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Utility of genetic testing for therapeutic decision-making in adults with epilepsy

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Abstract:

Objective: Genetic testing has become a routine part of the diagnostic workup in children with early onset epilepsies. In the present study, we sought to investigate a cohort of adult patients with epilepsy, to determine the diagnostic yield and explore the gain of personalized treatment approaches in adult patients.

Methods: 200 patients (age span: 18 to 80 years) referred for diagnostic gene panel testing at the Danish Epilepsy Centre were included. The vast majority (91%) suffered from comorbid intellectual disability. The medical records of genetically diagnosed patients were mined for data on epilepsy syndrome, cognition, treatment changes and seizure outcome following the genetic diagnosis.

Results: We found a genetic diagnosis in 46/200 (23%) patients. SCN1A, KCNT1 and STXBP1 accounted for the greatest number of positive findings (48%). More rare genetic findings included SLC2A1, ATP6A1V, HNRNPU, MEF2C and IRF2BP1. Gene specific treatment changes were initiated...
in 11/46 (17%) patients (1 with SLC2A1, 10 with SCN1A) following the genetic diagnosis. Ten patients improved either with seizure reduction and/or an increased alertness and general well-being.

**Significance:** With this study, we show that routine diagnostic testing is highly relevant in adults with epilepsy. The diagnostic yield is similar to previously reported pediatric cohorts, and the genetic findings can be useful for therapeutic decision-making, which may lead to better seizure control ultimately improving quality of life.

**Introduction:**

Within recent years, Next Generation Sequencing (NGS) technologies, including gene panels, exome sequencing (WES) or genome sequencing (WGS) have become a routine part of the diagnostic workup in children with early onset epilepsies, especially developmental and epileptic encephalopathies (DEEs)\(^1\). Initial NGS studies revealed high diagnostic yields, with up to 28% of pediatric epilepsy cohorts\(^2\), whereas a recent study on >8500 patients has revealed a diagnostic yield of approximately 15%\(^3\). In general, studies of selected cohorts of patients with for instance neonatal onset epilepsies or cohorts including patients with Dravet syndrome have very high diagnostic yields, but in broader, unselected cohorts the yield will likely be lower.

The diagnostic odyssey is often most intensive in early childhood. However, published data as well as our own personal experience, show that a diagnosis can still have great impact for both the patient and the parents, even when the patient has reached adulthood\(^4\). Furthermore, features of the epilepsy may evolve such that the characteristic epileptic syndromes present in childhood may not be easily recognizable in adulthood and thus the adult institutionalized patient may not be referred for genetic testing, even though he/she used to have a clinical recognizable genetic epilepsy. This could be the case for Dravet syndrome, and might lead to a less than optimal treatment in some of these patients\(^5\). Other limitations include the conception that a genetic diagnosis will not change the outcome for an adult patient or economic barriers, such as funding, where children are prioritized.

Besides getting a diagnosis, the advent of genetic testing has enabled precision medicine in approximately one quarter of patients, which is illustrating the enormous utility of genetic testing.
for therapeutic decision-making. The most common genes with precision medicine strategies include \(SCN1A\), \(SCN2A\), \(SCN8A\) and \(SLC2A1\)\(^7-10\).

Here we aimed to investigate a cohort of adult epilepsy patients to clarify the underlying genetic cause. Furthermore, we sought to investigate if a relevant genetic diagnosis would lead to treatment changes and eventually improve seizure outcome and quality of life in an adult epilepsy population.

**Methods:**

We included in this study all adult (18 years or above) patients referred for genetic testing at the Danish Epilepsy Centre during a six-year period (2013-2018). Patients were mainly referred for diagnostic purposes, especially if their early medical history suggested a possible genetic cause (such as early seizure onset, negative family history and intellectual disability (ID)). The majority (91%) of the patients were institutionalized and suffered from comorbid ID. Patients were tested using different gene panels consisting of 45-580 genes associated with epilepsy, ID or autism spectrum disorder. The vast majority of the patients were tested with panels consisting of at least 100 genes.

