient 5 (P5):

P5 was born to nonconsanguineous Finnish parents. He suffered from HSE at the ages of two and a half years, 22 years and 28 years. He presented hyperbilirubinemia during the first few days of his life. He had normal speech and motor development and no signs of chronic illness. He presented a first episode of HSE at the age of 2.5 years, with an acute onset of uncontrolled facial and ocular movements. By the afternoon of the day on which symptoms began, he became febrile, his speech was slurred and he developed generalized seizures. Viral encephalitis was suspected and intravenous acyclovir treatment was initiated. The patient was transferred to the pediatric intensive care unit of Oulu University Hospital (Oulu, Finland).

Electroencephalography (EEG) results were suggestive of HSE. Acute-phase CSF tested negative for HSV antibodies and the patient's white blood cell count was 3 x 10^6/L. Serum anti-HSV antibodies were undetectable on admission to hospital, but seroconversion for anti-HSV antibody was noted (1:128) one month later, in determinations with a quality-controlled in-house complement binding test. It was not possible to differentiate between anti-HSV1 and anti-HSV2 antibodies in this test. He developed aphasia during the episode of acute illness, but otherwise made a full recovery. He attended speech therapy sessions for several years, with good results. He attended regular school and, subsequently, began studying to become a car mechanic.

At the age of 22 years, he developed sudden epileptic seizures. Postictal EEG was normal, white blood cell counts were slightly high in the CSF (23 x 10^6/L), and the acute-phase CSF sample was negative for HSV nucleic acid. Brain CT and MRI scans showed lesions consistent with his history of HSE, with no evidence of acute infection. Two days later he developed high fever, headache, vomiting, confusion, somnolence, and repeated epileptic seizures. CSF analysis

showed high levels of protein (752 mg/mL), normal glucose concentration (3.1 mmol/L), and a high white blood cell count (296 x 10^6/L, 98% mononuclear). Bacterial cultures were negative and the CSF sample was positive for HSV1 nucleic acid. Acute-phase serum tested positive for IgG antibodies against HSV1. The patient was successfully treated with intravenous acyclovir for 14 days and was released from hospital in good condition. One month later, neuropsychological evaluation confirmed complete recovery from this second HSE episode and the patient's neurocognitive functions were similar to those of an age-matched control population.

At the age of 28 years, the patient had a low-grade fever for two days, followed by strong fever (39 °C), confusion and disorientation and, finally, generalized epileptic seizures. The acute-phase brain CT scan was unremarkable. Lumbar puncture revealed a high white blood cell count (388 x 10^6/L, 96% mononuclear) and the CSF sample tested positive for HSV1 nucleic acid. EEG results were abnormal, with left parieto-occipital disturbance consistent with HSE. The patient received intravenous acyclovir for 14 days and was released from hospital in good general condition. Two months later, neuropsychological evaluation revealed a mild deterioration of his cognitive functions with respect to previous findings. The patient has been treated with prophylactic oral acyclovir ever since.

The patient's father is currently 59 years old and has the same heterozygous TLR3 mutation as the patient, without developing HSE or other remarkable infectious disease courses till present.

Patient 6 (P6)

P6 was born to nonconsanguineous Finnish parents. He suffered a first episode of HSE at the age of 24 years. A tonic-clonic seizure occurred at onset and he remained confused and

agitated until the next day. Brain CT scan was normal, but the cerebrospinal fluid (CSF) displayed pleocytosis (42 x 10^6/l leukocytes, 95% monocytes). He received Intravenous fluctuating problems relating to memory and behavioral control (impulsiveness, impertinence). CSF leukocyte/total lymphocyte counts were 422 x10^6/L on the 7th day of treatment and 166 x10^6/l on the 15th day of treatment. Anti-HSV1 IgG antibodies were detected in the CSF on the 15th day of treatment.

The patient recovered well without neuropsycological sequelae until his first unprovoked seizure at the age of 28 years. CSF showed normal cell numbers and brain MRI showed right temporal polar and mesial atrophy and gliosis. Antiepileptic drug (AED) treatment was initiated with carbamazepine and no further seizures were observed until the patient developed severe hyponatremia in a triathlon race one year later. The AED was changed to valproate. Tonic-clonic seizures initially continued to occur about once every two months, their frequency subsequently decreasing to once every two to three years after the introduction of combination treatment with high-dose lamotrigine. In addition, seizures restricted to an uncomfortable feeling of fear and nausea continued twice to four times/monthly, with no response to either levetiracetam or topiramate add-on treatment.

