From diagnosis to treatment in genetic epilepsies : implementation of precision medicine in real-world clinical practice

# From diagnosis to treatment in genetic epilepsies: implementation of precision medicine in real-world clinical practice

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## Abstract

The implementation of whole exome sequencing (WES) has had a major impact on the diagnostic yield of genetic testing in individuals with epilepsy. The identification of a genetic etiology paves the way to precision medicine: an individualized treatment approach, based on the disease pathophysiology. The aim of this retrospective cohort study was to: (1) determine the diagnostic yield of WES in a heterogeneous cohort of individuals with epilepsy referred for genetic testing in a real-world clinical setting, (2) investigate the influence of epilepsy characteristics on the diagnostic yield, (3) determine the theoretical yield of treatment changes based on genetic diagnosis and (4) explore the barriers to implementation of precision medicine. WES was performed in 247 individuals with epilepsy, aged between 7 months and 68 years. In 34/247 (14%) a (likely) pathogenic variant was identified. In 7/34 (21%) of these individuals the variant was found using a HPO-based filtering. Diagnostic yield was highest for individuals with an early onset of epilepsy (39%) or in those with a developmental and epileptic encephalopathy (34%). Precision medicine was a theoretical possibility in 20/34 (59%) of the individuals with a (likely) pathogenic variant but implemented in only 11/34 (32%). The major

barrier to implementation of precision treatment was the limited availability or reimbursement of a given drug. These results confirm the potential impact of genetic analysis on treatment choices, but also highlight the hurdles to the implementation of precision medicine. To optimize precision medicine in real-world practice, additional endeavors are needed: unifying definitions of precision medicine, establishment of publicly accessible databases that include data on the functional effect of gene variants, increasing availability and reimbursement of precision therapeutics, and broadening access to innovative clinical trials.

Key words

(mono)genetic epilepsy

Whole exome sequencing

Diagnostic yield

Precision medicine

Abbreviations

WES: whole exome sequencing

PM: precision/personalized medicine

NGS: next generation sequencing

ASM: anti-seizure medication

ID: intellectual disability

ASD: autism spectrum disorder

MRI: magnetic resonance imaging

DEE: developmental and/or epileptic encephalopathy

GGE: genetic generalized epilepsy

PWE: people with epilepsy

LoF: loss-of-function

GoF: gain-of function

IGE: idiopathic generalized epilepsy

EEG: electroencephalography

HPO: Human Phenotype Ontology

GEFS+: genetic epilepsy and febrile seizures plus

AD(H)D: attention deficit (and hyperactivity) disorder

HTZ: heterozygous

HMZ: homozygous

AR: autosomal recessive

AD: autosomal dominant

DRE: drug-resistant epilepsy

## 1. Introduction

The etiology of epilepsy is diverse and includes genetic, structural, metabolic, immune and infectious causes.[2] Certain epilepsy syndromes have a high genetic contribution.[2-4] They can be caused either by single pathogenic genetic variants, as in developmental and/or epileptic encephalopathies (DEEs), or by a more complex oligogenic or polygenic predisposition, as assumed in genetic generalized epilepsy (GGE). In the last two decennia, next generation sequencing (NGS) techniques have led to a steep increase of novel gene discovery in epilepsy.[5,6] Whole-exome-sequencing (WES) facilitated the analysis of the complete protein-coding part of the genome in one single experiment. WES is often combined with a virtual gene panel analysis, meaning that the whole exome is sequenced, but only a specific set of genes is analysed. This method is faster and more flexible, giving the opportunity to adjust panels according to centre-specific preferences.[7]

The diagnostic yield of WES in people with epilepsy (PWE) varies widely, and depends highly on the characteristics of the target cohort. Two meta-analysis reported overall diagnostic yields for WES between 24 and 32%, with the highest diagnostic yields in individuals with early-onset seizures, intellectual disability, and/or a diagnosis of DEE.[8,9 9]

Until recently, the choice of anti-seizure medication (ASM) for genetic epilepsies was mainly guided by seizure type and epilepsy syndrome.[10] Contrary to conventional treatment, precision medicine follows the principle of providing more patient-centred and individualized care.[11-13] The concept of this approach was first coined by Jain in the early 2000s, defining precision medicine (or personalized medicine, PM) as: 'the prescription of specific therapeutics best suited for an individual, based on pharmacogenetic and pharmacogenomic information'.[14] This concept was soon successfully implemented for different epilepsies, e.g. sodium channel blockers in SCN2A-DEE or using ketogenic diet in glucose transporter 1 deficiency syndrome.[15-17] Recently, Byrne et al. proposed a six-tier-based PM framework for treating genetic epilepsies, arranged according to the degree to which the treatment addresses the underlying etiology: from therapies with a recognized response in certain epilepsy types to therapies targeting all genes and networks involved, with phenotype reversal as a result. Unfortunately, a genetic diagnosis does not equal PM for every patient, as for many genetic epilepsies a PM has not yet been determined, and many potential specific therapies are not yet available in clinical practice. Theoretical availability varies widely, from 40 to 72%, due to lack of a clear and an internationally accepted definition. Furthermore, implementation of already available PM differs based on the existing literature (table 1).[4,12,20-23]

The aim of the current study is to: (1) determine the diagnostic yield of WES in a heterogeneous cohort of PWE referred for diagnostic genetic testing in a real-world clinical setting, (2) investigate the influence of epilepsy characteristics on the diagnostic yield, (3) describe in what percentage of individuals with a genetic diagnosis PM is a theoretical option, and (4) explore the barriers to implementation of precision medicine.

## 2. Materials and methods

### *2.1 Study design*

The study cohort included all individuals with clinical suspicion of epilepsy referred for diagnostic WES to the Centre of Medical Genetics of Antwerp University Hospital. Inclusion started in January 2020 and ended in November 2021. The indication for genetic testing was determined by the referring physician, who was asked to provide a clinical summary. Individuals were eligible for inclusion, if diagnosis of febrile seizures or epilepsy was confirmed after reassessment of the patient records by the study team. Only people without a diagnosis were included, even if they underwent previous genetic testing. Records were systematically searched for the following data: age of onset of seizures, seizure type at onset, other seizures, seizure-frequency (daily, weekly, monthly, sporadic), seizure-freedom (6 months, 1 year, 2 years), eliciting factors for seizures, drug-resistance of epilepsy, use of ASM, developmental milestones, intellectual disability (ID), clinical neurological examination, dysmorphic features, electroencephalography (EEG) at onset, EEG abnormalities during follow-up, MRI reports, genetic analyses performed, personal history (including perinatal abnormalities, autism spectrum disorder (ASD) and febrile seizures) and family history of first- and second-degree relatives (including epilepsy, febrile seizures, ASD, ID and psychiatric disease).

Epilepsy was classified according to the 2022 classification of the International League Against Epilepsy (ILAE).[24] The overall diagnostic yield and epilepsy syndrome specific yield was determined. Febrile seizures were defined as recommended by the ILAE, and diagnostic yield was analysed for these individuals as well.[25] For individuals with a (likely) pathogenic variant the influence of the genetic diagnosis on therapy adjustments was analysed, first by performing a literature search for precision medicine availability, and second by analysing hurdles to their implementation. Precision medicine type was classified using the framework of Byrne et al. [12]

Age of epilepsy onset was divided in different categories: neonatal and early-infantile (≤3 months), infantile (≤1 year), between 1-5 years, 5-12 years, 12-18 years, 18-30 years, and after 30 years. Epilepsy was considered drug-resistant if 2 or more appropriately chosen anti-seizure medications/interventions were needed to control seizures.[26] ID was defined according to DSM-5 and based on formal intelligence coefficient testing (normal: ≥ 85, borderline: 70-84; mild 55-69; moderate 40-54; severe or profound < 39).[27] If these data were not available, classification was made based on clinical records and support needed. In individuals with normal to borderline intelligence, speech problems and

learning difficulties, either formally diagnosed or mentioned in the patient records by the treating physician, were reported as well.

