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Genetic cause of epilepsy in a Greek cohort of children and young adults with heterogeneous epilepsy syndromes

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ABSTRACT

We describe a cohort of 10 unrelated Greek patients (4 females, 6 males; median age 6.5 years, range 2–18 years) with heterogeneous epilepsy syndromes with a genetic basis. In these patients, causative genetic variants, including two novel ones, were identified in 9 known epilepsy-related genes through whole exome sequencing. A patient with glycine encephalopathy was a compound heterozygote for the p.Arg222Cys and the p.Ser77Leu *AMT* variant. A patient affected with Lafora disease carried the homozygous p.Arg171His *EPM2A* variant. A *de novo* heterozygous variant in the *GABRG2* gene (p.Pro282Thr) was found in one patient and a pathogenic variant in the *GRIN2B* gene (p.Gly820Val) in another patient. Infantile-onset lactic acidosis with seizures was associated with the p.Arg446Ter *PDHX* gene variant in one patient. In two additional epilepsy patients, the p.Ala1662Val and the novel non-sense p.Phe1330Ter *SCN1A* gene variants were found. Finally, in 3 patients we observed a novel heterozygous missense variant in *SCN2A* (p.Ala1874Thr), a heterozygous splice site variant in *SLC2A1* (c.517-2A>G), as a cause of Glut1 deficiency syndrome, and a pathogenic variant in *STXBP1* (p.Arg292Leu), respectively. In half of our cases (patients with variants in the *GRIN2B*, *SCN1A*, *SCN2A* and *SLC2A1* genes), a genetic cause with potential management implications was identified.

1. Introduction

An increasing number of genes is being associated with epilepsy, leading to a deeper understanding of epileptogenesis [1–5]. In genetically determined epilepsies, genotype-phenotype correlations are complex since variants in different genes can cause the same epilepsy syndrome and, inversely, pathogenic variants in the same gene can cause heterogeneous phenotypes [3,6]. Thus, based on the electroclinical, imaging and laboratory findings, it is not always feasible to predict the gene implicated in a genetic epilepsy syndrome and guide targeted sequencing. Next generation sequencing technologies, including whole exome sequencing (WES), have revolutionized the clinical practice of neurology, and especially epileptology, by allowing affordable concurrent sequencing of thousands of genes [4].

This improved ability for an accurate genetic diagnosis is important in clinical decision-making [1,7]. For example, patients diagnosed with the Glut1 deficiency syndrome due to variants in the *SCL2A1* gene could benefit from receiving the ketogenic diet [8]. Also, patients with Dravet syndrome due to *SCN1A* gene variants

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should avoid sodium channel blocking antiseizure medication, which, in contrast, are useful in early-onset encephalopathy due to variants in the *SCN8A* gene [8]. Furthermore, even when a treatable genetic cause of epilepsy is not identified, diagnostic certainty spares the patients and their families from a host of non-diagnostic tests, allows prognosis estimation, and ultimately improves overall patient care.

Our aim was to study the contribution, practicality, and efficiency of WES, as a straightforward approach, in the diagnosis and management of a Greek cohort of children and young adults with epilepsy/epileptic encephalopathy (epilepsy manifesting as developmental and/or epileptic encephalopathy). The results in our cohort extend further the genotypic and phenotypic spectrum of pediatric epilepsies, by identifying two novel causative variants (in the *SCN1A* and *SCN2A* genes, respectively), adding new phenotypic information on already described pathogenic variants, and showing that in half of our cases (patients with variants in the *GRIN2B*, *SCN1A*, *SCN2A* and *SLC2A1* genes) genetic findings were associated with potential management implications.

2. Patients and methods

2.1. Study subjects

Out of 44 consecutive patients with epilepsy/epileptic encephalopathy (21 females, 23 males, median age 6.5 years, range 0.5–30 years), with or without concurrent developmental delay, referred to the Neurology/Neurogenetics Laboratory of the University of Crete, Medical School, we included in this study 10 patients from 10 unrelated non-consanguineous families with a diagnostic result through WES. All 44 patients were suspected to suffer from genetic epilepsies, based on their clinical presentation, electrophysiological studies, and absence of known acquired causes of epilepsy. Patients were recruited from different clinical centers across Greece (Thessaloniki, Heraklion Crete, Athens). Informed consent was obtained from the patients and/or their legal guardians. The study protocol was approved by the Institutional Review Board of the University Hospital of Heraklion, Crete, Greece.

2.2. Whole exome sequencing

Since extensive work-up in our patients failed to identify an acquired cause of epilepsy, and genetic origin of the epilepsy syndrome was suspected, we proceeded to WES for these patients as the preferred approach. Due to the multiplicity of genes associated with epilepsy, the sequential targeted gene sequencing approach would be expensive and inefficient. Furthermore, an epilepsy gene panel approach would not allow identification, future re-analysis of the data, and new gene variants not included in panels.

Genomic DNA was extracted from peripheral blood. WES and initial bioinformatics analysis were performed in a CLIA-certified laboratory (Otogenetics Corporation, Norcross, GA, USA). Exome target enrichment was performed with the Agilent V5 (51 Mb) Sure-Select Target Enrichment System. For exome sequencing, a HiSeq 2500 (Illumina, USA) platform was used, with paired-ended reads of 100–125 bp and estimated average coverage of 50X.

The data were further analyzed using the Ingenuity Variant Analysis (IVA) software (Qiagen, USA), at the Neurology Laboratory, University of Crete. Initially, variants were filtered for minor allele frequencies < 0.1%. We then focused on the protein-altering variants that matched the patient's phenotype, based on literature and online database search. Confirmatory Sanger sequencing was performed for identified variants.

