Neurotherapeutics (2022) 19:1353–1367
https://doi.org/10.1007/s13311-022-01264-1

ORIGINAL ARTICLE

Impact of Genetic Testing on Therapeutic Decision-Making in Childhood-Onset Epilepsies—a Study in a Tertiary Epilepsy Center

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Accepted: 8 June 2022 / Published online: 20 June 2022

Abstract

We assessed the frequency of pediatric monogenic epilepsies and precision therapies at a tertiary epilepsy center. We analyzed medical records of children, born in 2006–2011 and followed at the Danish Epilepsy Center from January to December 2015; 357 patients were identified, of whom 27 without epilepsy and 35 with acquired brain damage were excluded. Of the remaining 295 children, 188 were consented for study inclusion and genetic testing. At inclusion, 86/188 had a preexisting genetic diagnosis and did not undergo further genetic testing. The 102 genetically unsolved patients underwent WES, which identified a (likely) pathogenic variant in eight patients and a highly relevant variant of unknown significance (VUS) in seven additional patients. Single nucleotide polymorphism array was performed in the remaining 87 patients and revealed no (likely) pathogenic copy number variants (CNVs). Patients with a genetic diagnosis had a significantly lower median age at seizure onset and more often had febrile seizures, status epilepticus, or neurodevelopmental impairment compared to those who remained genetically unsolved. Most common epilepsies were focal or multifocal epilepsies and developmental and epileptic encephalopathies (DDEs). Fifty-three patients, with a putative genetic diagnosis, were potentially eligible for precision therapy approaches. Indeed, genetic diagnosis enabled treatment adjustment in 32/53 (60%); 30/32 (93%) patients experienced at least a 50% reduction in seizure burden while only 4/32 (12.5%) became seizure-free. In summary, a genetic diagnosis was achieved in approximately 50% of patients with non-acquired epilepsy enabling precision therapy approaches in half of the patients, a strategy that results in > 50% reduction in seizure burden, in the majority of the treated patients.

Keywords Whole exome sequencing · SNP array · Precision therapy · Clinical outcome · Tertiary epilepsy center

Introduction

Epilepsy can derive from well-defined structural or metabolic disorders, some of which can have a monogenic cause. It is estimated that genetic factors can play a role approximately 70–80% of incidences of epilepsy [1]. These range from monogenic disorders caused by rare or ultra-rare variants with a high effect size to polygenic disorders with a complex genetic architecture [1]. During the past years, genetic testing for epilepsy has been increasingly incorporated into everyday clinical practice. This advance has primarily been driven by technological developments in next-generation sequencing, which has led to rapid discoveries in the etiology of rare monogenic epilepsies. Currently, at least 400 known genes have been associated with these disorders [2]. However, a recent modeling study of 31,058 parent–offspring trios estimated that more than 1000 genes associated with developmental disorders remain to be discovered, although their detection is increasingly difficult due to reduced penetrance and high pre- or perinatal mortality [3].

Obtaining a genetic diagnosis is of great importance for patients and their families. It provides an explanation and certainty and enables a more targeted genetic counseling,

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including knowledge about the prognosis and recurrence risk. Furthermore, it allows the patient and family to be connected to gene-specific networks of families with the same genetic condition. Last but definitely not least, in some cases the genetic diagnosis can help guide treatment. This type of personalized medication in epilepsy is constantly evolving, and knowledge about the pathomechanisms underlying difficult-to-treat epilepsies facilitates the development of new drugs and the repurposing of already available compounds [4]. Repurposing of already available drugs that are used for entirely different disorders means we can use compounds with known safety and tolerability profiles, thus shortening the time needed to set up clinical trials, as they require less time and resources compared to the development of new drugs [5]. One such example is the use of fenfluramine in Dravet syndrome [6]. Currently established personalized treatments based on knowledge about the pathophysiological effects of genetic variants include the use of sodium channel blockers in patients with disease-causing gain-of-function variants in *SCN2A* and *SCN8A* [7], ketogenic diet in patients with GLUT1 deficiency, and mammalian target of rapamycin (mTOR) inhibitors in mTORopathies [8].

A recently published Scottish population study tested children presenting with epilepsy before 36 months of age with a custom-designed 104-gene epilepsy panel [9]. The authors found that 80/333 (24%) children had a diagnostic genetic finding and the overall estimated annual incidence of monogenic epilepsies in their cohort was around 1 per 2120 live births [9]. The study also reported that a specific treatment approach was theoretically possible for 64/80 (80%) children with a monogenic diagnosis [9]. Peng et al. [10] used either exome or panel testing on 273 genetically unsolved children with drug-resistant epilepsy and achieved a genetic diagnosis in 86 patients (31%), of whom 34 (39%) benefited from an adjustment of their medication according to the genetic finding [10]. A recent multicenter systematic survey of 293 patients with a monogenic epilepsy found that a rational precision therapy approach was available for only 56 patients (19%) [11]. Such treatments were applied in 33/56 (59%) but were only successful (i.e., > 50% seizure reduction) in 10/33 (30%) patients [11]. The authors recommended "greater caution in raising expectation in people with epilepsy, clinicians and healthcare providers about the current impact of genetic findings in epilepsy" [11].

The present study explores the utility of stepwise genetic testing in children diagnosed with epilepsy and followed at the only tertiary epilepsy center in Denmark. The primary aim was to examine how often this would lead to a precision genetic diagnosis and thus facilitate precision therapy approaches.

Methods

Ethical Aspects

The study was approved by the local ethics committee in the Zealand region of Denmark (number SJ-91). Written informed consent was obtained from parents or legal guardians of each patient. Clinical information was collected from the patients' hospital records and their family members.

The Clinical Setting

The Danish Epilepsy Center (DEC), Filadelfia, is the only tertiary hospital in Denmark that is specialized in the treatment of epilepsy. The hospital has approximately 2600 annual outpatient consultations with children aged 0–18 years. The vast majority of patients referred to the DEC have intractable epilepsy, and thus, the patient population is biased towards the most difficult-to-treat cases.

Patient Analysis

This cross-sectional study was conceptualized in 2016 but was first initiated on May 1, 2019. The study population was children followed at DEC in 2015. During 2015, patients were referred to the DEC from all 18 Danish regional pediatric departments. Inclusion criteria for the study were as follows: (i) patients born in 2006–2011 (both years included) and followed at the DEC from January 1 to December 31, 2015; (ii) patients with a diagnosis of epilepsy (recurrent unprovoked seizures); and (iii) study consent provided by caregivers. Exclusion criteria were as follows: (i) a non-genetic etiology that would fully explain the seizures (e.g., hypoxic ischemic encephalopathy (HIE), perinatal stroke, or meningitis) and (ii) lack of study consent. Patients reaching a satisfying genetic diagnosis prior to referral to DEC and who met the inclusion criteria were also included. Patients fulfilling the study criteria were approached electronically in June 2019 and offered to be included in the study; non-responders were electronically approached a second time in November 2020.