*2.2 Whole exome sequencing and variant interpretation*

WES (Illumina) was performed on blood derived DNA after exome enrichment using the Twist Human Core Exome kit provided with additional probes for human RefSeq transcripts (Twist Bioscience) in PWE referred by their treating physician for genetic testing because of epilepsy. Variants were detected by means of an in-house pipeline (VariantDB software)[28] analyzing either a large set of genes associated with epilepsy with developmental delay phenotypes (often as trio analysis) or a more restricted set of genes known to cause familial epilepsy without developmental delay (often performed in singletons). During the study, two different versions of each panel were used. A list of genes included in the panels can be found in the supplementary material. The referring physician was responsible for choosing the appropriate gene set. In addition, exome-wide HPO (Human Phenotype Ontology)-based filtering (MOON software, Diploid/Invitae) was performed in all individuals to detect disease-associated variants in genes that were not included in the virtual gene panels. Variants were classified following the 'American College of Medical Genetics and Genomics' (ACMG) guidelines.[29] All results were discussed in monthly meetings of a multidisciplinary team consisting of molecular scientists, clinical geneticists, and (child) neurologists with specific expertise in genetic epilepsies. If necessary, additional clinical information was asked from the referring physician, or experts in the field were asked for their opinion about a specific variant. Definitive variant classification was made after consensus.

*2.3 Statistics*

Data were analysed using IBM SPSS Statistics software version 26 and with R Project for Statistical Computing, version 4.1.3. Normal distributed values were reported using means and standard deviations, non-normally distributed values were represented by their median with quartile 1 (Q1) and 3 (Q3). Fisher's exact test of independence was performed to compare diagnostic yields across different epilepsy types. Pearson's Chis-square test was used to assess relation between WES positivity and the following variables: epilepsy syndromes, prior genetic testing. Mann-Whitney U test was used to assess correlation between WES positivity and age. Variants were tested for being normally distributed using Shapiro-Wilk test. Logistic regression was used to assess correlation between WES positivity and epilepsy type for possible confounders (age, gender, ID, ASD).

*2.4 Ethical aspects*

WES was performed in line with the World Medical Association Declaration of Helsinki.[30]

All individuals, or parents/legal caregivers in case of minors, gave their written consent prior to WES analysis. The study was approved by the Ethical Committee of the University Hospital Antwerp (B300201316250).

*2.5 Literature review*

3. The selection of the articles on precision medicine for genetic epilepsies was based on a Pubmed search (September 2022) using the following search terms: (((precision medicine[MeSH Terms]) OR (personalised medicine[MeSH Terms])) AND (epilepsy[MeSH Terms]). Only articles published in 2017 or later were included. This search resulted in 122 papers, whose abstracts were screened based on population (not limited to a single genetic epilepsy) and genetic analysis used (NGS). The references of the retrieved papers were scanned for identification of additional manuscripts. This resulted in the final selection of 7 studies, listed in table 1.Results

*3.1 Case selection*

During the 22 months inclusion period, 257 individuals were referred for WES analysis for suspicion of epilepsy. Of these, 247 met clinical criteria for a diagnosis of epilepsy or febrile seizures and were included for further analysis.

*3.2 Background characteristics*

Fifty-five percent of cases were male. Median age at inclusion was 11 years (Q1: 6.2, Q3: 19.0), ranging from 7 months to 68 years. At inclusion, 154 (62%) individuals had normal to borderline intellectual ability, of which 14 (5% of total cohort) were reported to have either speech or learning problems.

Clinical neurological examination was normal in 174 (70%) individuals. Abnormalities varied from clumsiness to severe quadriparesis. Brain magnetic resonance imaging (MRI) was performed in 209 (84%) individuals and was normal in 80%. In 11 (4%) individuals MRI abnormalities were linked to their epilepsy (malformation of cortical development in 7, hypoxic-ischemic encephalopathy in 2, focal ischemic lesion in 1 and hippocampal sclerosis in 1).

A family history (first- or second-degree relatives) of epilepsy was present in 78 (32%) individuals, and of febrile seizures in 24 (9.7%).

Characteristics are summarized in table 2. Comparison of characteristics between individuals with a negative and positive WES are displayed in table 3.

*3.3 Epilepsy characteristics*

Age of onset of epilepsy varied widely and 180 (73%) individuals presented with epilepsy between 3 months and 12 years of age. Specific age categories at epilepsy onset are represented in figure 1.

Epilepsy was classified as follows: 74 (30%) had a DEE, 3 (1%) had a syndrome with progressive neurological deterioration, namely progressive myoclonus epilepsy, 70 (28%) had a focal epilepsy, 55 (22%) had a GGE, and 8 (3%) had a generalized and focal epilepsy syndrome, namely GEFS+. Further details are summarized in Table 4.

*3.4 Genetic analysis*

More than half of the individuals included in the cohort had received some form of genetic testing prior to WES. Approximately half of individuals (127, 51%) underwent genome-wide single nucleotide polymorphism (SNP) array-based copy number variant analysis. A targeted epilepsy-related gene panel (non-WES-based) was performed in 46 (19%) individuals, and in 11 (4%) another WES-based panel, analysing a set of genes not primarily associated with epilepsy, had been performed. Other analyses (e.g. single gene analysis, karyotyping, *FMR1* analysis) were performed in 65 (23%) individuals.

*3.4.1 Genetic variants*

Pathogenic or likely pathogenic variants were found in 28 different genes in 19 (7,7%) and 15 (6,1%) individuals respectively. The *ATP1A3, CDKL5, DLG4, GABRB3, GNAO1, GRIN2A, HECW2, KDM6B, KCNMA1, KMT2E, NEXMIF, PHF21A, PIGN, QARS1, RORA, SCN8A, SLC2A1, SLC6A1, STXBP1, TBCD, TPP1*, *TSC2, ZMYM2* and *ZNF142* genes were each found to be mutated in one individual. In the following genes (likely) pathogenic variants were found in multiple individuals: *CHD2* (n=2), *DEPDC5* (n=4), *PRRT2* (n=2) and *SCN2A* (n=2). Variants of unknown significance (VUS) were found in 21 individuals in 23 genes. An overview of all (likely) pathogenic variants, as well as a listing of the VUS, are given in table 5.

*3.4.2 Diagnostic yield*

The overall diagnostic yield (pathogenic or likely pathogenic variants) was 34 (14%). In an additional 21 (9%) individuals a class 3 variant was reported. Interestingly, of the 34 individuals with a clear molecular diagnosis, 7 (7/34: 21%, 3% of total cohort) were diagnosed solely through the exome-wide HPO-based filtering, as the variant was not identified by virtual gene panel analysis.

The diagnostic yield (figure 1) was significantly influenced by epilepsy type (Fisher exact test: p< 0.001): 26/77 (34%) DEE individuals had a genetic diagnosis, 5/70 (7%) individuals with a focal epilepsy, 2/55 (4%) individuals in the GGE group, 1/8 (12,5%) individuals with GEFS+. In individuals with only febrile seizures, no genetic diagnosis was found. In the DEE group, diagnostic yield raised to 37% after exclusion of structural causes and individuals with an epileptic encephalopathy, according to the 2022 ILAE classification.[24] To investigate whether the link between epilepsy type and diagnostic yield was purely driven by DEE, we performed a subanalysis excluding individuals with a DEE. Diagnostic yield indeed did not significantly differ between epilepsy types in this subgroup (Fisher exact test: p = 0.435).