Genes were included in the gene panels, if they were described in one or more patients with epilepsy, intellectual disability (ID) or autism spectrum disorders. Genomic DNA was extracted from EDTA blood using standard methods. Libraries were prepared with either an AmpliSeq custom targeted gene panel (ThermoFisher), a custom SureSelect XT gene panel, or a virtual gene panel extracted from SureSelect XT Human All Exon v6, SureSelect XT Human All Exon v7 or SureSelect XT Clinical Research Exome (Agilent Technologies). The libraries were sequenced on the Ion Torrent PGM system (ThermoFisher) for the custom panels, or the NextSeq 550 (Illumina) or the NovaSeq 6000 (Illumina) for virtual panels extracted from exomes. For the Ion Torrent PGM data, reads were mapped to hg19 in the torrent suite software (ThermoFisher) and variant calling was performed in Strand NGS software. For the NextSeq 550 and NovaSeq 6000 data, reads were aligned to hg19 with the Burrows-Wheeler Aligner\(^11\) and variants were called with the Freebayes software\(^12\). Variants were classified according to the ACMG classification\(^13\), and interpreted as pathogenic or likely pathogenic if they were truncating variants in a gene where this is the known pathomechanism, previously seen in an affected patient, \textit{de novo} or inherited from an affected
parent, absent in controls (in the gnomAD database), affecting highly conserved amino acids, and/or predicted damaging by prediction tools.

In genetically diagnosed patients, the medical records were mined for data on epilepsy syndrome, cognition, treatment changes following the genetic diagnosis as well as seizure outcome. Furthermore, the phenotype of the patients was further discussed with the treating clinicians. For the patients who had a positive response to treatment changes initiated based on the genetic diagnosis, parents or caretakers were contacted for additional details on cognition, attention and mood before and after the treatment change. Sodium channel blockers were defined as phenytoin (PHT), carbamazepine (CBZ), oxcarbazepine (OXC), lamotrigine (LTG), zonisamide (ZNS) and lacosamide (LCM).

The study was approved by the local ethical committee, and all patients or legal guardians signed informed consent.

Results:
We performed genetic testing on 200 adult patients (between 18 – 80 years) followed at the Danish Epilepsy Centre. All patients suffered from epilepsy, and the majority (91%) had various degrees of comorbid ID. We found a genetic cause in 46 patients (23%). Male/female ratio 61%/39%. Presumed pathogenic variants were found in 22 different genes and figure 1 depicts the distribution across genes. The median age at seizure onset for the genetically diagnosed patients was 10 months (range 1 month to 23 years).

Four variants were classified as variants of unknown significance (VUS) according to the ACMG criteria. We included them as their clinical history strongly suggested a genetic epilepsy or resembled other patients with pathogenic variants in the same gene. The majority of the genetically diagnosed patients (17/46) (36%) carried a *SCN1A* variant (median age of seizure six month, range 2.5 months to 2 years). Only two of the 17 *SCN1A* patients had a clinical diagnosis of Dravet Syndrome prior to genetic testing. Previous clinical diagnoses included DEE, focal epilepsy and Lennox-Gastaut syndrome (LGS). However, retrospectively the clinical diagnosis was changed to Dravet syndrome after the genetic diagnosis. Other prevalent genes
included \textit{KCNT1} (6\%) and \textit{STXBP1} (6\%), as well as \textit{CDKL5} (4\%), \textit{CHD2} (4\%) and \textit{PURA} (4\%). Forty-six percent arose \textit{de novo}, whereas nine percent were inherited from affected parents, for the remaining patients the inheritance was unknown.

**Therapeutic consequences:**

Precision medicine approaches exist for several of the genes identified in the cohort including \textit{SLC2A1}, \textit{SCN1A} and \textit{SCN8A} \cite{1, 7, 9, 10, 14}. In eight patients (17\% of those with a genetic finding, 4\% of the total cohort), the genetic diagnosis led to treatment changes and a subsequent improvement of seizure control and/or cognitive function. Two examples are given below, the remaining can be seen in supplementary table 1.

- Patient #24 is a 41-year old female with moderate to severe ID and onset of an intractable unclassified DEE with febrile seizures, status epilepticus, myoclonic, focal and generalized tonic-clonic (GTC) seizures and atypical absences at five months of age. At the time of referral for genetic testing the patient had between 2-11 GTCs as well as an unknown number atypical absences per month despite treatment with carbamazepine (CBZ), valproate (VPA), clobazam (CLB). Genetic testing revealed a non-paternal (mother deceased) missense variant in \textit{SCN1A} (c.949T>C, p.(Tyr317His)), and retrospectively she was diagnosed with Dravet syndrome. After the genetic diagnosis the patient was tapered off CBZ, after which she experienced a slight seizure reduction, primarily in the atypical absences. Her GTCs remained at a frequency of 2-3 per month but appeared during daytime instead of nighttime. Her caretakers describe a marked improvement in her communication and social skills, and she seems less affected by side effects of the antiepileptic drugs (AEDs), such as drowsiness and fatigue. Furthermore her language skills has improved, and she has learnt several new words. The patient was scheduled for a VNS surgery but this was cancelled after the genetic diagnosis. After the treatment change, she displayed a tendency to turn her circadian rhythm around, but with firm guiding this has resolved.