Exome sequencing was performed from August 2019 to June 2021 and single nucleotide polymorphism (SNP) array analysis was carried out from August 2021 to December 2021. When possible, treatments were adjusted based on the genetic diagnosis. This was carried out continuously until December 2021; the effect of treatment adjustment on seizure burden was evaluated in February 2022. At this point, the burden of seizures was compared to the burden at time of reaching a genetic diagnosis.

The records of included patients were systematically reviewed for data on seizure type(s), epilepsy type, EEG,

epilepsy syndrome, cognitive skills, comorbidities, neuroimaging, surgery, preexisting genetic diagnosis, and medical treatment adjustments as a result of a genetic diagnosis.

Seizures and epilepsies were classified according to the International League Against Epilepsy (ILAE) 2017 position papers [12, 13]. Seizure types were classified as focal, generalized, or unknown while epilepsy types were classified as focal, generalized, combined focal and generalized, or unknown. Seizure types were determined by the treating epileptologist at the DEC, based on videos and parental descriptions in addition to ictal (video)EEG recordings. When possible, an electroclinical epilepsy syndrome was determined based on age at seizure onset, seizure and epilepsy types, (video)EEG recordings, and the neurodevelopmental trajectory. The diagnostic criteria used are available on the ILAE website http://www.epilepsydiagnosis.org and the ILAE 2022 position papers [14–16]. The term "developmental and epileptic encephalopathies" (DEE) embraced disorders where the developmental impairment is related to both the epileptiform activity and the underlying genetic etiology [12, 17, 18]. The term "early infantile developmental and epileptic encephalopathy" (EI-DEE) was used for patients categorized as either Ohtahara syndrome or early myoclonic encephalopathy [14]. "Genetic generalized epilepsy" was classified accordingly to the ILAE recommendations from 2022 [15, 19] and included "genetic epilepsy with febrile seizures plus" (GEFS +).

Global developmental delay (GDD) was based on both formal and functional assessments and classified according to the Diagnostic and Statistical Manual of Mental Disorders 5th edition [20]. GDD was classified as mild, moderate, severe, or profound by a pediatric neurologist (AB). The term N/A (not available) was used if the level of GDD was unknown or difficult to determine. Antiseizure medications (ASM) were considered to have efficacy if they resulted in > 50% reduction in seizures for a period longer than 6 months. Patients were classified as treatment-resistant if they failed to become and remain seizure-free for at least 6 months with adequate trials of two ASMs.

Variant Identification

Some patients had already received a genetic diagnosis that could fully explain their symptoms. This diagnosis had been reached as part of a clinical workup undertaken prior to study inclusion (Figs. 1 and 2) using chromosomal karyotyping, comparative genomic hybridization (CGH) array, targeted panel sequencing with 45–600 genes, or whole exome sequencing (WES) (Fig. 2). We offered stepwise genetic testing to patients without a genetic diagnosis at study inclusion. This was done using DNA from peripheral blood lymphocytes, first with WES and then SNP microarray if WES did not provide a genetic diagnosis. Segregational analysis was performed if parental DNA was available. Figure 1 visualizes the different steps of the present study.

WES

Singleton WES was initially performed, and segregational analysis was used for (likely) pathogenic and highly relevant variants of uncertain significance (VUSs). If a subject harbored three or more variants of interest, then parental WES was performed. DNA from peripheral blood lymphocytes was extracted using standard procedures. Library preparation and enrichment for WES was performed on PBL DNA following the standard TWIST procedure for the Twist Library Preparation EF Kit 1.0, Enzymatic Fragmentation, Twist Universal Adapter System, and TWIST Human Core Exome Kit (TWIST Bioscience, San Francisco, CA, USA). The TWIST libraries were loaded to a S1 flow cell and sequenced in a paired end 110 cycles on a NovaSeq 6000 system (Illumina, Inc., San Diego, CA, USA). For all included subjects, the genomic regions targeted by the respective enrichment design had an average coverage of > 100 reads, and > 97% were covered by at least 20 reads. The NGS method and the variant analysis pipeline was validated internally by analyzing five control samples per every 350 samples and externally by participating in the EMQN program.

Bioinformatics Following WES

Sequences were mapped to hg19, and variant calling was achieved with BWA-MEM and Freebayes. The variant filtering was performed using VarSeq software (Golden Helix, Bozeman, USA). Common SNPs with a variant allele frequency ≥ 25% and SNPs observed in more than one sample for each analyzed sample batch were filtered out. All possible modes of inheritance (sporadic de novo, dominant, recessive, X-linked) were analyzed using sensible minor population allele frequency cutoffs ≤ 0.01% in the Genome Aggregation Database (gnomAD) [21]. Genetic nonsynonymous missense/inframe insertions/inframe deletions/frameshift/stop_lost/stop_grain/5-prime UTR (< 10 base pair upstream)/splice site variants were evaluated through database searches such as dbSNP155, ClinVar, the Exome Aggregation Consortium database (ExAC), gnomAD, and Human Gene Mutation Database (HGMD) Professional [22]. Missense variants were also submitted to prediction softwares such as PolyPhen2 [23], SIFT [24], MutationTaster [25], MutationAssessor [26], FATHMM or FATHMM MKL Coding [27], CADD [28], and pathogenic variant enriched regions viewer while splice site variants were evaluated by the PWM, MaxEntScan, GeneSplicer, and NNSplice prediction tools. Variants analyzed under a dominant inheritance model that were observed more than three times in ExAC