As expected, the diagnostic yield differed significantly according to the age of seizure onset (Fisher's exact test: p= 0.010) and declined with increasing age of onset. Median age of epilepsy onset was 17 months (Q1: 4.75; Q3: 49.5) for PWE with a positive WES compared to a median age of 60 months (Q1: 17, Q3: 120) for PWE with a negative WES (Mann-Whitney U test: p=0.029). Presence of intellectual disability was associated with higher diagnostic yield (Pearson's Chi-square test: 7/135; 5% vs. 27/104; 26%, p=0.001). Remarkably, overall diagnostic yield was significantly higher in females than in males (Pearson's Chi-square test: 22/111, 20% vs. 12/136, 9%, p= 0.013), although gender among epilepsy groups and age did not differ significantly (Fisher's exact test: p=0.426, Mann-Whitney U test: p=0.636). Logistic regression still showed that gender was an independent predictor of WES positivity after correction for age, epilepsy group, ID and ASD.

The diagnostic yield was not different for individuals who already had prior negative or inconclusive genetic testing compared with individuals in which WES was the first genetic test performed (Pearson's Chi-Square test 26/182 vs. 8/65, p=0.835). For individuals that specifically had prior testing with a targeted epilepsy-related gene panel or a WES-based panel for diseases other than epilepsy, the diagnostic yield of WES was 5/54 (9,3%). The diagnostic yield was

not different for these individuals compared to those without prior genetic testing (Pearson's Chi-Square test: 5/54 vs. 29/193 , p = 0.277).

Rapid WES (mean turnaround time was 21 days) was performed in 7 individuals resulting in a high diagnostic yield of 86% (6/7). Indication for rapid WES included neonatal therapy-resistant seizures in 6 individuals, and a childhood-onset DEE with neurological regression in 1 individual.

*3.5 Precision medicine*

In 20/34 (59%) individuals with a genetic diagnosis PM was a theoretical possibility. In 5/20 (25%) individuals, this treatment was already implemented based on clinical suspicion of genetic diagnosis before genetic confirmation. In these 5 individuals ASM known to be beneficial were started. In 6/20 (30%) individuals genetic diagnosis led to treatment changes. In case 5 with a *PIGN*-DEE, pyridoxin (13 mg/kg/day) was started, leading to seizure reduction. In case 19, a boy with a *SCN8A*-DEE, introduction of carbamazepine (17mg/kg/day) led to a seizure-free interval of 3 months until now. In case 30 with neuronal ceroid lipofuscinosis type 2, due to a compound heterozygous *TPP1* mutation, enzyme replacement therapy was started (cerliponase alfa). A 300 mg dose was administered intrathecally with a 2-week interval. There was a decrease of disease progression compared with historical cohorts. Developmental assessment, using Bayley scales of infant and toddler development III, showed a cognitive developmental age of 19 months (at age 5, 17 months after initiation of treatment). This was a slight decline compared with testing before start (cognitive developmental age of 22 months at age of 4 years). Unfortunately, no clear effect was seen on her seizures, which continued at a daily basis. In case 31, a 14-month-old boy with a DEE due to a *GABRB3* gain of function (GoF) variant, vigabatrine was stopped because of potential hazardous effects.[31] There was no clear effect on seizure frequency, but alertness improved. In case 26 a glucose transporter 1 deficiency syndrome was diagnosed at adolescent age, and modified Atkins ketogenic diet was introduced, due to persistent absences and concentration disturbances despite lamotrigine. Absences were clearly reduced initially, but at this moment, adherence to ketogenic diet is limited, making evaluation of its effectiveness impossible. In the remaining patient (case 23) with a *SCN2A*-DEE, there was a clear reduction of seizures with carbamazepine, eliminating seizure during the day and improving alertness.

Reasons for not implementing treatment changes in the remaining 9 individuals were: seizure-control (case 2), no reimbursement of the drug for the indication of epilepsy (case 4,8,11,13), drug only available in clinical trials (case 22, 32,33) and unclear functional effect of the gene variant (case 20). Detailed summary of these individuals, their genetic variants and PM options, according to the framework of Byrne et al can be found in table 5.[12]

## 4. Discussion

The importance of genetic testing in epilepsy is well established and WES is increasingly becoming the cornerstone of genetic diagnosis in epilepsy. The first aims of the current study were to determine the real-world diagnostic yield of WES and the influences of epilepsy characteristics on this diagnostic yield. In this real-world cohort of 247 PWE, a diagnostic yield of 14% was seen, which is low compared to other studies.[8] A likely reason for the lower diagnostic yield in our study is the heterogeneous cohort of PWE, reflecting the characteristics of PWE that are currently referred for WES in countries with good access to diagnostic genetic testing. First, two-thirds of our cohort had already undergone genetic testing at some point during their disease history, and only those without genetic diagnosis were referred for WES. This means that many of the more common genetic causes of epilepsy will have been detected earlier, at least in those with longer-standing epilepsy. Importantly, the diagnostic yield in individuals who had previously undergone targeted panel analysis was still 9%. Given the continuous advances in gene discovery, gene panels are quickly outdated, and our results underscore the recommendations of the ILAE to use WES as a first-tier genetic test if possible.[32] Second, only 30% of our cohort had a DEE, only 23% had an onset of epilepsy before the age of 1 year, and the proportion of individuals with intellectual disability was only 42%. All three factors have been shown to contribute to a higher yield of genetic testing.[3,22,23] Third, a large proportion of the cohort had an epilepsy type in which yields of genetic testing are traditionally low: around one quarter of the study cohort were individuals with GGE, an entity generally having a complex polygenic background, explaining the low diagnostic yield of 4% in these individuals.[33] Our cohort also included a relatively high number of individuals with complex febrile seizures (24/247, 10%). We did not find any (likely) pathogenic variants in these patients, contributing to the lower overall diagnostic yield in this cohort. To fully reflect the real-world setting, we also did not exclude structural epilepsies (4/247, 1%), not associated with malformations of cortical development. In many studies, these patients would be excluded. In clinical practice, however, these cases are sometimes referred for genetic testing as well, as it is not always clear to what extent structural abnormalities on imaging contribute to the phenotype.

As already established by previous research, the highest diagnostic yield was seen in individuals with early-onset seizures or a DEE.[8] In this respect, diagnostic yield of rapid WES, provided to patients with a selection of patients with neonatal onset epilepsy and/or progressive neurodevelopmental problems, was extremely high (86%), highlighting the

value of this procedure, provided that test indications are carefully chosen. Remarkable, female gender was associated with a higher diagnostic yield, which is not reported until now, and could not be explained by overrepresentation of the female sex in certain epilepsy types, age of onset or ID. This needs to be confirmed in larger cohorts, as unknown confounders can be the reason for this unexpected correlation.

Our results also highlight the added value of exome-wide HPO based analysis complementary to the use of virtual WES-based epilepsy panels. Seven PWE (7/34: 21%, 3% of total cohort) had a genetic diagnosis of a pathogenic variant in a gene not included in the epilepsy panel (*KDM6B*, *ZMYM2*, *ZNF142*, *DLG4* and *PHF21A* variants in respectively case 9, 16, 24, 27 and 28), but perfectly matching phenotype.