3. Cohort description (Tables 1-3, supplemental Information)

In this Greek cohort of 10 patients with epilepsy/epileptic encephalopathy (4 females, 6 males; median age 6.5 years, range 2–18 years) and a broad range of phenotypes, with or without developmental delay, we identified causative variants in the *AMT, EPM2A, GABRG2, GRIN2B, PDHX, SCN1A* (2 patients), *SCN2A, SLC2A1* and *STXBP1* genes, respectively. The types of seizures in our patient cohort were very heterogeneous, both between patients and even between time periods for the same patient (Table 1). Concerning additional clinical features and outcomes, 8 of the 10 patients showed developmental delay (Table 1), while of the remaining 2 patients, one with Lafora disease developed cognitive decline after normal development. In 8 of the patients, we observed hypotonia and movement disorders, including extrapyramidal syndrome, choreoathetosis and ocular motility disorder (Table 1). Only the patient with the *SCN2A* variant had a past family history of a similar neurologic disorder in his brother (Table 1). All 10 patients had received multiple antiseizure medications and in 4 of them the ketogenic diet was attempted, with mixed treatment results (Table 1).

EEG findings were quite variable, both between patients and in the same patient at different ages and under different treatment modalities (Table 2). For example, as shown in Fig. 1 for patient #5 (with the *PDHX* pathogenic variant) the EEG features of hypsarrhythmia improved after the initiation of the ketogenic diet. In three of the patients, MRI brain scans were unrevealing. In the remaining patients, imaging findings ranged from mild brain atrophy to severe structural lesions (e.g. hematomas) and developmental abnormalities (e.g. delayed myelination and dysgenesis of corpus callosum; Table 2). For instance, brain MRI of patient # 5 (harboring the p.Arg446Ter *PDHX* gene pathogenic variant in homozygous state) showed corpus callosum dysgenesis, mild dilatation of the occipital horns bilaterally and adjacent leukoencephalopathy (Fig. 2). Brain MRI for patient #1 (with the *AMT* gene variants) showed intraventricular hemorrhage, subdural hematomas, ventriculomegaly, delayed myelination, bi-thalamic lesions and atrophy and abnormal signal intensity at the internal capsule, as well as a glycine peak in MR spectroscopy (Fig. 3). In 2 of the patients, prominent metabolic abnormalities were noted: elevated CSF/plasma glycine and lactic acidosis in the patients with the *AMT* and the *PDHX* variants, respectively (Table 2). Finally, for patient #2, the diagnosis of Lafora disease due to the pathogenic p. Arg171His *EPM2A* gene variant, was verified by an axillary biopsy that revealed Lafora bodies (PAS positive intracytoplasmic inclusions) in the cells lining the apocrine sweat glands (Fig. 4).

4. Discussion

In this work, we identified the cause of epilepsy/epileptic encephalopathy in a Greek cohort of 10 pediatric patients, across a broad range of phenotypes. Specifically, we found pathogenic variants in the following genes: *AMT*, *EPM2A*, *GABRG2, GRIN2B, PDHX, SCN1A* (2 patients), *SCN2A*, *SLC2A1* and *STXBP1*, as the cause of epilepsy in this cohort with heterogeneous phenotypic manifestations Table 3. Two variants, the p.Phe1330Ter *SCN1A* and the p. Ala1874Thr *SCN2A* gene variants were novel, adding to the genotypic spectrum of genetic epilepsy. Even though there is evidence [9] that in a sizeable proportion of patients analyzed with WES there could be multiple molecular diagnoses (i.e. pathogenic variants in more than one gene underlying the disease phenotype), causative variants in a single gene were identified in our patients. In the patients with homozygous causative genetic variants (in the *EPM2A* and *PDHX* genes, respectively), consanguinity was consid-

Table 1
Summary of clinical features and family history of patients included in the study.