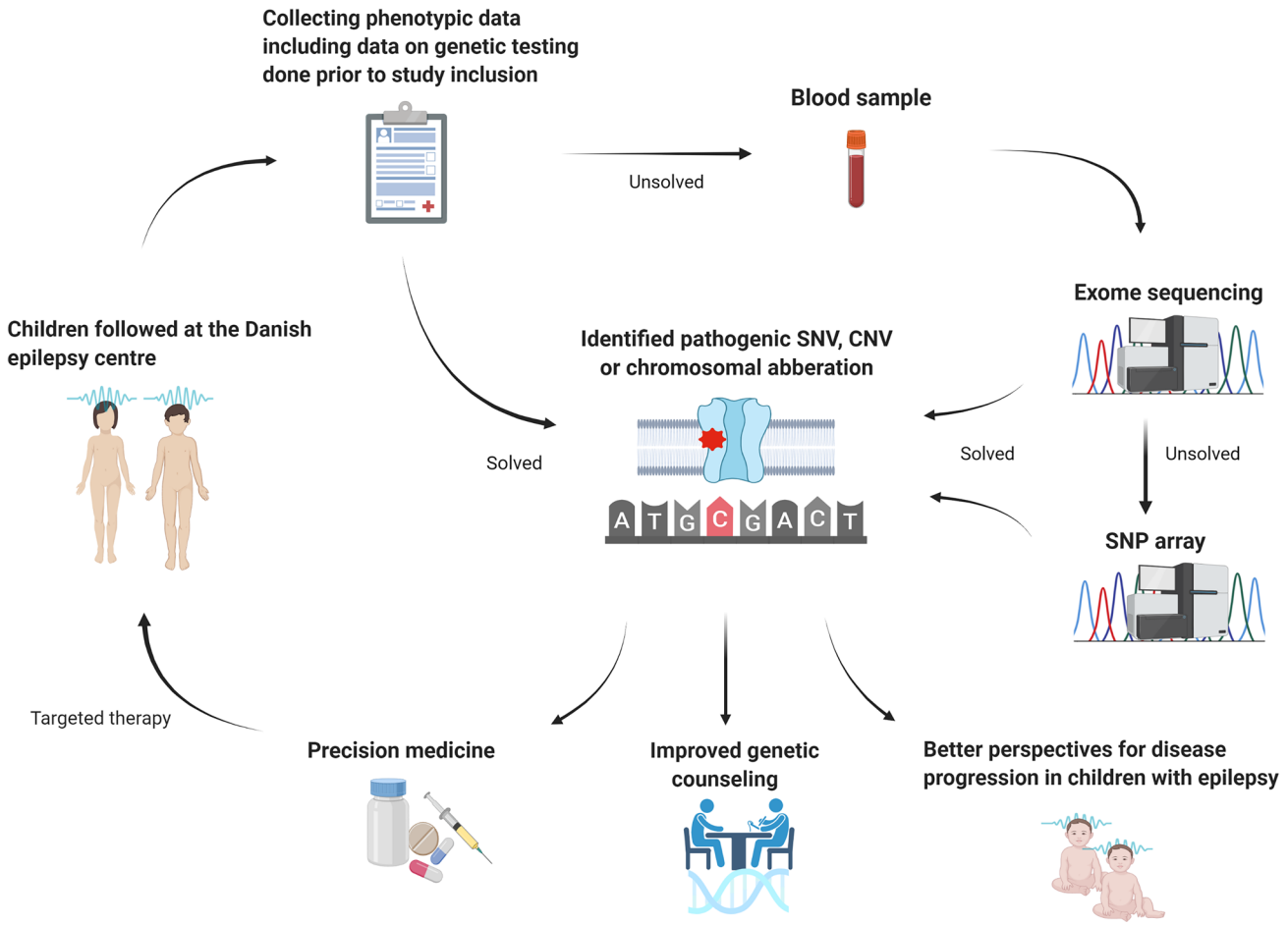

Fig. 1 Overview of the different steps in the present study, starting with selection of the study cohort at the tertiary epilepsy center and followed by stepwise genetic testing and identification of disease-causing variants. The diagram illustrates how identification of genetic epilepsies can contribute to improved genetic counseling and precision therapy approaches

[29] and gnomAD [21] were considered too common and discarded. Potentially pathogenic variants were validated through conventional Sanger sequencing and, if possible, parents were included for segregation analysis.

Criteria for Pathogenicity of Rare Variants Detected by WES

Variants were classified for pathogenicity using the 2015 American College of Medical Genetics (ACMG) and Genomics Guidelines [30]. The web-based tool WInterVar (https://wintervar.wglab.org) was used to classify missense variations according to the ACMG criteria. A test was considered diagnostic when the subject was found to have one or two (likely) pathogenic variants in a single gene, depending on the mode of inheritance. Variants were classified as pathogenic if they (1) caused nonsynonymous, splice-site altering, or truncating changes; (2) were predicted as damaging by three or more prediction software programs; (3) were not present in controls in the ExAC or gnomAD control cohorts [21]; (4) had previously been classified as (L)PATH in either ClinVar [31] or HGMD; (5) were de novo changes or were inherited from an unaffected mosaic parent or an affected parent; or (6) showed a strong and specific correlation between the gene and the patient's phenotype. We used Sanger sequencing to confirm variants and to perform segregation analysis.

In case of genes not yet linked to a human disorder, a VUS was considered highly relevant if (1) the variant was de novo and either extremely rare or never reported before in gnomAD [21] or the patient was compound heterozygous, homozygous, or hemizygous for a variant never reported in homo- or hemizygous form in gnomAD; (2) in silico predictions supported pathogenicity or the amino acid position in question was highly conserved in mammals and evolutionary more distant species, suggesting that the position does not tolerate variation; (3) animal models showed phenotype similar to the human subjects; and/or (4) in vitro studies revealed a

pivotal role of the gene in the growth, differentiation, and/or function of neurons.

SNP Array

Patients genetically unsolved after WES were investigated using Illumina GSA V2 SNP-array of genomic DNA. CNVs were called by the PennCNV [32] version 1.0.5. PennCNV is a hidden Markov model (HMM)-based CNV calling algorithm that incorporates LogR ratio, B-allele frequency, population allele frequency, and the distance between adjacent SNPs into the HMM model. We used the joint CNV calling algorithm for trios and the standard CNV calling algorithm for single individuals, duos, and quartets. CNV calls with fewer than 30 consecutive SNPs (disregarding multi-allelic calls) were excluded. We visually inspected de novo CNVs as well as CNV where inheritance was unknown as both parents were not available for genetic testing. Visual inspection was done depicting LogR ratio and the B-allele frequency by genomic positional mapping on hg 38. We excluded CNVs in which general fluctuation in LogR or B-allele frequency was inconsistent with that of a true CNV.

CNVs were classified as either pathogenic, VUS, or benign according to the AMCG guidelines [33]. A CNV was considered pathogenic if (1) it occurred de novo, (2) it harbored one or more known epilepsy genes, (3) the proband's phenotype was similar to published cases, and (4) it was absent or rarely reported in public CNV databases. If a known epilepsy gene was not present, additional factors were taken into consideration such as gene function and tissue expression, established haploinsufficiency or triplosensitivity, and the phenotype of knockout animal model.

Statistical Analyses

Quantitative statistics were analyzed using IBM SPSS version 24. Two-sided *T*-test was used to determine the association between age at seizure onset and the ability to achieve a genetic diagnosis. *P* value of 0.05 was considered significant. χ^2 analyses were used to explore differences in degree of (i) cognitive impairment between genetically solved and unsolved patients and (ii) diagnostic yield of genetic testing based on age at seizure onset.

Results

We identified 357 patients who were born in 2006–2011 and followed at DEC in 2015. Figure 2 gives an overview of the flow of these patients through the present study. Twenty-seven patients (7.5%) turned out not to have epilepsy, and 35 (11%) had epilepsy due to acquired brain damage; both these groups were excluded from further analysis. Within the 35 patients with presumed acquired brain damage, the majority were believed to have perinatal HIE ($n=20$) while less common etiologies included neonatal hypoglycemia ($n=4$), prematurity ($n=4$), postnatal hypoxia ($n=3$), brain tumor ($n=2$), congenital herpes infection ($n=2$), and fetal alcohol syndrome ($n=1$). We considered the remaining 295 children to have a potential monogenic epilepsy and offered them genetic testing as part of the present study. Parents of 188 patients from 186 unrelated families were willing to participate and gave consent for study inclusion and genetic testing as part of this study.

The clinical characteristics of these 188 patients are given in Table 1. The male to female ratio was 1.2:1. Of the 188 patients, 140 (74%) had some degree of developmental or cognitive impairment ranging from mild to profound (Table 1). The most common epilepsies were focal epilepsies (49/188; 26%), multifocal DEEs (36/188; 19%), and electroclinical syndromes such as epileptic spasms syndrome (41/188; 22%) and Dravet syndrome (20/188; 11%) (Table 2). Associated seizure types were either focal or combined focal and generalized seizures. Of the genetically tested patients, only 8.5% (16/188) were categorized as generalized epilepsies as they exclusively experienced generalized seizures with generalized ictal EEG discharges (Table 2). These included nine patients with GEFS + , four with childhood absence epilepsies (CAE), one patient with myoclonic absence epilepsy, and six patients with a generalized epilepsy that could not be further classified.