We further wanted to define the theoretical possibility and difficulties of implementing PM in clinical practice. PM was a theoretical possibility for 59% of individuals in this study cohort for whom a genetic diagnosis was established. Genetic diagnosis led to treatment adjustments in 30%. In another 28% PM was already applied before genetic diagnosis, and genetic diagnosis subsequently confirmed clinical suspicion. It illustrates the importance of a careful clinical diagnosis and epilepsy classification. For example, in case 3 *(TSC2)*, vigabatrine was started because of clinical diagnosis of infantile epileptic spasms syndrome. Another example is case 18, a neonate, who was treated early with carbamazepine because of clinical suspicion (neonatal clusters of tonic seizures) of a GoF mutation in *SCN2A*. Main barriers to implementation were mainly practical, being the lack of reimbursement of the drug for this indication (and therefore the high costs) or because the drug is currently only available in a clinical trial setting. For example, individuals with an epilepsy due to a *DEPDC5* (likely) pathogenic variant (case 4,8,11,13) could theoretically benefit from mTOR inhibition, but can only be used off-label for this indication.[34] As evidence for the cost-effectiveness of certain repurposed drugs for genetic epilepsies increases, reimbursement policies will hopefully be adapted. For individuals with epilepsy due to a *SLC6A1* or *STXBP1* (likely) pathogenic variant, phenylbutyrate is currently being investigated in clinical trials, but inclusion is currently not possible for Belgian (and many other) patients. In case 2, seizure-control was considered adequate, and adjustment of treatment was therefore not made. Finally, the last barrier to implementation was the lack of knowledge about the functional consequences of a specific gene variant, crucial to select the right PM, especially for genetic epilepsies due to variants in ion channel genes. In case 20 *(GRIN2A)*, for example, different precision medicine options would be available in case of a LoF (loss-of-function) or

GoF effect. Because of lack of knowledge about the functional effect of this specific variant, no specific treatment choice could be made.

Fortunately, the increasing knowledge of the functional effects of gene variants will eventually increase implementation of PM. Public databases collating all available information about disease-associated variants further facilitate the implementation of PM. Examples of such databases are the *SCN1A* mutation database and prediction model, the curated RIKEE-database of variants in the genes *KCNQ2/3/5*, the *KCNA2* 4-AP treatment website for *KCNA2* variants, and the *GRIN* variants database for variants in the *GRIN* genes..[35-39]

It is still unclear to what extent new therapies prove to be disease-modifying in genetic epilepsies, and determining their impact will require long-term follow-up studies. The promising results of gene therapy in mouse models of Dravet syndrome as well as the successful use of gene therapy in other neurological diseases, offer hopeful perspectives for the future.[40,41] Apart from the need of multicentric clinical trials, given the rare disease population, definition of patient-centred outcome measures will be essential to target relevant clinical features that matter most to patients and caregivers.[42,43] Study teams and clinicians should not focus solely on seizure reduction, but also consider composite endpoints to evaluate treatment. To learn about the individual response of individuals to a certain PM, it is essential that these studies also report on the failures of therapies.[12,44,45] Because of the very low incidences of some of these monogenic epilepsies, rationally designed n-of-1 trials, in which a single patient serves both as a case and a control, will be needed to further widen the horizon of PM.[46]

Our study also has limitations. Because of the real-world setting, our cohort included individuals who had previously undergone genetic testing, so our study approach does not allow us to define the diagnostic yield of WES in genetic testing-naive cases with otherwise similar disease characteristics. This most likely explains why no variants were detected in some of the more frequently affected genes in PWE including *SCN1A*, *PCDH19* or *KCNQ2*. Because individuals were sometimes referred for genetic testing from external centers, clinical records were not always completely available to the study team, possibly leading to incorrect epilepsy or level of ID classification. We also acknowledge that indications for genetic testing in epilepsy differ between countries, influencing study population. Therefore, caution is required when comparing the diagnostic yield of genetic testing. In this regard, the ILAE task force on clinical genetic testing recently issued an opinion paper which can guide clinicians to select the right PWE for genetic

testing.[32] In parallel, genetic testing should not be performed in individuals with simple febrile seizures, but in some cases of recurrent and/or complex febrile seizures, genetic analysis to exclude monogenic underlying etiology (e.g. *SCN1A*) can be justified.

Another limitation of this study, and in general for many studies about PM, is the lack of a clear definition of PM. We chose to use the tier classification by Byrne et al.[12], to define possible PM in the study cohort. It can however be debated whether therapies belonging to the first two tiers are indeed a form of PM, as they do not target the gene dysfunction per se. Differences in definition of PM should therefore be taken into account when comparing our results with those of other studies.

To conclude, WES can certainly shorten 'the diagnostic odyssey' in epilepsy, but for a significant number of individuals (213/247, 86%) this journey continues. This cohort emphasizes the wide variation of PWE that are referred for genetic testing in clinical practice, resulting in a lower diagnostic yield compared to other more selected cohorts. We confirm that in individuals with early-onset epilepsy or epilepsy with concomitant ID, and particularly DEE, diagnostic yield of WES is high, and WES should be prioritized as an early tool in the diagnostic approach. We further showed that establishing a genetic diagnosis had potential treatment implications in about half of the cohort. Practical implementation of PM is however challenging. In only 30% of individuals with a genetic diagnosis, this led to treatment changes, acknowledging that an additional 20% already received a PM approach based on clinical suspicion. To increase implementation of PM, we first need a strong collaboration between patient stakeholders, treating clinicians and researchers, which invests in documentation of detailed phenotype and genotype information including the available functional evidence of associated gene defects, and in making this information publicly available for clinical practice. Second, we need to define standards for innovative trial designs that lower thresholds for participation of broader groups of often severely affected children. Together, this will lead to an increase in PM possibilities, making them available for an increasing number of patients, and hopefully reducing costs and expending reimbursement criteria.

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8. Appendices

**Table 1: Precision medicine in genetic epilepsies: highlights of the literature.**

| Author, year of publication | Cohort | Theoretical precision medicine (%) | Implementation (%) | Outcome (n) | Barriers to implementation (n) | Authors' conclusions | Remarks |
|---|---|---|---|---|---|---|---|
| Snoeijen-Schouwenaars et al., 2017[23] | 25 PWE + ID (tertiary centres) | 10/25 (40%) | 1/10 (10%)<br>4/10 (40%) effective treatment prior to genetic diagnosis | Reducing lamotrigine with significant changes in mood/behavior. | - Not available in clinical setting (5) | WES diagnostics might be relevant for the treatment strategy. | Individuals considered for PM included 3 variants in *SLC*-genes, for which availability of PM is doubtful (but can be considered as tier 1).[12,48] |
| Peng et al, 2018[20] | 86 DRE (tertiary center) | 62/86 (72%) | 34/62 (55%) | - Seizure-freedom during at least 6 months (18/34, 53%)<br>- Seizure reduction during at least 6 months (13/34, 38%) | - Patient/parental refusal (28) | NGS can benefit individuals by improving diagnosis accuracy and treatment efficacy. | - Highly selected cohort of drug-resistant epilepsy, with high number of infantile spasms, and presumable DEE<br>- Broad interpretation of treatment impact. Inclusion in tier 1 is questionable for some.[12]<br>- Short follow-up, no clear definition of seizure-reduction |
| Truty et al, 2019[22] | 1502 PWE (secondary and tertiary centers) | 869/1502 (58%) | Not mentioned | Not mentioned | Not mentioned | NGS can possibly enable precision medicine approaches in a significant number of individuals with epilepsy. | - Theoretical precision medicine included: introduction of specific ASM (198/491, 40%), withdrawal of contraindicated ASM (242/491, 49%), metabolic/diet adjustments (51/491, 10%), additional 377/1502 (25%) possible clinical trial available. |
| Demos et al., 2019[4] | 59 PWE, onset before 5 years of age | 27/59 (46%) | 22/27 (81%)<br>1/27 ((4%) effective treatment prior to genetic diagnosis | - Not mentioned (10)<br>- No effect (3)<br>- Withdrawal of ASM due to benign course (1)<br>- Seizure-freedom (4)<br>- Seizure reduction (2)<br>- Early palliative care (1) | - Unknown (3)<br>- Deceased prior to implementation (1) | Early diagnosis and intervention are important, but advances in precision medicine are also required | Of the 27 patients in whom PM was a theoretical option, 8/27 (30%) had a *SCN1A*-mutation, potentially explaining the high implementation rate in this cohort, as treatment strategies in *SCN1A* are well-defined. |
| Balestrini et al., 2020[21] | 293 PWE | PM group: 33/56 (59%)<br><br>General treatment change group: not mentioned | Overall 94/293 (32%)<br>- PM group: 33/56 (59%)<br>- General treatment change group: 73/237 (31%) | - PM group: (10/33, 30%)<br>- General treatment change group: (24/73, 33%) | - Seizure-control or acceptance (9)<br>- Other effective treatment (5)<br>- No (or not yet) follow-up after diagnosis (3)<br>- Refusal by parents (2)<br>- Deceased patient (1)<br>- Funding difficulties (1)<br>- Unknown (2) | Limited reach of PM in epilepsies | - Authors made differentiation between precision medicine and general treatment changes due to genetic diagnosis.<br>- Improvement in quality of life in 114 individuals (39%) |
| Zou et al., 2021[49] | 320 PWE, suspicion of underlying genetic disease | 42/320 (13%) | Not mentioned | Seizure-freedom in 2 individuals (2/42,5%) | Not mentioned | Genetic results can improve therapy. However, change of clinical managements still relies on patient data or clinical studies. | - Possibly higher success rate, but not clearly mentioned<br>- Broad interpretation of treatment impact. Inclusion in tier 1 is questionable for some (e.g. benzodiazepines).[12] |
| Bayat et al., 2022[50] | 101 PWE | 53/101 (52%) | 32/53 (60%) | Seizure reduction (> 50%) in 30/32 (93%) of which 4 became seizure-free. | Seizure-control (12/53, 40%) | Genetic diagnosis enables PM in 50% of patients and results is seizure reduction in the majority of them | - Extensive documentation of PM strategies used, all to be considered tier 1 or higher.[12]<br>- High proportion of individuals with DEE |