Patient #	Sex	Age at WES diagnosis	Age at onset of epilepsy	Type of seizures	Frequency of Seizures	Additional clinical features	Epilepsy/ epilepsy syndrome	Family History	Anti-seizure medications used	Outcome (seizures)
1	F	6 mo.	Neonate	Tonic spasms in clusters	Multiple per day	Encephalopathy, hypotonia	Ohtahara syndrome	No	Levetiracetam, clobazam, valproic acid, phenobarbital, zonisamide	Developmental delay (improvement of seizures)
2	F	18 y.	14 y.	Generalized tonic clonic, absences, myoclonic	Multiple per day	Visual hallucinations, myoclonus	Progressive myoclonus epilepsy	No	Levetiracetam, valproate, lamotrigine, clobazam, topiramate, ketogenic diet, zonisamide, phenobarbital	Dementia (partial response of seizures)
3	M	18 mo.	4 mo.	Infantile spasms	Multiple per day	Encephalopathy, hypotonia, abnormal ocular motility, nystagmus, extrapyramidal syndrome	Early infantile epileptic encephalopathy	No	Vigabatrin, steroids, valproic acid, pyridoxal phosphate	Developmental delay (improvement of seizures)
4	F	17.5 y.	Neonate	Tonic spasms, myoclonic	Multiple per day	Encephalopathy, hypotonia	Early infantile epileptic encephalopathy	No	Vigabatrin, ACTH, Valproate	Developmental delay (improvement of seizures)
5	F	2 y.	Neonate	Early spasms	Multiple per day	Encephalopathy, hypotonia	Early infantile epileptic encephalopathy	No	Vigabatrin, high dose prednisone, ketogenic diet	Developmental delay (seizure free with ketogenic diet) (KD)
6	M	18 mo.	5.5 mo.	Generalized tonic clonic	Variable	Mild early hypotonia	Generalized	No	Phenobarbital, oxcarbazepine, levetiracetam, valproate	Normal development (partial seizure control)
7	M	15 y.	8 mo.	Generalized, focal to bilateral tonic clonic	High	-	Unclassified	No	Valproate, tpopiramate, lamotrigine, ketogenic diet	Developmental delay (poor outcome of seizure control)
8	M	13 y.	5 mo.	Focal seizures, tonic, myoclonic	High	Hypotonia	Early infantile epileptic encephalopathy	Yes (brother)	Levetiracetam, valproic acid, ketogenic diet	Developmental delay (poor outcome of seizure control)
9	M	15 y.	3 mo.	Non motor behavior arrest, myoclonic, atonic	High	-	Myoclonic-astatic epilepsy (Doose syndrome)	*No*	Valproate, levetiracetam,topiramate, lamotrigine, vigabatrin, clobazam, rufinamide, ethosuximide, ketogenic diet	Developmental delay (poor outcome of seizure control)
10	M	5 y.	Unknown	Focal tonic spasms, myoclonic, generalized tonic clonic	Multiple per day	Macrosomia, axial hypotonia, limb hypertonia, movement disorder (dyskinesia, choreoathetosis)	Early infantile epileptic encephalopathy	*No*	ACTH, prednisone, valproate, topiramate	Developmental delay (poor seizure control)

Abbreviations: mo.: months, y.: years, ACTH: adrenocorticotropic hormone.

ered as a possibility; however, we were not able to identify parental relationships using family history.

In most cases, we were able to reach a diagnosis in children and adolescents that had not been formally diagnosed, despite several non-diagnostic tests, including metabolic studies, EEG recordings, brain imaging and cerebrospinal fluid analyses. In addition, in all patients, genetic diagnosis led to management adjustments, including genetic counseling, abandonment of unnecessary diagnostic tests and treatments and establishment of targeted therapies and follow-up.

There have been several recent studies using a WES approach to investigate patients with presumed genetic epilepsy, either isolated or with concomitant developmental/neurocognitive delay [10–12]. The diagnostic yield in these studies ranges from 10 to 25% [12–15], similarly to the 22.7% observed in our study (10 out of 44 patients diagnosed with a causative variant). Among the NGS methods used in the diagnostic investigation of genetic epilepsy, WES has the advantage of more extended coverage compared to gene panels, without significant increase in cost, allowing the detection of variants in not yet described epilepsy genes in future re-analyses [10]. Also, it has a greater diagnostic potential compared to chromosomal micro-arrays, that target exclusively copy number variants [10]. With the wider availability and the decreasing cost of whole genome sequencing in the future, it is predicted that it will surpass WES for the genetic diagnosis of epilepsy; however, at present WES remains the preferred diagnostic method for most patients with epilepsy.

The clinical and biochemical diagnosis of glycine encephalopathy was confirmed by the identification of the p.Ser77Leu and p.Arg222Cys ***AMT*** gene variants in compound heterozygosity in **patient #1**. Pathogenic variants in the *AMT* gene cause epileptic encephalopathy associated with nonketotic hyperglycinemia [16]. In the typical neonatal form of the disease, patients show decreased level of consciousness, hypotonia, respiratory

Table 2
Summary of EEG, MRI and lab/biopsy results of patients included in the study.

Patient #	EEG	MRI	Lab/biopsy results
1	Burst suppression pattern	Intraventricular hemorrhage, subdural hematomas, ventriculomegaly. delayed myelination, bi-thalamic lesions, abnormal signal intensity/restricted diffusion at myelinated areas including the posterior limb of the internal capsule	Elevated plasma and CSF glycine
2	Encephalopathic pattern, generalized spike-wave complexes	Normal	Lafora bodies in axillary skin biopsy
3	Encephalopathic pattern	Cerebral atrophy	-
4	Poor organization, generalized sharp/wave complexes and slow sharp delta waves of high voltage, hypsarrhythmia	Delay of myelination, decrease in cerebral volume	-
5	Hypsarrhythmia	Corpus callosum dysgenesis, colpocephaly with dilatation of the occipital horns bilaterally	Lactic acidosis
6	Generalized sharp wave complexes	Unrevealing	-
7	Compatible with generalized epilepsy	Generalized atrophy, especially at frontal lobes	-
8	Interictal epileptic activity on left dorsal frontocentral region, midline	Abnormal periventricular and occipital white matter, old infarct of the left basal ganglia	-
9	Generalized sharp wave complexes	Hypoplasia of posterior part of corpus callosum.	-
10	Burst suppression pattern, hypsarrhythmia, bilateral occipital slow waves, focal epileptic discharges	Normal	-

Abbreviations: mo.: months, y.: years, MRI: Magnetic resonance Imaging, EEG: Electroencephalogram, CSF: cerebrospinal fluid.

insufficiency, drug-resistant seizures, and increased CSF glycine. Infantile and late-onset forms of nonketotic hyperglycinemia convey a better prognosis. The *AMT* gene encodes for an aminomethyltransferase that forms part of the mitochondrial tetrameric glycine cleavage system [17]. Concerning the *AMT* gene variants of our patient, the p.Ser77Leu variant has previously been found in two patients with neonatal nonketotic hyperglycinemia [18]. Moreover, the p.Arg222Cys *AMT* gene variant has also been found in two other patients with neonatal nonketotic hyperglycinemia [19].