At study inclusion, 86/188 patients already had a genetic diagnosis that could fully explain their symptoms; 69 patients had a SNV, 16 had a CNV, and one patient had a chromosomal aberration (Supplementary Table 1). These diagnoses had been established during prior clinical workup (Figs. 1 and 2) using primarily gene panel sequencing (in 124/188 patients), followed by karyotyping, CGH-array, or WES in a minority of patients (Fig. 2). Although these 86 patients were included in the study, they did not undergo further genetic testing.

The 102/188 genetically unsolved patients underwent WES, comprising singleton WES in 89 patients and trio sequencing in 13 patients. This approach identified a (likely) pathogenic variant in eight patients (*CWF19L1*, *IQSEC2*, *IRF2BPL*, *KCNMA1*, *POU3F3*, and *STAMBP*) and a highly relevant VUS in seven patients (*ADGRL1*, *CELSR1*, *CUL4B*, *KCNH5*, *NEXMIF*, *SLITRK2*, and *TRA2B*) (Table 3). SNP array was performed in the remaining 87 patients and revealed neither (likely) pathogenic nor any highly relevant CNVs.

Next-generation sequencing approaches (pre-study panels and study-WES) thus identified a clinically relevant SNV or indel explaining the clinical picture in 77 patients, in addition to the highly relevant indel or SNV of unknown

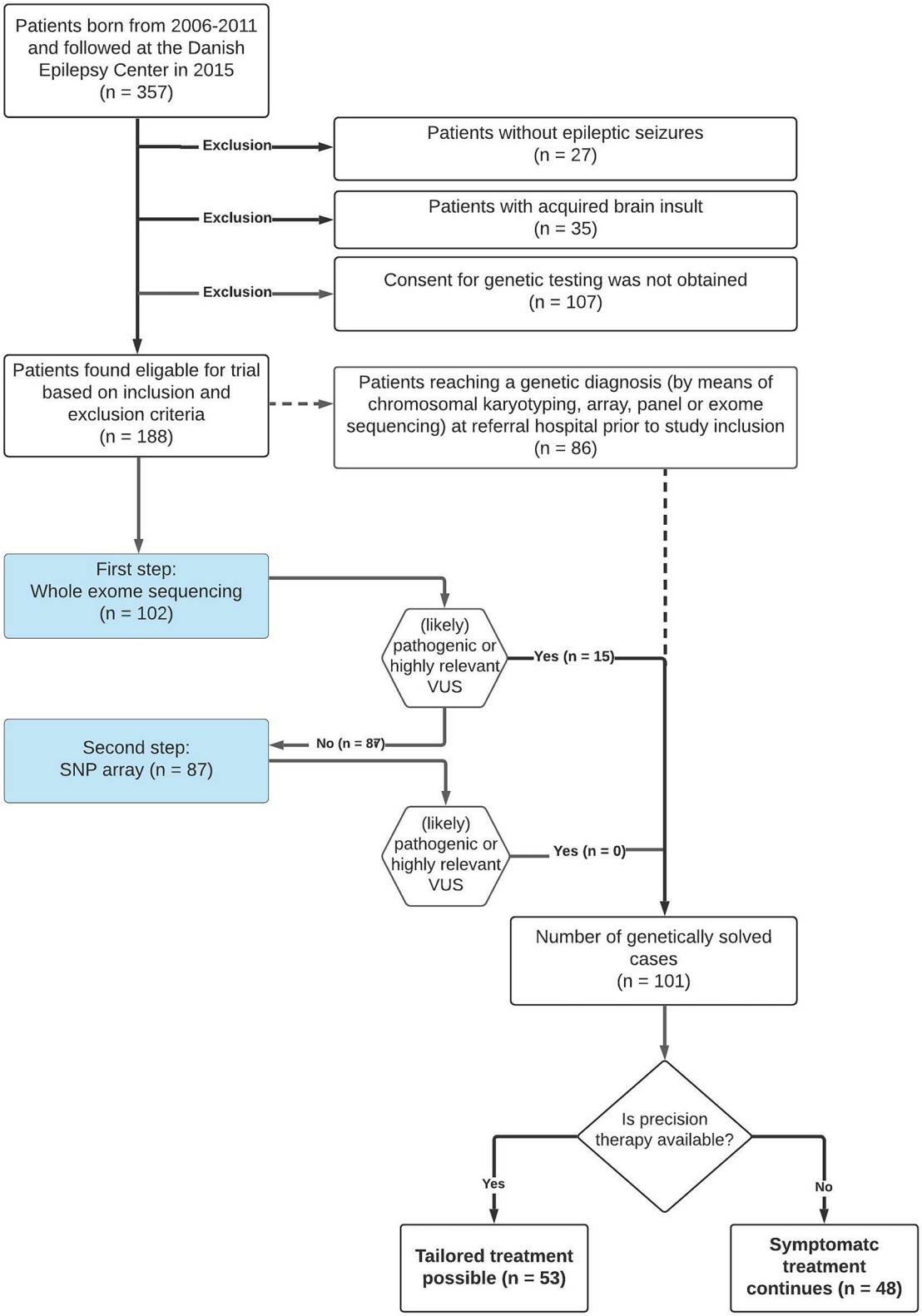

Fig. 2 Detailed overview of the outcome of participants included in the present study. Full lines show the patients' path through the study while dotted lines indicate procedures performed prior to study inclusion. Number of patients at each step is indicated by "*n*". SNP, single nucleotide polymorphism; VUS, variant of unknown significance

significance detected in seven patients. This encompassed variants across 41 genes (Table 3), and none of the patients harbored a dual genetic diagnosis (Table 3). The most common genetic causes were pathogenic variants in *SCN1A* causing Dravet syndrome or *TSC2* causing tuberous sclerosis. Genes encoding ion channels were commonly affected, and variants in these genes explained the phenotype in 34 of the 101 patients with a putative genetic diagnosis. Only one of the diagnosed patients (ID-36) had an inborn error of metabolism related to pyridoxine 5'-phosphate oxidase deficiency.

The highest diagnostic yield based on electroclinical syndromes was in patients with EI-DEE (100%), Dravet syndrome (90%), multifocal DEE (66%), and epileptic spasms syndrome (61%) (Table 2). The numbers were lower in groups with developmental and/or epileptic encephalopathy with Spike-Wave activation in sleep (40%) and epilepsy with myoclonic-atonic seizures (EMA) (37%). There was a significant difference in diagnostic yield based on age at seizure onset ($p < 0.001$). The yield was highest if seizures started before 2 months of life (93%) and reached 59% in patients having first seizures between 2 months and 2 years of life. If onset of first seizure was between 2 and 9 years of life, the yield fell to 29% (Table 2). Of the 16 patients with a genetic generalized epilepsy, nine were classified as GEFS + while four had a childhood absence epilepsy (CAE). Of those with GEFS + , 55% (5/9) achieved a genetic diagnosis due to variants in *SCN1A* ($n = 2$), *SCN1B* ($n = 1$), *GABRD* ($n = 1$), and *CUL4B* ($n = 1$) while one patient with CAE was found to harbor a likely pathogenic variant in *SLC6A1* (Supplementary Table 1).