WES: Whole exome sequencing, PWE: people with epilepsy; ID: intellectual disability; e.g.: for example; PM: precision medicine; DRE: drug-resistant therapy; NGS: next generation sequencing.

**Table 2: Background characteristics, family history, genetic testing and epileptic characteristics of individuals.**

| | | n | % |
|---|---|---|---|
| Total cohort | | 247 | 100 |
| Gender | Male | 136 | 55.1 |
| | Female | 111 | 44.9 |
| WES | Positive* | 34 | 13.8 |
| | Negative | 189 | 76.5 |
| | Class 3 variant | 21 | 8.5 |
| | Others (heterozygous carrier of AR disease; incidental finding) | 3 | 1.2 |
| Perinatal problems | Absent | 215 | 87 |
| | Present | 12 | 4.9 |
| | Unknown | 20 | 8.1 |
| Early milestones | Delayed | 57 | 23.1 |
| | Normal | 176 | 71.3 |
| | Unclear | 4 | 6.0 |
| Cognition | Normal | 122 | 49.4 |
| | Learning problems | 8 | 3.2 |
| | Isolated speech problems | 5 | 2.0 |
| | Borderline | 19 | 7.7 |
| | Mild | 34 | 13.8 |
| | Mild to moderate | 11 | 4.5 |
| | Moderate | 12 | 4.9 |
| | Severe to profound | 28 | 11.3 |
| | Unknown | 8 | 3.2 |
| ASD | Yes | 22 | 8.9 |
| | No | 225 | 91.1 |
| Dysmorphic features | Yes | 24 | 9.7 |
| | No | 212 | 85.8 |
| | Unknown | 11 | 4.5 |
| Neurologic examination | Normal | 174 | 70.4 |
| | Abnormal | 62 | 25.1 |
| | Unknown | 11 | 4.5 |
| Brain MRI | Normal | 168 | 68 |
| | Abnormal | 41 | 16.6 |
| | Unknown | 38 | 15.4 |
| Febrile seizures in personal history | Yes | 51 | 20.6 |
| | No | 189 | 76.5 |
| | Unknown | 7 | 2.8 |
| Psychiatric/behavior problems in personal history | Yes | 37 | 15 |
| | No | 203 | 82.2 |
| | Unknown | 7 | 2.8 |
| Family history of epilepsy | Yes | 78 | 31.6 |
| | No | 161 | 65.2 |
| | Unknown | 8 | 3.2 |
| Family history of febrile seizures | YES | 24 | 9.7 |
| | NO | 215 | 87.0 |
| | Unknown | 8 | 3.2 |
| Family history of ASD | Yes | 9 | 3.6 |
| | No | 230 | 93.1 |
| | Unknown | 8 | 3.2 |
| Family history of intellectual disability | Yes | 13 | 5.3 |
| | No | 226 | 91.5 |
| | Unknown | 8 | 3.2 |
| Family history of psychiatric disorders and AD(H)D | Yes | 8 | 3.2 |
| | No | 225 | 91.1 |
| | Unknown | 14 | 5.7 |
| Micro-array performed | Yes | 127 | 51.4 |
| | No | 120 | 48.6 |
| Targeted epilepsy gene panel performed | Yes | 46 | 18.6 |
| | No | 201 | 81.4 |
| Other WES performed | Yes | 11 | 4.5 |
| | No | 236 | 95.5 |
| Other genetic analysis° | Yes | 65 | 26.3 |
| | No | 182 | 73.7 |
| Seizure frequency at inclusion | Sporadic | 33 | 13.4 |
| | Daily | 29 | 11.7 |
| | Weekly | 18 | 7.3 |
| | Monthly | 13 | 5.3 |

| | | | |
|---|---|---|---|
| | Seizure-free (more than 6 months) | 144 | 58.3 |
| | Unknown | 10 | 4.0 |
| Duration of seizure-freedom at time of inclusion | 6 months | 20 | 8.1 |
| | 1 year | 54 | 21.9 |
| | 2 years | 70 | 28.3 |
| | Not seizure-free | 93 | 37.7 |
| | Unknown | 10 | 4.0 |
| Drug-resistant epilepsy[#] | Yes | 91 | 36.8 |
| | No | 147 | 59.5 |
| | Unknown | 9 | 3.6 |

* Positive: individuals in which WES showed a class 4 or 5 variant according to the ACMG-classification, either found by the WES-based epilepsy panel or by the HPO-based variant filtering;

°Other genetic analysis: karyotyping, *FMR1*-analysis and specific single gene analysis (Sanger).

[#] Valproic acid was used in 106 (43%) individuals and was the most prescribed ASM. Vagal nerve stimulation was used in 5 individuals, deep brain stimulation in 1 and ketogenic diet in 1.

WES: Whole exome sequencing; N.A: not applicable. AD(H)D: attention deficit (and hyperactivity) disorder GEFS+: genetic epilepsy and febrile seizures plus; IGE: idiopathic generalized epilepsy. ASD: autism spectrum disorder.

**Table 3: Comparison of characteristics between individuals with positive vs. negative WES**

| | | WES | | |
|---|---|---|---|---|
| | | Positive* (n,%) | Negative (n,%) | Statistics (p-value) |
| **Onset of epilepsy** | < 1 year | 17 (50%) | 40 (19%) | Pearson Chi-square (p < 0.001) |
| | > 1 year | 17 (50%) | 173 (81%) | |
| | Total | 34 | 213 | |
| **Intellectual disability** | Yes | 7 (8%) | 77 (38%) | Pearson Chi-square (p < 0.001) |
| | No | 27 (17%) | 128 (62%) | |
| | Total | 34 | 205 | |
| **Drug-resistant epilepsy** | Yes | 21 (62%) | 70 (34%) | Pearson Chi-square (p = 0.004) |
| | No | 13 (38%) | 134 (66%) | |
| | Total | 34 | 204 | |
| **Autism spectrum disorder** | Yes | 3 (9%) | 19 (9%) | Fisher's exact test (p = 1) |
| | No | 31 (91%) | 194 (91%) | |
| | Total | 34 | 213 | |
| **Febrile seizures** | Yes | 8 (24%) | 43 (21%) | Pearson Chi-square (p = 0.726) |
| | No | 26 (66%) | 163 (79%) | |
| | Total | 34 | 206 | |
| **Familial history of febrile seizures** | Yes | 3 (9%) | 21 (10%) | Fisher's exact test (p = 1) |
| | No | 29 (91%) | 185 (90%) | |
| | Total | 32 | 206 | |
| **Familial history of epilepsy** | Yes | 7 (21%) | 71 (34%) | Pearson Chi-square (p = 0.132) |
| | No | 26 (79%) | 135 (66%) | |
| | Total | 33 | 206 | |