WES identified the homozygous p.Arg171His ***EPM2A*** variant in **patient #2** with drug-resistant progressive myoclonic epilepsy and dementia. The diagnosis of Lafora disease was later confirmed by axillary biopsy and spared the patient from unnecessary treatment modalities. Lafora disease causes progressive myoclonic epilepsy presenting in adolescence and leading to death usually within a decade from symptom onset [20,21]. As in our patient, antiepileptic medications are of limited value in controlling intractable seizures [21]. The loss of function of laforin (encoded by the *EPM2A* gene) leads to the accumulation of poorly branched dysfunctional glycogen, forming Lafora bodies within neurons and other cells, such as apocrine gland cells in the axilla (Fig. 4). The p.Arg171His *EPM2A* variant found in our patient has been repeatedly described in the literature [22–26]. This variant resides in the dual-specificity phosphatase domain of laforin, leads to cytoplasmic clump formation, and decreases phosphatase activity against glycogen [22,27,28].

WES linked the heterozygous *de novo* p.Pro282Thr ***GABRG2*** pathogenic variant to epileptic encephalopathy in **patient #3**. Variants in this gene are associated with autosomal dominant generalized epilepsy with febrile seizures plus and early infantile epileptic encephalopathy [29,30]. The *GABRG2* gene encodes for the γ subunit of the $GABA_A$ heteromeric ion channel receptors, composed of five subunits surrounding a chloride channel [31]. The $GABA_A$ receptors are the main inhibitory human brain neurotransmitter receptors. They are the target of barbiturates and benzodiazepines, used as antiepileptics, sedatives and anxiolytics [31]. The p.Pro282Thr variant, as a *de novo* occurrence, has recently been described in a 12-year-old girl with drug-resistant Lennox-type epilepsy, developmental delay, dysmorphic features, hypotonia, nystagmus and cerebral atrophy on MRI [32], a clinical picture similar to that of our patient. The p.Pro282Thr variant, which results in a non-conservative amino acid substitution, is located in a conserved position across species, in the pore region of the $GABA_A$ receptor and is predicted *in silico* to damage protein function (CADD score 27.2). Also, a pathogenic variant at the same residue (p.Pro282Ser), with proven profound functional consequences on the $GABA_A$ receptor, has been described in a 10-year old female patient with global developmental delay, hypotonia and drug-resistant seizures [30].

The p.Gly820Val ***GRIN2B*** pathogenic variant was identified in **patient #4**. Pathogenic *GRIN2B* variants cause autosomal dominant epileptic encephalopathy and other phenotypes, collectively known as *GRIN2B*-related neurodevelopmental disorders [33–38].

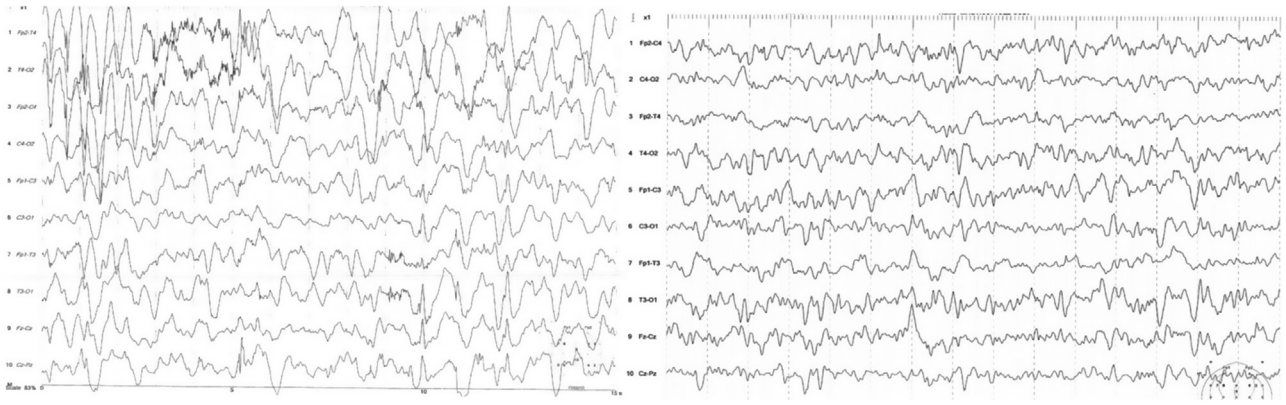

Fig. 1. EEG recordings of patient # 5. A. Hypsarrhythmia pattern before initiation of ketogenic diet (KD). B. Improvement of the hypsarrhythmia pattern 2 years after initiation of KD. WES revealed the p.Arg446Ter (c.1336C>T) *PDHX* gene pathogenic variant in homozygous state.