Amongst the presumed genetically solved cases, causative variants were almost exclusively SNVs and CNVs as only a single patient had a complex chromosomal rearrangement. Seventy-eight percent of these patients had variants consistent with an autosomal dominant inheritance, 13% with an X-linked inheritance, and 6% with an autosomal recessive inheritance. Amongst the 84 patients with SNVs or indels, the most common variant types were missense variants (56%), followed by 22% frameshift variants, 18% splice-altering variants, and 4% inframe deletions. Larger CNVs were identified in 16 patients and included deletions/duplications that affected a number of genes. The complex chromosomal rearrangement was caused by a ring chromosome 20 detected in a girl (ID-101) with profound DD and cognitive impairment and treatment-resistant early-onset atypical absence seizures that often progressed into status epilepticus. Supplementary Table 1 shows the genetic variants and associated clinical phenotypes at individual level.

After dividing patients into those who were genetically solved and those who were not, we found that the proportion of patients with developmental delay or cognitive impairment was 89% in the genetically solved group and 56% in the unsolved group. Development/cognition was often more severely affected in the genetically solved group but was often within normal boundaries in the unsolved group

Table 1 Clinical characteristics of study participants undergoing genetic testing. *n*, number; *VUS*, variant of unknown significance

	Patients consented to genetic testing	Genetically solved cases (including those with suspicious VUS)	Genetically unsolved cases	*p*-value (solved vs unsolved)
	$n = 188$	$n = 101$	$n = 87$	-
Male	101/188 (55%)	48/101 (47%)	42/87 (48%)	-
Female	86/188 (45%)	54/101 (53%)	44/87 (52%)	-
Epilepsy diagnosis	188/188 (100%)	101/101 (100%)	87/87 (100%)	-
Developmental delay or cognitive impairment	140/188 (74%)	90/101 (89%)	49/87 (56%)	$p < 0.001$
	Degree: none = 48 mild = 40 moderate = 24 severe = 25 profound = 42 Unable to classify = 9	Degree: none = 11 mild = 22 moderate = 13 severe = 16 profound = 30 Present but unable to classify = 9	Degree: none = 38 mild = 18 moderate = 9 severe = 9 profound = 12 Present but unable to classify = 1	
Abnormal brain MRI	61/188 (32%)	38/101 (37%)	23/87 (26%)	$p = 0.298$
History of febrile seizures	99/188 (51%)	74/101 (72%)	31/87 (35%)	$p = 0.002$
History of status epilepticus	97/188 (51%)	55/101 (54%)	17/87 (19%)	$p < 0.001$
Treatment resistant epilepsy	98/188 (51%)	44/101 (43%)	41/87 (47%)	$p = 0.787$
Median age at onset of seizures	12 months (range: 1st day of life to 8th year of life)	6 months (range: 1st day of life to 8th year of life)	24 months (range: 1st day of life to 8th year of life)	$p < 0.001$

(Table 1). In the solved group, 90% (91/102) had an impairment and this was classified as severe-profound in 47 cases; in the unsolved group, 44% (38/86) were not reported to have an impairment. These represented statistically significant differences between the two groups ($p < 0.001$, Table 1). We also found significant differences between the two groups regarding presence of febrile seizures or status epilepticus ($p = 0.002$ and $p < 0.001$, respectively, Table 1). There was no significant difference between the two groups in terms of treatment resistant seizures ($p = 0.787$, Table 1) and also the frequency of brain MRI abnormalities, and the overall frequency of such abnormalities was around 32% (61/188) ($p = 0.298$, Table 1). The distribution of epilepsy syndromes differed between the genetically solved group and the unsolved group (Table 2). Although the distribution of GGEs was fairly similar in the two groups, the genetically solved group had a larger proportion of DEEs (63%, 78/123) and fewer focal (non-DEE) epilepsies (32%, 16/49).

Table 2 Diagnostic yield from genetic testing of study participants (i.e., whether achieved a genetic diagnosis or not) according to epilepsy syndrome classification and age at seizure onset

Diagnostic yield from genetic testing according to epilepsy syndrome classification (at seizure onset)					
		Subjects consented to genetic testing	Genetically solved cases	Genetically unsolved cases	Diagnostic yield
Developmental and/or epileptic encephalopathies	Epileptic spasms syndrome	41	25	16	25/41 (61%)
	Dravet syndrome	20	18	2	18/20 (90%)
	Epilepsy with myoclonic-atonic seizures	8	3	5	3/8 (37%)
	With spike-wave activation in sleep	5	2	3	2/5 (40%)
	EI-DEEs	4	4	0	4/4 (100%)
	Lennox-Gastaut syndrome	3	0	3	0/3 (0%)
	Multifocal DEEs	36	25	11	25/36 (69%)
	With early atypical absences	1	1	0	1/1 (100%)
	Not further classified DEEs	5	0	5	0/5 (0%)
	Total	**123**	**78**	**45**	**78/123 (63%)**
Focal epilepsies (non-DEEs)	Self-limited epilepsy with centro-temporal spikes	5	0	5	0/5 (0%)
	Frontal lobe epilepsy	3	0	3	0/3 (0%)
	Not further classified focal epilepsies	41	16	25	16/41 (39%)
	Total	**49**	**16**	**33**	**16/49 (32%)**
Genetic generalized epilepsies	Childhood absence epilepsy	4	1	3	1/4 (25%)
	Epilepsy with myoclonic absences	1	1	0	1/1 (100%)
	GEFS +	9	5	4	5/9 (55%)
	Not further classified generalized epilepsies	2	0	2	0/2 (0%)
	Total	**16**	**7**	**9**	**7/16 (43%)**
Diagnostic yield from genetic testing according to age at seizure onset					
Time of seizure onset		Subjects consented to genetic testing	Genetically solved cases	Genetically unsolved cases	Diagnostic yield
< 2 months of life		15	14	1	14/15 (93%)
≥ 2 months but < 2 years of life		128	75	53	76/128 (59%)
≥ 2 years but ≤ 9 years of life		45	13	32	13/45 (29%)

DEEs, early infantile developmental and/or epileptic encephalopathies; *EI-DEEs*, early infantile developmental and epileptic encephalopathies; *GEFS +*, genetic epilepsy with febrile seizures plus

Table 3 Overview and frequency of genetic diagnosis included in the present study and the diagnostic method used to reach the diagnosis.