* Positive: individuals in which WES showed a class 4 or 5 variant according to the ACMG-classification, either found by the WES-based panel or by the HPO-filtering
WES: Whole exome sequencing;

## Table 4: Epilepsy classification of individuals according to the ILAE classification 2022

| ***Syndrome and epilepsy classification*** | **Subtype/ etiology** | **n** |
|---|---|---|
| *Syndromes with developmental and/or epileptic encephalopathy and syndromes with progressive neurological deterioration* | | **77** |
| Developmental and/or epileptic encephalopathy | | 74 |
| | Structural | 3 |
| | Epilepsy with myoclonic-atonic seizures | 4 |
| | Infantile epileptic spasm syndrome | 8 |
| | Epilepsy in infancy with migrating focal seizures | 1 |
| | Metabolic | 3 |
| | Lennox-Gastaut | 9 |
| | EE-SWAS | 2 |
| | FIRES | 1 |
| | DEE: not further specified | 43 |
| Progressive myoclonic epilepsy | | 3 |
| *Generalized and focal epilepsy syndromes* | | **8** |
| Genetic epilepsy with febrile seizures plus | | 8 |
| *Focal epilepsies* | | **70** |
| Focal: mri-negative | | 38 |
| | Sleep-related hypermotor epilepsy | 2 |
| | Frontal lobe epilepsy | 3 |
| | Temporal lobe epilepsy | 2 |
| | Focal, mri negative: not further specified | 31 |
| Focal: self-limiting | | 25 |
| | Self-limited epilepsy with centrotemporal spikes | 15 |
| | Childhood occipital visual epilepsy | 1 |
| | Familial focal epilepsy with variable foci | 3 |
| | Self-limited (familial) infantile epilepsy | 2 |
| | Self-limited (familial) neonatal epilepsy | 1 |
| | Focal, self-limiting: not further specified | 3 |
| Focal: structural | | 7 |
| | Malformation of cortical development | 6 |
| | Hippocampal sclerosis | 1 |
| *Genetic generalized epilepsy* | | **55** |
| Idiopathic generalized epilepsy | | 38 |
| | Childhood absence epilepsy | 5 |
| | Childhood/ Juvenile absence epilepsy | 8 |
| | Juvenile absence epilepsy | 12 |
| | Juvenile myoclonic epilepsy | 5 |
| | Generalized tonic clonic seizures only | 8 |
| Myoclonic epilepsy in infancy | | 2 |
| Epilepsy with eyelid myoclonia | | 1 |
| GGE: not further specified | | 14 |
| *Epilepsy: not possible to classify more specifically* | | **13** |
| *Febrile seizures only* | | **24** |
| | | **247** |

DEE: developmental and/or epileptic encephalopathy, GGE: genetic generalized epilepsy; EE-SWAS: epileptic encephalopathy with spike-and-wave activation in sleep; FIRES: febrile infection-related epilepsy syndrome.

**Table 5: Characteristics and precision medicine options of individuals with pathogenic and likely pathogenic variants**

| Case | Age at inclusion | Age at epilepsy onset | Time to genetic diagnosis | Gender | Gene (NM number) | Variant | Inheritance (parental origin) | Class[#] | Functional effect | Epilepsy classifica-tion | Degree of ID | Precision medicine options, tier according to Byrne et al.[12] | Implementation/barriers for implementation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 31y | 4m | 23y9m | female | *PRRT2* (NM_145239.2) | c.824C>T; p.(Ser275Phe) | HTZ AD (unknown) | 4 | LOF[51] | Self-limited familial neonatal epilepsy | None | Carbamazepine[52]; Tier 2 | No treatment changes Already started prior to genetic diagnosis |
| 2 | 26y | 19m | 22y8m | female | *KMT2E* (NM_182931.2) | c.1729_1733del; p.(Glu577Lysfs*14) | HTZ AD (*de novo*) | 5 | LOF | DEE | Mild | N-acetylcysteine or antioxidantia.[53] | No treatment changes Seizure control |
| 3 | 2y | 6m | 1y1m | male | *TSC2* (NM_000548.4) | c.4351dupC; p.(Arg1451Profs*73) | HTZ AD (*de novo*) | 5 | LOF | TSC | Mild | Early vigabatrin; Tier 2 mTOR-inihibition[54]; Tier 3 | No treatment changes Already started on vigabatrin prior to genetic diagnosis |
| 4 | 50y | 14y | 22y5m | female | *DEPDC5* (NM_001242896.1) | c.2760C>A; p.(Tyr920*) | HTZ AD (unknown) | 5 | LOF | Familial focal epilepsy with variable foci | None | mTOR-inihibition[34]; Tier 3 | No reimbursement for this indication |
| 5 | 1y5m | 6m | 7m | male | *PIGN* (NM_176787.4) | c.932T>G; p.(Leu311Trp) | HMZ AR (paternal + maternal) | 5 | LOF[55] | DEE | Severe to profound | Pyridoxine[56] | Implemented |
| 6 | 21y | 4y | 13y2m | female | *NEXMIF* (NM_001008537.2) | c.3734dup; p.(Ser1246Lysfs*15) | HTZ XL (*de novo*) | 5 | LOF | DEE | Moderate | None | N.A. |
| 7 | 17m | 7m | 5m | female | *PRRT2* (NM_145239.2) | c.649dup; p.(Arg217Profs*8) | HTZ AD (unknown) | 5 | LOF | Self-limited familial neonatal-infantile epilepsy | None | Carbamazepine[52]; Tier 2 | No treatment changes Already started prior to genetic diagnosis |
| 8 | 9y | 6y6m | 1y9m | male | *DEPDC5* (NM_001242896.1) | c.2760C>A; p.(Tyr920*) | HTZ AD (paternal) | 5 | LOF | Familial focal epilepsy with variable foci | Borderline | mTOR-inihibition[34]; Tier 3 | No reimbursement for this indication |
| 9 | 11y | 9y | 2y3m | female | *KDM6B* (NM_001348716.1) | c.1471_1487delinsGGGCTG; p.(Cys491Glyfs*1) | HTZ AD (*de novo*) | 4 | LOF | DEE | Borderline | None | N.A. |
| 10 | 9y | 5y | 3y6m | female | *CHD2* (NM_001271.3) | c.3922_3926delinsC; p.(Lys1308Argfs*10) | HTZ AD (*de novo*) | 5 | LOF | IGE: childhood/ juvenile absence epilepsy | Borderline | None | N.A. |
| 11 | 11y | 10y | 9m | female | *DEPDC5* (NM_001242896.1) | c.2512C>T; p.(Arg838*) | HTZ AD (unknown) | 5 | LOF | Familial focal epilepsy with variable foci | None | mTOR-inihibition[34]; Tier 3 | No reimbursement for this indication |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | 21m | 4m | 1y3m | female | *TBCD* (NM_005993.4) | c.2314C>T; p.(Arg772Cys) | HMZ AR (paternal + maternal) | 4 | Probably LOF*,[57] | DEE | Severe | None | N.A. |
| 13 | 18y | 2y10m | 12y7m | female | *DEPDC5* (NM_001242896.1) | c.2760C>A; p.(Tyr920*) | HTZ AD (unknown) | 5 | LOF | GEFS+ | None | mTOR-inihibition[34]; Tier 3 | No reimbursement for this indication |
| 14 | 4y | 5m | 3y6m | male | *HECW2* (NM_020760.2) | c.4471G>C; p.(Glu1491Gln) | HTZ AD (*de novo*) | 5 | unknown | DEE | Severe | None | N.A. |
| 15 | 7y | 3y | 1y2m | male | *KCNMA1* (NM_001014797.2*)* | c.2563C>T; p.(Arg855Trp) | HTZ AD (*de novo*) | 4 | unknown | DEE | Moderate | None | Selective BK activator[58] only investigated in experimental animal models. |
| 16 | 29y | 3y | 23y9m | male | *ZMYM2 (*NM_003453.4) | c.2479C>T; p.(Arg827*) | HTZ AD (unknown) | 4 | LOF | DEE (Lennox-Gastaut) | Severe to profound | None | N.A. |
| 17 | 15y | 1y | 13y11m | female | *ATP1A3* (NM_152296.4) | c.2525T>A; p.(Met842Lys) | HTZ AD (*de novo*) | 4 | unknown | DEE | Mild | Flunarazine[59]; Tier 2 | No treatment changes Already started prior to genetic diagnosis |
| 18 | 7m | 1st day | 9m | female | *SCN2A* (NM_021007.2) | c.5408A>T; p.(Glu1803Val) | HTZ AD (*de novo*) | 4 | Probably GOF$ | DEE | Mild | Sodium channel blocker[60]; Tier 3 | No treatment changes Already started on carbamazepine prior to genetic diagnosis |
| 19 | 2y | 1y | 2m | male | *SCN8A* (NM_014191.3) | c.3967G>A; p.(Ala1323Thr) | HTZ AD (*de novo*) | 4 | Probably GOF$ | DEE (Infantile epileptic spasm syndrome) | Mild | Sodium channel blocker[60]; Tier 3 | Implemented<br><br>Specific sodium channel blocker ($Na_v1.6$ channel)[61] ; Tier 3, currently investigated in clinical trials. |
| 20 | 6y | 3y | 9m | female | *GRIN2A* (NM_000833.4) | c.1513G>A; p.(Ala505Thr) | HTZ AD (*de novo*) | 4 | Unknown | DEE | Mild | Memantine[62] (if GoF); Tier 3 L-Serine[63] (if LoF); Tier 3 | No treatment changes Functional effect unclear. |
| 21 | 17y | 9y | 7y4m | female | *RORA* (NM_134260.2) | c.325T>C; p.(Cys109Arg) | HTZ AD (*de novo*) | 4 | Probably LOF[64] | IGE: Epilepsy with eyelid myoclonia | Borderline | None | N.A. |
| 22 | 16y | 2y | 12y10m | male | *SLC6A1* (NM_003042.3) | c.131G>A; p.(Arg44Gln) | HTZ AD (absent in mother, father unknown) | 5 | LOF[65] | DEE | Moderate | phenylbutyrate[66]; Tier 3 | No treatment changes Only available in clinical trials, currently no enrollment possible |
| 23 | 10y | 11 days | 10y9m | female | *SCN2A* (NM_001040143.1) | c.629T>C; p.(Leu210Pro) | HTZ AD (*de novo*) | 4 | Probably GOF$ | DEE | Severe to profound | Sodium channel blocker[60]; Tier 3 | Recently switched. |
| 24[67] | 3y | 1y | 1y2m | female | *ZNF142* (NM_001379659.1) | c.2506C>T; p.(Arg836*) | Compound HTZ AR (absent in mother, father unknown = donor) | 4 | LOF | DEE | Mild | None | N.A. |