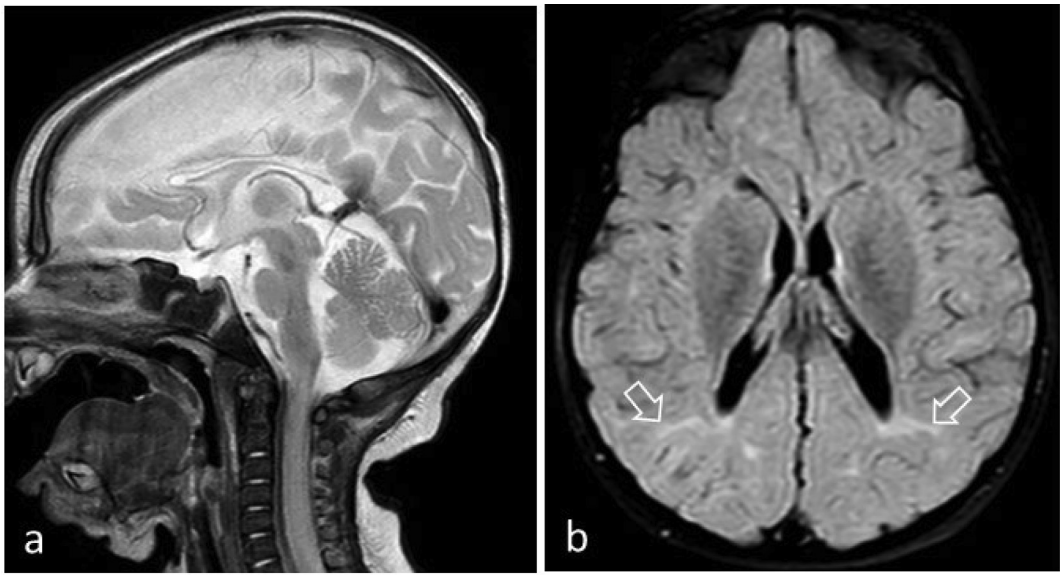

Fig. 2. MRI imaging of patient # 5 at 3 years of age. a. Sagittal T2-WI showing corpus callosum dysgenesis. b. Axial T2-FLAIR showing colpocephaly with mild dilatation of the occipital horns bilaterally and adjacent restricted leukoencephalopathy (arrows). WES revealed the p.Arg446Ter (c.1336C>T) *PDHX* gene pathogenic variant in homozygous state.

For patients with pathogenic variants in *GRIN2B*, therapy with N-methyl-D-aspartate (NMDA) receptor inhibitors e.g. by memantine may be of value [39]. The *GRIN2B* gene encodes for the glutamate binding NR2B subunit of the NMDA receptors, which are the main mediators of excitatory neurotransmission in human brain, being especially important for neuronal development and memory formation [34,35]. The p.Gly820Val *GRIN2B* variant found in our patient has already been described in the literature as a cause of epileptic encephalopathy [35,40,41]. Also, other changes at the same residue (p.Gly820Glu, p.Gly820Ala) have similar phenotypic manifestations [41–43]. The Gly820 residue lies in the 4th helix of the transmembrane domain of the NMDA receptor, in a highly conserved area where changes are expected to be functionally harmful.

The p.Arg446Ter ***PDHX*** pathogenic variant was found in **patient # 5** with lactic acidosis and seizures since birth. Variants in the *PDHX* gene have been associated with congenital lactic acidosis [44–47]. The *PDHX* gene encodes for component X of the pyruvate dehydrogenase (PDH) complex, a highly regulated complex interconnecting glycolytic processes with the tricarboxylic acid cycle, and thus playing a crucial role in energy homeostasis. The PDH complex catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA, CO_2 and NADH(H^+) through the sequential action of pyruvate dehydrogenase (E1), dihydrolipoamide acetyltransferase (E2) and dihydrolipoamide dehydrogenase (E3) [48], with component X functionally interconnecting the E2 and E3 enzymes. The p.Arg446Ter pathogenic variant results in a truncated protein product and has been initially described in two siblings from the UK [49] and a patient from France [47] showing neurodevelopmental delay and lactic acidosis. Later, it has been found in patients with congenital lactic acidosis, psychomotor delay, progressive encephalopathy and seizures from Bulgaria, Hungary, Romania and Slovakia showing that this is a common variant across Europe [50].

In patients **#6** and **#7**, WES revealed the p.Ala1662Val and p.Phe1330Ter ***SCN1A*** gene variants, respectively. *SCN1A* is the gene most commonly associated with genetic epilepsy, with more than 1200 pathogenic variants described thus far [51]. Variants in the *SCN1A* gene are associated with generalized epilepsy with febrile seizures plus, early infantile epileptic encephalopathy or Dravet syndrome, familial hemiplegic migraine and autism, among other phenotypes [52,53]. Also, the identification of a loss of function *SCN1A* variant could have therapeutic implications, since in this case sodium channel blockers should be avoided [39,54]. The *SCN1A* gene encodes for the large α-subunit of the neuronal voltage-gated sodium channel type 1 (NaV1.1) [52]. The six transmembrane domains of this α-subunit form the pore of the sodium channel, whereas the auxiliary β-subunits assist in the regulation of the channel properties and mediate its interaction with other proteins [52]. The p.Ala1662Val *SCN1A* variant found in our patient has already been described in a patient with severe myoclonic epilepsy of infancy [55]. This variant is in a mutational hot spot, with no benign variation. Also, it is part of a critical functional domain and *in silico* evidence suggests it is pathogenic (CADD = 28.5). In *patient #7*, the p.Phe1330Ter *SCN1A* gene variant was identified, that has not been previously described in the literature. However, it is predicted *in silico* to be deleterious. Also, it leads to a truncated protein product in a gene that loss of function due to similar variants is a known pathogenetic mechanism. In addition, the p.Phe1330Ter variant has not been found in normal individuals.