Gene name	Number of patients	Inheritance	Diagnostic test
SCN1A	18	AD	Panel
TSC1/TSC2	10	AD	Panel/sanger sequencing
PCDH19	4	XL	Panel
SCN2A	4	AD	Panel
SCN8A	4	AD	Panel
GABRB3	3	AD	Panel
CACNA1A	2	AD	Panel
DEPDC5	2	AD	Panel
IQSEC2	2	XLD	WES
PIGA	2	AD	Panel
SLC6A1	2	AD	Panel
STAMBP	2	AD	WES
UBE3A	1	AD	Panel
ADGRL1	1	Epilepsy candidate gene	WES
ATP1A3	1	AD	Panel
CDKL5	1	XLD	Panel
CELSR1	1	Epilepsy candidate gene	WES
CUL4B	1	XLR	WES
CWF19L1	1	AR	WES
FOXG1	1	AD	Panel
GABRB2	1	AD	Panel
GABRD	1	AD	Panel
IRF2BPL	1	AD	WES
KCNH5	1	Epilepsy candidate gene	WES
KCNMA1	1	AD	WES
KCNQ2	1	AD	Panel
KCNQ3	1	AD	Panel
MED12	1	AD	Panel
NEXMIF	1	XLD	WES
MECP2	1	XL	Panel
NPRL3	1	AD	Panel
PIGN	1	AR	Panel
PIGT	1	AR	Panel
PNPO	1	AR	Panel
POU3F3	1	AD	WES
SCN1B	1	AD	Panel
SLC1A2	1	AD	Panel
SLITRK2	1	Epilepsy candidate gene	WES
STXBP1	1	AD	Panel
SYNGAP1	1	AD	Panel
TRA2B	1	Epilepsy candidate gene	WES

AD, autosomal dominant; *AR*, autosomal recessive; *WES*, whole exome sequencing; *XL*, X-linked; *XLD*, X-linked dominant; *XLR*, X-linked recessive

For the full cohort of 188 patients, median age at seizure onset was 12 months (ranged from 1 day to 9 years of life). Median age at seizure onset was significantly lower in the group reaching a genetic diagnosis than in those who remained genetically unsolved (6 months vs 24 months, $t(186) = 4.587$, $p = < 0.001$) (Table 1). We also found a significantly higher proportion of patients with neurodevelopmental impairment of any kind in the solved group ($\chi^2(2188) = 58.18$, $p < 0.001$).

Ultimately, (likely) pathogenic variant(s) were present in 94/188 patients and a highly relevant VUS was found in 7/188 patients. Of these 101/188 patients, 53 were potentially eligible for precision therapy approaches (Supplementary Table 1). Treatment was adjusted according to the genetic findings in 32/53 (60%). More than 50% seizure reduction was reported in 30/32 (93%) but only 4 of these 30 patients became seizure-free. Everolimus in the treatment of mTORopathies and fenfluramine in the treatment of Dravet Syndrome were the most commonly used precision therapies (Supplementary Table 1). In 12/53 (40%) patients, satisfactory seizure control was obtained prior to genetic diagnosis, thus preventing further treatment adjustment; however, the ASMs used are not considered precision therapy for the underlying genetic condition. An overview of these ASMs is available in Supplementary Table 1; they include levetiracetam, valproate, stiripentol, ethosuzimide, vigabatrin, and four different sodium channel blockers (oxcarbamazapine, lamotrigine, zonisamide, and rufinamide). In this context, sodium channel blockers were effective in five patients with a disease-causing variant in *DEPDC5*, *NPRL3*, *PIGN*, *SLC6A1*, and *TSC2*, respectively. Levetirazetam and valproate were most commonly used either alone or in combination with the other ASMs. Table 4 shows number of patients with specific genetic disorders, how often therapy was adjusted based on the genetic diagnosis and the efficacy of the therapeutic adjustment. Further clinical details on a patient specific level are available in Supplementary Table 1.

Discussion

Of the 357 patients, 101 (28%) reached a genetic result that prevented further genetic testing, corresponding to 28% of the entire cohort. We classified the variant(s) found in 94 patients as (likely) pathogenic according to the ACMG guidelines [30], while seven had a highly relevant SNV/indel. SNP-array analysis did not identify a genetic diagnosis for any of the participants. Comparing the group of genetically solved patients (101/188, 54%) with those who remained unsolved (87/188, 46%), we found significant differences in median age at seizure onset ($p < 0.001$), presence of febrile seizure ($p = 0.002$), status epilepticus ($p < 0.001$), and degree of developmental and/or cognitive impairment

Table 4 Overview of genes detected in this study and clinically available precision therapies. The table shows number of patients with specific genetic disorders, how often therapy was adjusted based on the genetic diagnosis and the efficacy of the therapeutic adjustment. Further clinical details on a patient specific level are available in Supplementary table 1

Gene name	Clinically available precision therapy	Number of patients	Number of patients adjusted in treatment following the genetic diagnosis	No effect of precision therapy	50–90% reduction in seizure frequency upon therapy adjustment	Seizure free upon therapy adjustment
SCN1A	Lof: Stiripentol (+ valproate + clobazam) Fenfluramine Cannabidiol Avoid sodium channel blockers	18	17/18	0/17	15/17	2/17
TSC1/TSC2	Everolimus and other mTOR inhibitors Resective epilepsy surgery of a focal dyspastic lesion is not contraindicative	10	5/10	0/5	3/5	2/5
PCDH19	None	4	-	-	-	-
SCN2A	GoF: Sodium channel blockers LoF: Avoid sodium channel blockers	4	2/4	0/2	0/2	2/2
SCN8A	GoF: Sodium channel blockers LoF: Avoid sodium channel blockers	4	4/4	0/4	2/4	2/4
GABRB3	LoF: GABAergic enhancers, e.g., phenobarbital or vigabatrin GoF: avoid GABAergic enhancers	3	0/3	-	-	-
CACNA1A	None	2	-	-	-	-
DEPDC5	Everolimus and other mTOR inhibitors Resective epilepsy surgery of a focal dyspastic lesion is not contraindicative	2	1/2	0/1	0/1	1/1 (upon surgery)
IQSEC2	None	2	-	-	-	-
PIGA	High-dose pyridoxine	2	2/2	0/2	0/2	0/2
SLC6A1	Avoid gaba potentiating drugs	2	0/2	-	-	-
STAMBP	None	2	-	-	-	-
UBE3A	None	1	-	-	-	-
ADGRL1	None	1	-	-	-	-
ATP1A3	None	1	-	-	-	-
CDKL5	Ganaxolone	1	0/1	-	-	-
CELSR1	None	1	-	-	-	-
CUL4B	None	1	-	-	-	-
CWF19L1	None	1	-	-	-	-
FOXG1	None	1	-	-	-	-
GABRB2	LoF: GABAergic enhancers, e.g., phenobarbital or vigabatrin GoF: avoid GABAergic enhancers	1	0/1	-	-	-
GABRD	In case of a gain-of-function variant then avoid vigabatrin	1	0/1	-	-	-
IRF2BPL	None	1	-	-	-	-
KCNH5	None	1	-	-	-	-

Table 4 (continued)