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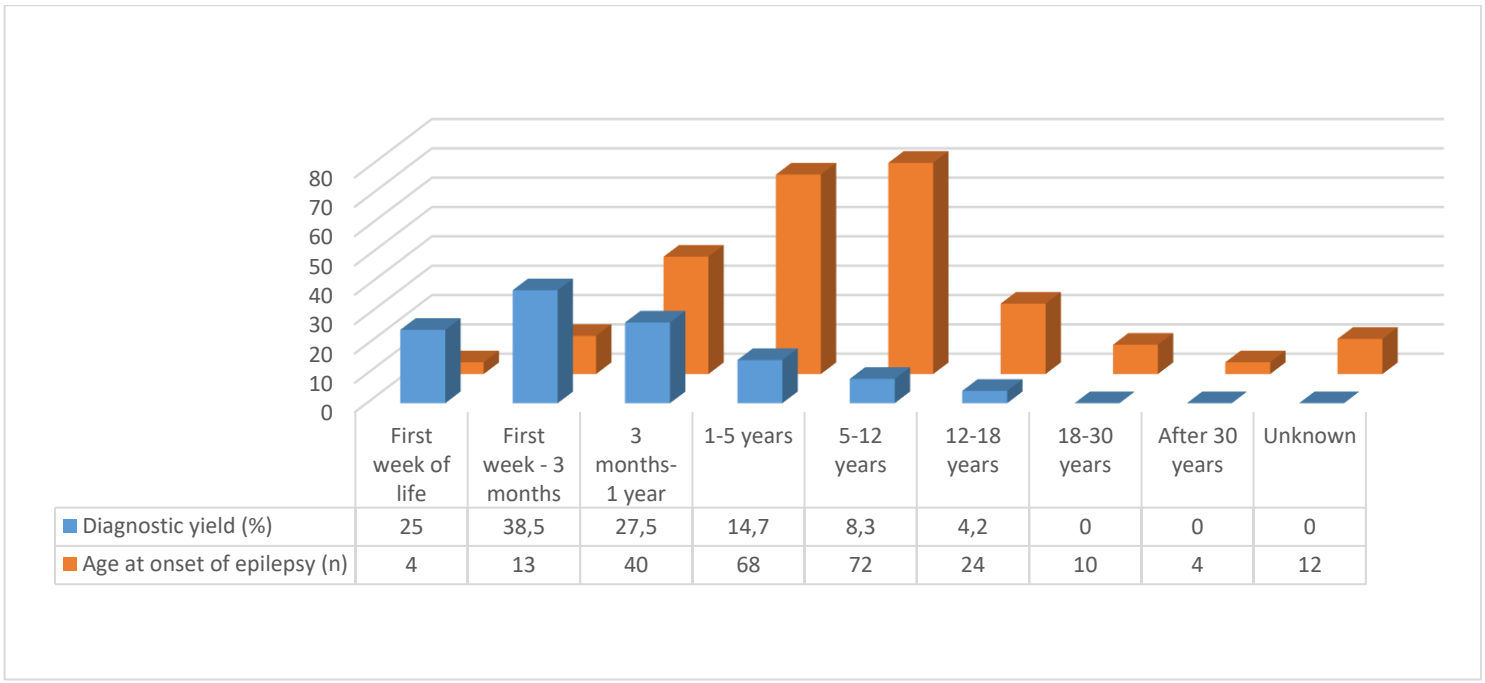
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | c.4485C>A ; p.(Phe1495Leu) | Compound HTZ AR (maternal) | 3 | LOF[68] | | | | |
| 25 | 18y | 18m | 15y4m | male | *CHD2* (NM_001271.3) | c.611dup; p.(Gln205Alafs*5) | HTZ AD (*de novo*) | 5 | LOF | DEE | Severe to profound | None | N.A. |
| 26 | 20y | 3y | 13y3m | female | *SLC2A1* (NM_006516.2) | c.26C>T; p.(Thr9Met) | HTZ AD (unknown) | 4 | Probably LOF[$] | Glucose transporter 1 deficiency syndrome | None | Ketogenic diet[69]; Tier 3 | Implemented |
| 27 | 9y | 7y2m | 1y4m | female | *DLG4 (*NM_001365.4) | c.1510_1513del; p.(His504Serfs*41) | HTZ AD (*de novo*) | 5 | LOF | DEE | Mild | None | N.A. |
| 28 | 6y | 4y6m | 1y2m | male | *PHF21A* (NM_001352025.3) | c.1702C>T; p.(Gln568*) | HTZ AD (*de novo*) | 4 | LOF | DEE | Moderate | None | N.A. |
| 29 | 12y | 1y | 9y5m | male | *QARS1* (NM_005051.3) | c.1133G>A; p.(Arg378His) | HMZ AR (maternal + paternal) | 4 | Probably LOF[§] | DEE | Severe to profound | None | N.A. |
| 30 | 5y | 2y11m | 1y3m | female | *TPP1* (NM_000391.3) | c.622C>T p.(Arg208*) | Compound HTZ AR (maternal) | 5 | LOF | DEE | Moderate | Enzyme replacement therapy (cerliponase alfa)[70]; Tier 3 | implemented |
| | | | | | | c.509-1G>C (disruption 3' splice site) | Compound HTZ AR (paternal) | 5 | LOF[71] | | | | |
| 31 | 14m | 2m | 9m | male | *GABRB3* (NM_000814.6) | c.914C>T p.(Ala305Val) | HTZ AD (*de novo*) | 5 | GOF | DEE | Severe to profound | Avoiding vigabatrine[31]; Tier 3 | implemented |
| 32 | 8m | 2w | 6m2w | female | *CDKL5* (NM_003159.2) | c.1648C>T p.(Arg550*) | HTZ XLD (*de novo*) | 5 | LOF | DEE | Severe to profound | Ganaxolone[72]; Tier 3 Soticlestat[73]; Tier 3 | No treatment changes Only available in clinical trials, currently no enrollment possible |
| 33 | 5m | 5w | 4m | female | *STXBP1* (NM_003165.3) | c.875G>A, p.Arg292His | HTZ AD (*de novo*) | 5 | LOF[74] | DEE | Severe to profound | phenylbutyrate[75]; Tier 3 | No treatment changes Only available in clinical trials, currently no enrollment possible |
| 34 | 5m | 10 days | 3m2w | female | *GNAO1 (*NM_020988.2) | c.607G>A p.(Gly203Arg) | HTZ AD (*de novo*) | 5 | LOF[76] | DEE | Severe to profound | None | N.A. |