The p.Ala1874Thr ***SCN2A*** gene variant was identified in **patient # 8**. Variants in the *SCN2A* gene are known to cause benign familial neonatal infantile seizures, epilepsy of infancy with migrating focal seizures, epileptic encephalopathies, including Ohtahara syndrome, and other epileptic and non-epileptic phenotypes [56–58]. It is thought that gain of function mutations lead to early-onset (<3 months) epilepsy or epileptic encephalopathy, whereas loss of function variants are the cause of late-onset epilepsy or intellectual disability syndromes [54,59]. There is evidence that favors the aggressive use of sodium-channel blockers in patients with gain of function *SCN2A* variants and, inversely, the avoidance of these anti-epileptics in the case of loss of function variants [39,54,60]. The *SCN2A* gene encodes for the neuronal voltage-gated sodium channel NaV1.2, a paralog of the NaV1.1 channel described above, with which it shares a similar structure and role in neuronal action potential propagation [59]. The α-subunit encoded by the *SCN2A* gene forms the pore of the sodium channel, being assisted in its function by the β-subunits [59]. Concerning the *SCN2A* variant (c.5620G>A; p.Ala1874Thr) identified in our patient, it has not been previously described.

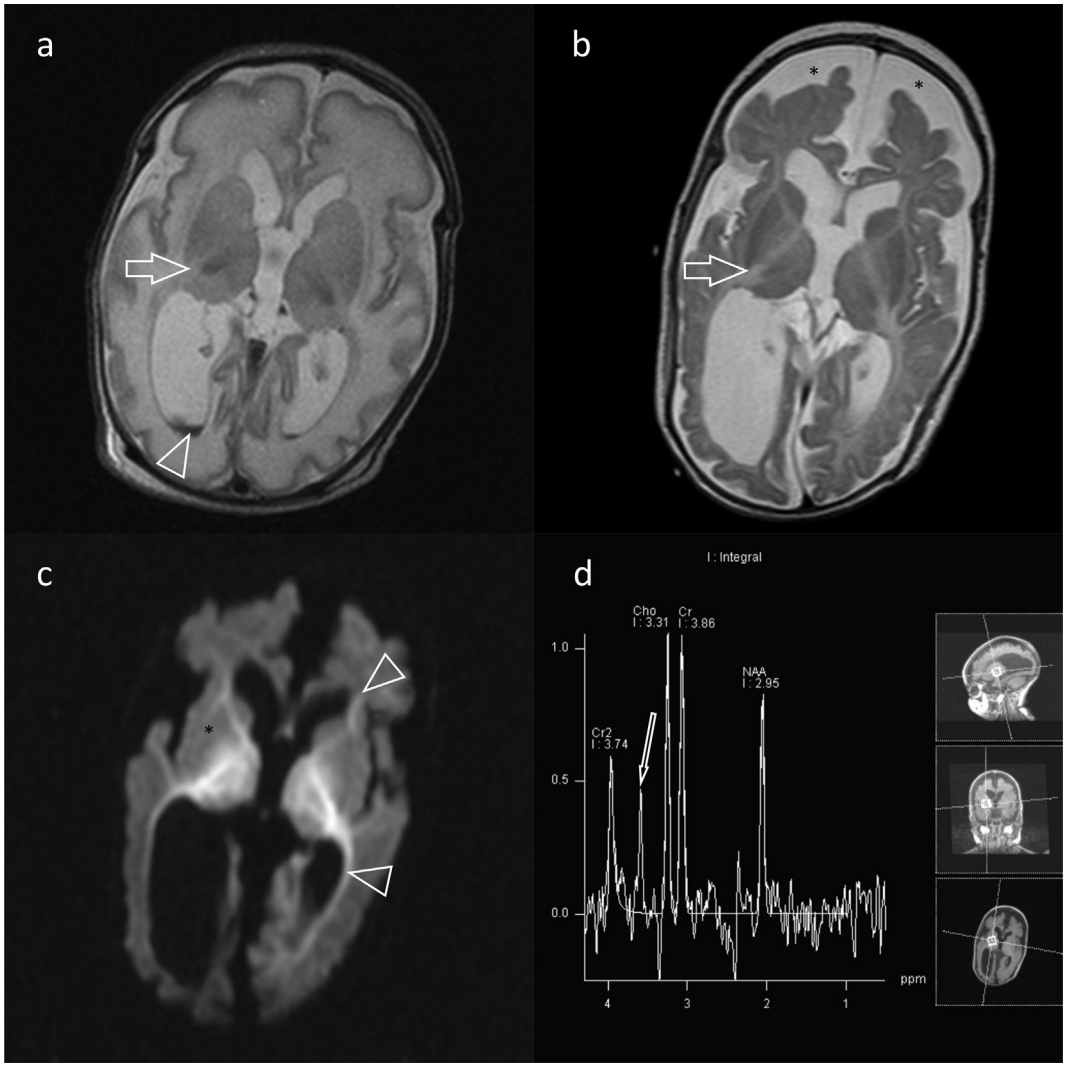

Fig. 3. MRI of patient #1 at various ages. a. T2-weighted sequence, axial image at age 13 days, shows "very white" white matter and a rather rounded cortex, consistent with a diffuse encephalopathy. Intraventricular haemorrhage (arrowhead) is a change frequently encountered in the vulnerable neonatal brain, especially in premature babies. Absence of hypointense posterior limb of internal capsule (PLIC) (arrow) was an early indication of leukoencephalopathy. b. T2-weighted sequence, axial image at age 3.5 months and with reduced head circumference, shows increased extra-axial cerebrospinal fluid space frontally (*) with dilated ventricular system, consistent with reduced brain volume. Note small hyperintense thalami and lack of myelination with persisting absence of PLIC (arrow). C. Diffusion sequence, axial plane at age 3.5 months. There is restricted diffusion at areas where myelinated white matter should exist. This is evident as hyperintensity at the internal capsules (*) and at periventricular white matter around the frontal and occipital horns (arrowheads). D. Spectroscopy at 145 TE shows a glycine peak at 3.35 ppm (arrow). The clinical, imaging and biochemical diagnosis of glycine encephalopathy was confirmed by the identification of the p.Arg222Cys (c.664C>T) and p.Ser77Leu (c.230C>T) *AMT* gene variants in compound heterozygosity.