Gene name	Clinically available precision therapy	Number of patients	Number of patients adjusted in treatment following the genetic diagnosis	No effect of precision therapy	50–90% reduction in seizure frequency upon therapy adjustment	Seizure free upon therapy adjustment
KCNMA1	None	1	-	-	-	-
KCNQ2	LoF: Sodium channel blockers Retigabine	1	1/1	0/1	0/1	0/1
KCNQ3	None	1	-	-	-	-
MED12	None	1	-	-	-	-
NEXMIF	None	1	-	-	-	-
MECP2	None	1	-	-	-	-
NPRL3	Everolimus and other mTOR inhibitors Resective epilepsy surgery of a focal dyspastic lesion is not contraindicative	1	0/1	-	-	-
PIGN	High-dose pyridoxine	1	0/1	-	-	-
PIGT	High-dose pyridoxine	1	1/1	0/1	0/1	0/1
PNPO	Pyridoxine or pyridoxal 5'-phosphate	1	1/1	0/1	1/1	0/1
POU3F3	None	1	-	-	-	-
SCN1B	Scn1b null neurons show a paradoxical increase in persistent sodium currents when treated with carbamazapin, suggesting that sodium channel blockers should be avoided Compounds enhancing GABA activity should be preferred	1	0/1	-	-	-
SLC1A2	Ketogenic diet	1	1/1	0/1	1/1	0/1
SLITRK2	None	1	-	-	-	-
STXBP1	None	1	-	-	-	-
SYNGAP1	None	1	-	-	-	-
TRA2B	None	1	-	-	-	-

(Table 1). This difference is likely caused by the higher proportion of DEEs in the solved group (63%, 78/123) compared to the unsolved group (37%, 45/123). In contrast, the unsolved group had more patients with focal (non-DEE) epilepsies (68%, 33/49) compared to the solved group (32%, 16/49), and such epilepsies are more likely to have a more complex genetic background [34]. The diagnostic yield was particularly high amongst patients with seizure onset before the second year of life. Causative variants were almost exclusively SNVs/indels (82%) and CNVs (17%). Based on the 94 patients with a (likely) pathogenic variant(s) and the seven patients with a highly relevant VUS, precision therapy was available in 53 (52%) patients but was implemented in only 32/53 (60%). Satisfactory seizure control using alternative treatments was obtained prior to genetic diagnosis in 12/53 (40%) patients, thus preventing further treatment adjustments. Precision therapy was efficient in 30/32 (93%) patients, but only 4/30 (12.5%) became seizure-free.

We found that *SCN1A* or *TSC2* were the most commonly affected genes, causing a clinical spectrum ranging from GEFS + to Dravet syndrome in 18 patients (*SCN1A*) and tuberous sclerosis in 10 patients (*TSC2*). Although less frequent, pathogenic variants in ten genes, including *PCDH19*, *SCN2A*, *SCN8A*, *CACNA1A*, *DEPDC5*, *GABRB3*, and *SLC6A1*, were identified in 2–4 patients each, while disease-causing variants in 29 genes were only detected in single patients. In comparison, the Scottish population study [9] reported *PRRT2*, *SCN1A*, and *KCNQ2* to be the most prevalent genes followed by *SLC2A1*, *CDKL5*, *PCDH19*, *SLC6A1*, *DEPDC5*, *CACNA1A*, *KCNA2*, and *KCNQ3*. These findings are more representative of the epilepsies presenting at a general pediatric department and not at a tertiary epilepsy center. *PRRT2* causes self-limiting and treatable infantile-onset epilepsies [18] while *KCNQ2* is associated with self-limiting/benign neonatal-onset seizures as well as neonatal onset DEEs [18]. In the Scottish study [9], only 2/10 patients with a pathogenic variant in *KCNQ2* were

diagnosed with a DEE; this is compatible with our findings as we only found a single patient with KCNQ2-related DEE. Self-limiting neonatal or infantile seizures are not expected to be followed at a tertiary epilepsy center, and we found no patients with *PPRT2*- and only one patient with *KCNQ2*-DEE. Precision therapy approaches used in the present study included mTOR-inhibitors in epilepsies caused by pathogenic variants in the mTOR pathway such as *TSC1*, *TSC2*, *DEPDC5*, *NPRL2*, and *NPRL3* [8]; repurposing of fenfluramine (a serotonin reuptake inhibitor) in the treatment of Dravet syndrome [6]; pyridoxine in GPI anchoring disorders [35]; avoiding ASMs that may worsen seizure activity (i.e., sodium channel blockers in epilepsies due to loss-of-function (LoF) variants in *SCN1A*); or deploying sodium channel blockers in epilepsies caused by gain-of-function variants in *SCN2A* and *SCN8A* [7].

Our data support a role for early genetic testing to provide a diagnosis that can enable personalized therapies. However, there is still a gap between reaching a genetic diagnosis and getting a disease-specific treatment. Often, a precision therapy does not exist or is limited to the option of avoiding certain drugs (e.g., sodium channel blockers in LoF *SCN2A*-related disorders or gaba-potentiating drugs in GoF *GABA*-related disorders) [18, 36]. Sometimes promising drugs, such as fenfluramine or ganaxolone, are in the horizon but remain difficult or impossible to prescribe. Ganaxolone reduces seizure frequency in *CDKL5*-related epilepsies [37] but is currently only approved by the Food and Drug Administration and not the European Medicines Agency. Fenfluramine is authorized in all EU countries but still needs to be approved in Denmark (unless a "compassionate use" permit is obtained from the Danish Medicines Agency). Finally, it must be pointed out that when available, the treatment often only targets seizures while leaving the developmental problems and comorbidities unchanged.

The obvious questions are as follows: How to bridge the gap and how do we develop therapies that not only target seizures but also tackle neurodevelopmental issues? A possible solution may come from promising therapies targeting DNA, RNA translation, and protein modulation. Such treatments are more likely to treat seizures as well as comorbidities since they are acting directly on the underlying pathomechanism causing the different phenotypic features [18, 38]. Gene therapies include DNA targeting treatments but also single-stranded antisense oligonucleotides (ASOs) that bind and alter RNA translation and ultimately protein expression [39, 40]. Several ASOs are in preclinical development in translational mice models including *SCN1A*- and *SCN8A*-encephalopathy and DS [41, 42] and *MECP2* duplication syndrome [43]. Some have reached clinical trials, including an ASO designed to treat patients with DS by preventing inclusion of a poison exon thereby upregulating the will-type allele (https://clinicaltrials.gov/ct2/show/NCT04442295).