Table 5: Characteristics and precision medicine options of individuals with pathogenic and likely pathogenic variants. AD: autosomal dominant; AR: autosomal recessive; HMZ: homozygous; HTZ: heterozygous; DEE: developmental and epileptic encephalopathy; GGE: genetic generalized epilepsy. M: months; Y: years; LOF: loss-of-function variant; GOF: gain-of-function variant; TSC: tuberous sclerosis complex; DEE: developmental and epileptic encephalopathy; N.A.: not applicable.

[#] Apart from the listed pathogenic and likely pathogenic variants, variants of unknown significance (21 individuals) were reported in *CACNA1G*, *CAMK4*, *CCDC32*, *CHRNB2*, *CPS1* (compound heterozygous in 1 individual), *CUX2*, *DEPDC5* (2 individuals), *DNMT1*, *HECW2*, *KCNAB2*, *KCNT1, KDM5A, MACF1, NPRL3, NTRK2, RFT1, SCN1A* (2 individuals), *SLC32A1, STX1B, TFE3, TSC1, TSC2* and *ZNF142* (found on the other allele in the same patient with a likely pathogenic ZNF142 variant).

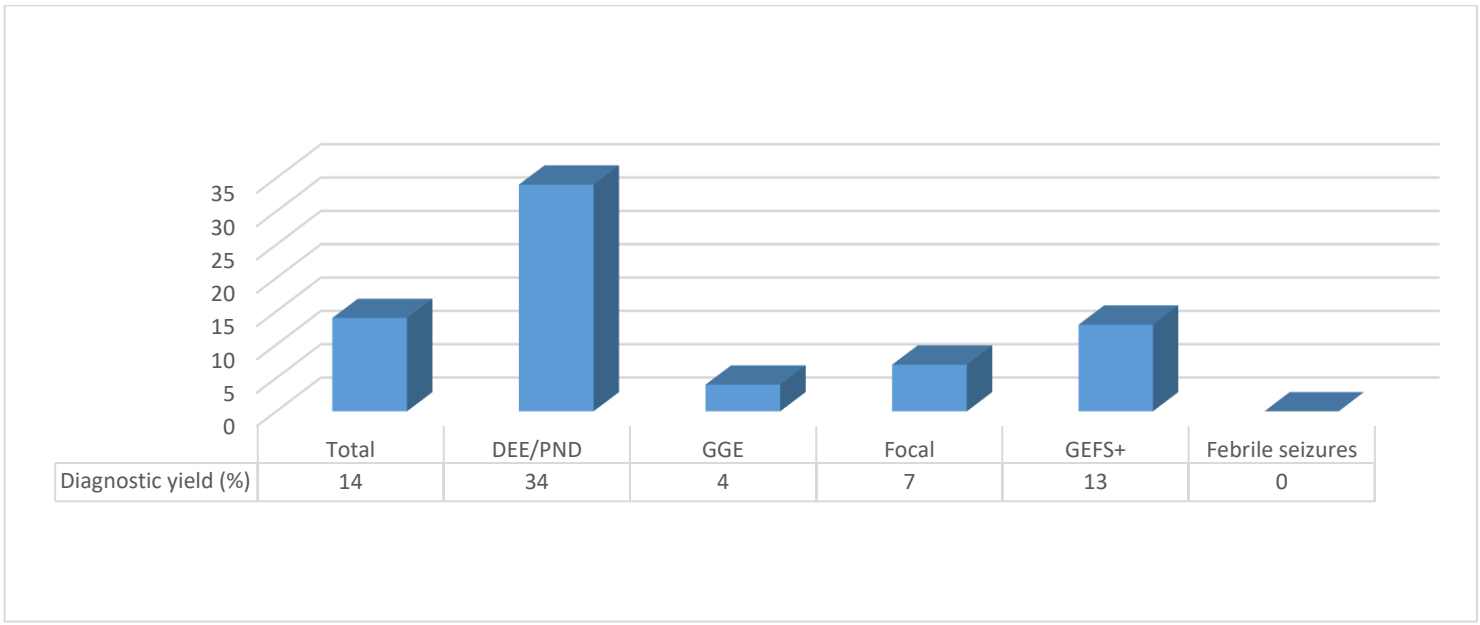
[$] Variant not functionally investigated, but effect based on available information of similar variants and clinical presentation.

**Figure 1: Diagnostic yield according to age at onset of epilepsy and epilepsy type**




| | First week of life | First week - 3 months | 3 months-1 year | 1-5 years | 5-12 years | 12-18 years | 18-30 years | After 30 years | Unknown |
|---|---|---|---|---|---|---|---|---|---|
| Diagnostic yield (%) | 25 | 38,5 | 27,5 | 14,7 | 8,3 | 4,2 | 0 | 0 | 0 |
| Age at onset of epilepsy (n) | 4 | 13 | 40 | 68 | 72 | 24 | 10 | 4 | 12 |

A




| | Total | DEE/PND | GGE | Focal | GEFS+ | Febrile seizures |
|---|---|---|---|---|---|---|
| Diagnostic yield (%) | 14 | 34 | 4 | 7 | 13 | 0 |

B

Figure 1: Diagnostic yield of whole exome sequencing in individuals with epilepsy. A: blue: diagnostic yield according to age at onset of epilepsy; red: number of individuals according to age at onset of epilepsy. B: diagnostic yield according to epilepsy type. GEFS+: genetic epilepsy and febrile seizures plus; GGE: genetic generalized epilepsy; DEE/PND: Syndromes with developmental and/or epileptic encephalopathy and syndromes with progressive neurological deterioration.