However, it is a missense variant in a gene with a low rate of benign missense variants and strong computational evidence suggests a deleterious effect on the protein product (CADD = 22.1).

The heterozygous c.517-2A>G splice site variant in the ***SLC2A1*** gene was found in **patient #9** with myoclonic astatic epilepsy. Variants in the *SLC2A1* gene are associated with the Glut1 deficiency syndrome (infantile seizures, developmental delay, microcephaly, and ataxia), paroxysmal dyskinesia, absence epilepsies, myoclonic astatic epilepsy, episodic choreoathetosis, spasticity, and focal epilepsy [61–63]. In these Glut1 deficiency syndromes, ketogenic diet, when initiated early, leads to a favorable therapeutic response[39,62]. However, there have been cases where the ketogenic diet failed, either due to poor tolerability or true inefficiency, as was probably the case in our patient [64]. The *SLC2A1* gene encodes for Glut1 (glucose transporter type 1), which transports glucose across the blood-brain barrier. Since brain metabolism relies on glucose utilization, Glut1 is the most important energy carrier across the blood brain barrier. Glut1 deficiency due to *SLC2A1* pathogenic variants leads to poor availability of glucose, causing early-onset encephalopathy [65]. The c.517-2A>G *SLC2A1* gene variant leads to splice-site loss, in a gene that loss of function is a known pathogenetic mechanism. In addition, it is predicted *in silico* to be functionally deleterious (CADD = 34.0). The *SLC2A1* c.517-2A>G variant has been described before, again in heterozygous state, in a 4-year old patient with recurrent generalized tonic seizures and reversible brain white matter lesions [66].

Finally, WES identified the pathogenic p.Arg292Leu variant in the ***STXBP1*** gene as the cause of epileptic encephalopathy in

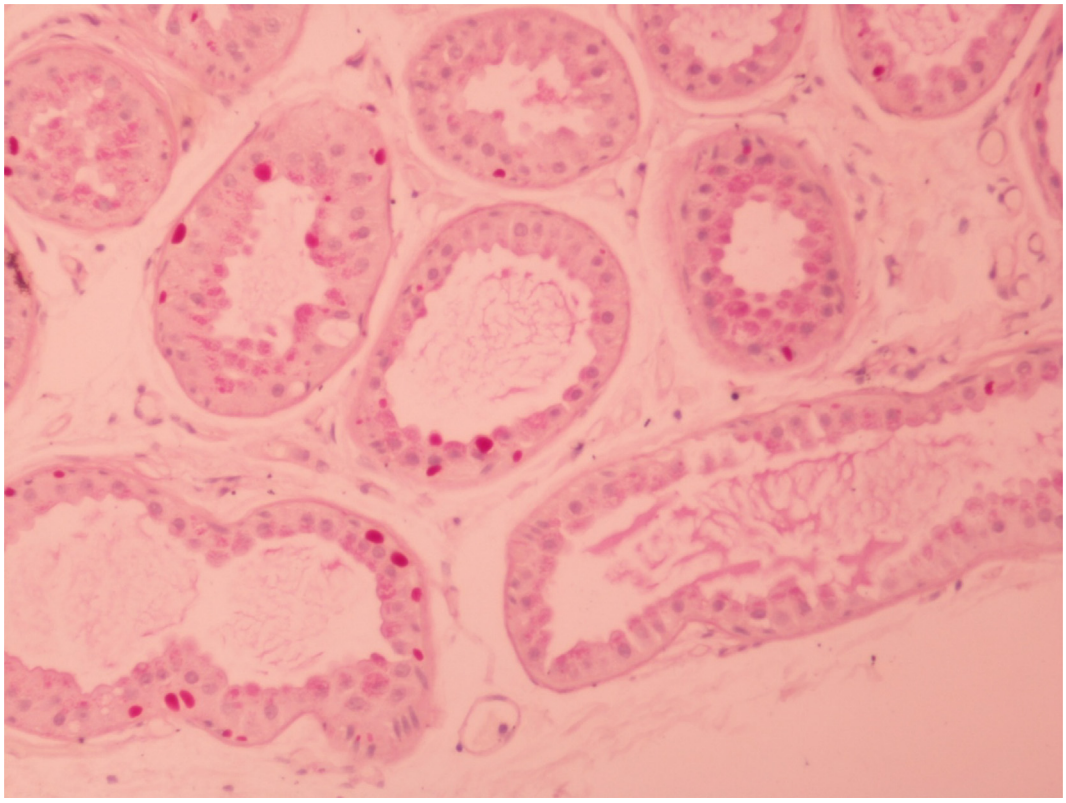

Fig. 4. Lafora bodies in axillary skin biopsy from patient #2. Lafora bodies (PAS positive intracytoplasmic inclusions) are found in the cells lining the apocrine sweat glands. In this patient, we identified by WES a homozygous pathogenic *EPM2A* variant (p.Arg171His; c.512G>A).

patient #10 [67,68]. The *STXB1* gene encodes for the syntaxin binding protein, which belongs to the SEC1 family of membrane trafficking proteins, contributes to synaptic vesicle release and may play a role in neurodegenerative disorders [69]. The p.Arg292Leu variant found in our patient has been described before as occurring *de novo* in several patients with epilepsy [70–72]. This variant has strong computational evidence favoring its pathogenicity (CADD = 25.4) and is located in a mutational hotspot, at an amino acid residue where another missense change has been characterized as pathogenic (p.Arg292His, ClinVar VCV000207424.2).