A 3T MRI scan at the age of 36 years showed the right hippocampus to have a less well-defined internal structure than the left hippocampus. On video EEG recordings, ictal EEG activity was detected in the right frontotemporal electrodes, correlating with clinical seizure signs. Standard resection of the right temporal lobe was carried out at the age of 37 years, without complications. Histopathological investigation revealed inflammation, atrophy and gliosis in the anterolateral neocortical sample, with mild inflammation and local focal atrophy in

the hippocampus. Subsequent HSV PCR on paraffin-embedded tissue was positive for the neocortical sample, but negative for the hippocampal sample.

The patient was treated with valacyclovir (1000 mg x 3 p.o.) thereafter, with continuation of the AED prescribed before surgery. He has remained seizure-free. Neuropsychological examinations six months and two years after surgery showed an absence of change with respect to the presurgical evaluation. Minor problems were found in the maintenance of attention and executive functions, with impulsiveness and rigidity/inflexibility. Verbal intelligence was well above average, and visual intelligence remained average-high, with problems in more demanding visuoconstructive tasks and executive functions. Basic immunological evaluation revealed an absence of abnormalities.

The sister of the patient is homozygous for the same TLR3 mutation as found in P6. She is currently 39 years old, with no remarkable infectious disease courses recorded.

Supplementary Text e-2: No mutations found in other TLR3 pathway genes through whole exome sequencing

In addition to Sanger sequence all exons of *TLR3*, we further performed WES in the six patients investigated in this study, to check for mutations in other known TLR3 pathway genes. WES revealed a total of 373, 232, 413, 564, 257 and 438 rare nonsynonymous variations (with a MAF <0.001 in EVS, <0.001 in dbSNP, <0.001 in our in-house WES database for non-HSE patients, and not seen in 1000 genomes), in a total of 347, 220, 390, 542, 257 and 426 genes, in P1, P2, P3, P4, P5 and P6, respectively (Supplementary Table e-1). For each patient, we applied the recently developed human gene connectome (HGC) method [1], to prioritize these genes according to their predicted biological distance from *TLR3*. No mutation was identified in the top 1% of genes

(which comprises 120 genes) in the *TLR3* connectome and with previously reported experimental evidence (which includes all six known HSE-causing genes), with the exception of *TLR3* itself, on WES of the six patients. We also carried out a more detailed study of the WES results of P1 and P2, who were born to consanguineous families unrelated to each other, to search for any possible HSE-relevant homozygous mutations, as homozygous mutations are frequently found in patients from consanguineous families. WES showed that three genes in P1 and five genes in P2 harbored in each one homozygous rare missense variant of reliable sequencing quality (Supplementary Table e-2). None of these genes has been reported to be specifically involved in host antiviral immunity. Neither do they have any particular pattern of expression in the CNS. Thus, the possible pathogenic role of these mutations remains unclear.

Reference:

1. Itan Y, Zhang SY, Vogt G, et al. The human gene connectome as a map of short cuts for morbid allele discovery. *Proc Natl Acad Sci U S A* 2013; **110**(14): 5558-5563.

Supplementary Table e-1. Number of genes harboring rare variants in the six patients, as revealed by whole-exome sequencing

			Number of variants			Inheritance model		
Patient	Number of genes	Number of variants	Sub	Del	Ins	He	'Comp'	Ho
P1	347	373	366	5	2	355	25	18
P2	220	232	226	6	0	221	10	11
P3	390	413	389	13	11	375	30	38
P4	542	564	559	3	2	556	30	8
P5	257	257	251	4	2	248	4	9
P6	426	438	425	7	6	431	32	7

Note: Variants were considered rare if they were not seen in 1000Genome, and had a MAF <0.001 in EVS, <0.001 in dbSNP, and <0.001 in our in-house WES database of non-HSE patients.

Supplementary Table e-2. List of genes possibly harboring rare homozygous variants in P1 and P2, as revealed by whole-exome sequencing

	Genes harboring rare homozygous variants in P1	Genes harboring rare homozygous variants in P2
Nonsense (stop gained)	*none*	*none*
Readthrough (stop lost)	*none*	*none*
Missense	ZP3 AKAP9 TCOF1	LY75; CCDC140 MAP2K1; POLG ZNRF4
Silent	ME2	none
Frameshift	*none*	*none*
Inframe	*none*	*none*
UTR	*none*	CALML4
Splice	EPHB4; ME2 FUK; TAP2 RAPGEF3	*none*
ncRNA	*none*	*none*

Note: * Variants were considered rare if they were not seen in 1000Genome, and had a MAF <0.001 in EVS, <0.001 in dbSNP, and <0.001 in our in-house WES database of non-HSE patients.

* * Based on WES results shown in Supplementary Tables 1 and 2, homozygous variations were further checked individually for their sequence quality. Only when the mutant allele is present in >80% of the reads, out of at least five reads detected in the sequenced region of a gene, this gene was considered to possibly carry a homozygous variation and presented in this table.