A recent meta-analysis showed that for diagnostic purposes the highest yield was in whole genome sequencing (WGS) (48%, 95% CI = 28–70%) followed by WES (24%, 95% CI = 18–30%), panel sequencing (19%, 95% CI = 16–24%), and array CGH (9%, 95% CI = 7–11%) [44]. Although any patient with treatment-refractory epilepsy could potentially benefit from genetic testing, it would probably be of most importance in those with seizures starting before 3 years of age, and in our cohort, a genetic diagnosis was reached in 86/137 (67%) of patients whose seizures started within this age. It would also likely benefit those with a family history of seizures or those with associated neurological deficit such as developmental and/or cognitive impairment. Several studies have shown that the overall diagnostic yield of targeted panels and WES is dependent on the epilepsy phenotype and age at seizure onset [44–46]. The highest yield is in DEEs (45, 46), and the lowest is in cohorts with focal epilepsy [44]. In 2016, Møller et al. [47] reported on 216 patients consecutively referred for genetic testing for epilepsies ranging from benign neonatal seizures to DEEs. Patients underwent genetic testing using a panel with 46 genes, and a presumed disease-causing variant was detected in 23% of all cases. Neonatal-onset epilepsies were associated with the highest rate of positive findings (57%), followed by 26% amongst those who started seizures between 2 months and 2 years of life, and only 14% in those with onset between 2 and 9 years of life [47]. Although the two cohorts from the current study are not directly comparable with this previous study, and our data may be biased as they are obtained from a tertiary epilepsy center, we found that an overall diagnostic yield of about 60% if seizures started within the first 2 years of life, and 29% when seizure onset was between the 2nd and 9th years of life. In the Møller et al. study [47], the yield for patients with genetic generalized epilepsies was 17% compared to 43% (7/16, Table 1) found in the present study; the genetically solved generalized epilepsy syndromes in our study included GEFS + (5 patients), myoclonic absences (1 patient), and childhood absence epilepsy (1 patient). This was mostly attributed to the high numbers of children with GEFS + in the present study as this electroclinical syndrome is presumed to have an underlying monogenic architecture [18]. The number of CNVs in our study was approximately 9% while the diagnostic yield of panel combined with WES reached 44% (84/188); the high number of mendelian inherited disorders was likely attributed by the high number of DEEs, as these are more likely to have a monogenic etiology [48]. Although our study design was not able to compare the yield of multigene panels to that of WES, we found that WES detected (likely) pathogenic variant(s) in nine patients (9/188; 4.8%) who were genetically unsolved at study inclusion despite having been tested with a multigene panel.

WGS and WES are the most comprehensive tests in genetic diagnostics of monogenic epilepsies, and WGS is currently considered the ultimate diagnostic tool. Although increasingly considered as first-line tests, some clinicians still consider exome and genome sequencing strategies as a last resort [49].

Early use of these sequencing approaches enable a precise genetic diagnosis and can potentially end the diagnostic odyssey that many patients face. On average, a molecular cause of complex neurological disorders of suspected genetic origin in the field of pediatric neurology is determined after three misdiagnoses and 16 physician visits over several years [50, 51]. A clear benefit of WES/WGS is the possibility of reanalyzing the data annually or every second year, especially in an era when new epilepsy genes are constantly being identified [45]. WGS has a higher diagnostic yield compared to WES [2, 44], firstly, due to a more evenly distributed coverage depth [52], and secondly, because WGS captures SNVs and indels in coding and noncoding regions, CNVs, and chromosomal alterations including inversions and transpositions [45]. A clear disadvantage is the large volume of data provided by these methods, most of which can be misleading or useless.

One of the strengths of the current study was the availability of parental samples and the access to clinical data on probands and their families. This meant we could ensure that variants of interest occurred de novo and could also compare the phenotypes with those described for the implicated genes. Furthermore, all genetically unsolved patients underwent a stepwise genetic investigation; the rationale behind this approach is that although WES is a powerful diagnostic tool, it might miss CNVs that are otherwise easily detectable by microarray. Chromosomal karyotyping and *FMR1* testing were not included in the genetic workup of the present study as their diagnostic yield in a tertiary epilepsy center is limited to 2% and 0.6%, respectively [53]. A limitation of our study was that a trio WES was not offered to all patients. We suspect that this could have increased the likelihood of detecting further potential de novo disease-causing variants.

Of the 35 patients with a presumed acquired brain damage, 20 where believed to have a perinatal HIE and were subsequently not offered genetic testing. A subset of patients with perinatal HIE may actually have an underlying genetic diagnosis. A recent study exome sequenced 113 encephalopathic neonates with an acute peripartum/intrapartum event or Apgar score ≤ 7 and found that 19 patients carried disease causing genetic variations [54]. If we extrapolate these results to our study, less than five patients with HIE may have an underlying monogenic disorder; although, this is important for individual patients, it is unlikely to affect our overall results.

We considered that 295 children could potentially have a monogenic epilepsy and offered them genetic testing by electronically approaching their parents; despite two attempts to achieve consent parents of 107 patients never responded. The first study invitation was sent in 2019 and at that time patients were 10–13 years old; it is possible that many parents, after enduring a long diagnostic and therapeutic journey, were less anxious for the future and had settled with not knowing the underlying cause of the illness. Some parents might previously have received negative or uncertain results, leaving them unwilling to pursue updated genetic testing. Some patients may have had a milder and treatable epilepsy and parents might have reached a point where they felt, they no longer needed genetic testing in order to provide the best care for their child, to get information for their family, and to gather information for childbearing decisions.

Although the yield of genetic testing in difficult-to-treat childhood-onset epilepsies has been extensively investigated [2, 44], the impact of genetic testing on therapeutic decision-making has been less studied [9, 11, 53, 55]. Study results are not directly comparable due to varying study designs and study cohorts, but all papers offer an estimate of how often genetic testing enables a precision therapy approach, i.e., 44% [11], 47% [53], 63% [55], and 80% [9] of genetically solved patients. The high availability of precision therapy in the Symonds et al. study [9] was due to the high number of patients with self-limiting familial seizures caused by variants in *PRRT2* and *KCNQ2*. The impact of genetic diagnosis on precision therapy implementation and efficacy has only been studied once; although a targeted therapy was available in approximately half of the patients, a treatment change was prompted in 36% (106/293) and was effective in only 32% (34/106) [11]. A younger age at genetic testing was also associated with a more favorable seizure outcome, suggesting that the chance of improving outcome could diminish with increasing age [11]. An age effect was also reported in precision medicine studies where a drug-repurposing approach showed that better results and even seizure freedom could be achieved when the targeted treatment was started in early childhood rather than adolescence or adulthood [56]. Before we can fully understand the failure of some precision therapies, we first need to explore their efficacy as first-line treatments in prospective and preferably nationwide studies once a genetic diagnosis has been established.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13311-022-01264-1.

Acknowledgements The authors would like to thank the participants and their families for their participation in our research. Illustrations were created with BioRender.com. AB received financial support through the Disposition foundation (D51), the A.P. Møller foundation (20-L-0241), the Beckett foundation (21-2-7182), and the Grosserer L.F. Foghts foundation (22.030). The authors thank Dr. Claire Gudex for her contribution to editing the final draft of the article. The Danish Cytogenetic Central Registry study group consists of the following members: Mathilde Faurholdt Lauridsen, Christina Fagerberg, Tina Duelund Hjortshøj, Iben Bache, and Ida Vogel.

Author Contribution Conceptualization, AB and RSM; methodology, AB, RSM, and TFH; software, CDF, IN, and TRT; formal analysis, AB and CDF; data curation, AB, CDF, and TRT; writing—original draft preparation, AB; writing—review and editing, AB, CDF, TRT, IN, TFH, GR, and RSM; supervision, GR, RSM, and TFH; project

administration, AB; funding acquisition, AB and RSM. All authors have read and agreed to the published version of the manuscript.

Data Availability Anonymized data not published in this article will be made available by request from any qualified investigator.

Declarations

Ethical Publication Statement We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Disclosure CDF and IN were employed by the company Amplexa. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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