5. Conclusions

Overall, we identified disease causing variants in 9 epilepsy-related genes in 10 pediatric patients affected with epilepsy/epileptic encephalopathy, including two variants (one each in the *SCN1A* and *SCN2A* genes, respectively) that have not been previously described as causative for epilepsy, but have strong evidence favoring their pathogenicity. These 10 patients were part of a larger cohort of 44 patients with epilepsy/epileptic encephalopathy referred to us for WES, yielding a diagnostic rate of 22.7%. In half of our 10 diagnosed patients (5/10), bearing pathogenic variants in the *GRIN2B*, *SCN1A*, *SCN2A*, and *SLC2A1* genes, this improved diagnostic accuracy carried potential implications for their management. The final diagnosis obtained by WES, being either confirmatory or contributing to the diagnosis, spared all the patients and their families from unnecessary further investigations, inappropriate treatment approaches and offered the possibility of appropriate genetic counseling. Our results, in addition to those from other studies, further attest to the practicality and real-world utility of a straightforward approach involving WES, instead of targeted gene-sequencing or gene panel testing, as the initial genetic test in patients with epilepsy/epileptic encephalopathy of presumed genetic etiology.

Declarations of interest

None.

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Ethics statement

All authors meet the criteria for authorship stated in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals. Also, the authors have no relevant conflicts of interest and all contributors have read and approved the manuscript before submission.

Informed consent was obtained from the patients and/or their legal guardians. The study protocol was approved by the Institutional Review Board of the University Hospital of Heraklion, Crete, Greece.

Table 3
Summary of causative genetic variants included in this study.

Patient #	Gene	Variants	rs	Inheritance	CADD score	gnomAD frequency (%)	Pathogenicity evidence (ACMG criteria)	Epilepsy Type (Genetic Diagnosis)	Previously reported or novel
1	*AMT*	p.Ser77Leu (c.230C>T)/p.Arg222Cys (c.664C>T)	386833680/ 781466698	AR	24.0/ 32.0	≤0.001/ ≤0.001	PS4, PM2, PM3, PP3, PP5/PS4, PM1, PM3, PM5, PP3, PP5	Glycine encephalopathy (OMIM 605899)	[18,19]
2	*EPM2A*	p.Arg171His (c.512G>A)- homozygosity	137852916	AR	31.0	0.002	PS4, PM1, PM2, PP3, PP5	Epilepsy, progressive myoclonic 2A (Lafora) (OMIM 254780)	[24,25]
3	*GABRG2*	p.Pro282Thr (c.844C>A)	796052508	AD *(de novo)*	27.2	0.000	PM2, PP3	Early infantile epileptic encephalopathy type 74 (OMIM 618396)	[32]
4	*GRIN2B*	p.Gly820Val (c.2459G>T)	797044849	AD	26.1	0.000	PM2, PP2, PP3	Epileptic encephalopathy, early infantile, 27 (OMIM 616139)	[35,41]
5	*PDHX*	p.Arg446Ter (c.1336C>T)- homozygosity	1135402725	AR	38.0	≤0.001	PS4, PM2, PP3	Lactic acidemia due to PDX1 deficiency (OMIM 245349)	[44,45]
6	*SCN1A*	p.Ala1662Val (c.4985C>T)	794726839	AD	28.5	0.000	PM1, PM2, PM6, PP3, PP5	*SCN1A* gene (OMIIM 182389) related epilepsy	[55]
7	*SCN1A*	p.Phe1330Ter (c.3988_3989insGAGGTGATGGGATACCTTACCC)	-	AD	-	0.000	PVS1, PM2	*SCN1A* gene (OMIIM 182389) related epilepsy	Novel
8	*SCN2A*	p.Ala1874Thr (c.5620G>A)	753977894	AD	22.1	0.002	PP2, PP3/BS1	Epileptic encephalopathy, early infantile, 11 (OMIM 613721)	Novel
9	*SLC2A1*	c.517-2A>G (Splice site)	-	AD	34.0	0.000	PVS1, PM2	Glut1 deficiency syndrome (OMIM 612126, 606777)	[66]
10	*STXBP1*	p.Arg292Leu (c.875G>T)	796053361	AD	25.4	0.000	PS4, PM1, PM2, PM5, PM6, PP3	Epileptic encephalopathy, early infantile, 4 (OMIM 612164)	[70,71]

PS4 - The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.
PVS1 - Null variant in a gene where loss of function is a known mechanism of disease.
PM1 - Located in a mutational hot spot.
PM2 - Absent from controls (or at extremely low frequency if recessive) in gnomAD.
PM3 - For recessive disorders, detected in trans with a pathogenic variant.
PM5 - Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.
PM6 - Assumed de novo, but without confirmation of paternity and maternity.
PP2 - Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease.
PP3 - Multiple lines of computational evidence support a deleterious effect on the gene or gene product.
PP5 - Reputable source recently reports variant as pathogenic.
BS1 - Allele frequency is greater than expected for disorder.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebr.2021.100477.

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