- Patient #42 is a 34-year old female with severe ID limited language and no walking ability and onset of a generalized epilepsy with absences and GTCS at seven weeks of age. Genetic
testing revealed a de novo truncating variant in *SLC2A1* (c.1058_1061delTCGC, p.(Ile353SerfsTer2)), causing GLUT1 deficiency syndrome. Following the genetic diagnosis ketogenic diet was initiated, and the patient went from daily seizures to seizure freedom with only a few breakthrough seizures. Additionally, her energy levels have increased and she responds much more relevant to events and social interactions. She has learnt one new word (has about 50 words in her vocabulary). She has subsequently been tapered off all AEDs, except a small dose of valproate (VPA).

In addition to the patients described above and in supplementary table 1, three patients with an *SCN1A* variant was started on cannabidiol (CBD), of which two had an improvement, which included shorter seizure duration and frequency (#25), improved cognition (assessed in cognitive tests (EpiTrack Junior and BBT test, #38) and improved alertness and attention (#25). One patient (#36) was tapered off due to lack of effect.

**Discussion:**

During a six year period, we investigated 200 adults with severe epilepsy, more than 90% institutionalized with co-morbid intellectual disability. A genetic diagnosis was made in 46 (23%) of the patients which is comparable to the diagnostic yield found in cohorts of children, and similar to a recent study in adults by Borlot et al., in which they found a genetic cause in 22% (14/64). The high yield likely reflects the severity of the underlying neurological disorder, and it is doubtful that such a yield would be found in a more broadly based population cohort.

Previously, only very few studies have focused on genetic testing in adults; one study investigated copy number variations (CNVs) in 143 adult patients with childhood-onset epilepsy and ID, and found a pathogenic or likely pathogenic variant in 16.1% of the cohort. In eight of the patients, the CNV contained one or more genes previously associated with epilepsy and/or ID. In the above study the diagnostic yield is lower than what we achieved in the present study, perhaps because point variants are a more common genetic cause of epilepsy than CNVs, as suggested and discussed in Møller et al.

A very recent gene panel study investigated a cohort of 64 adult probands and two relatives, in which they found a pathogenic or likely pathogenic variant in 14 (22%). The most frequently
mutated genes in their cohort were **SCN1A**, **GABRB3**, and **UBE3A**. For eight of the genetically diagnosed patients, the diagnosis was changed accordingly, including three patients with Dravet Syndrome, who had previously been diagnosed with alleged vaccine encephalitis. The same was the case in the present study, where the diagnosis was changed in several patients after a positive genetic finding. The study by Borlot et al. did not include any follow-up data on changes in treatment. The epilepsy panel used in that study comprised 185 genes, suggesting that expanding the number of genes investigated (such as a larger panel or exome) does not significantly enhance the diagnostic yield. However, it must be kept in mind that for the individual patient receiving a genetic diagnosis may have a large impact on quality of life and ultimately socioeconomic costs, and these facts may be reason enough to use larger panels or WES/WGS.

In general, the genes and their distribution in this study resembled findings from recent NGS studies, investigating larger cohorts, as well as the recent study in adults. However, when comparing with a solely pediatric cohort, the genetic findings differ. In the pediatric cohort **PPRT2** and **KCNQ2** are among the most common genetic findings, whereas we did not see these genes at all in our cohort. Additionally, we found just a single patient with a variant in **SCN8A**, and none with variants in **SCN2A**, even though these genes are some of the most common genetic causes of epilepsy (Johannesen et al., manuscript in preparation). The same characteristics are found in a recent study by Borlot et al., where they found one patient with a **SCN2A** variant, one patient with a pathogenic variant in **KCNQ2** (who had been seizure free for several years, illustrating a stable epilepsy) and none with **SCN8A**. We speculate, that this might be due to inclusion bias. At the Danish Epilepsy Centre, which is a tertiary epilepsy center, we primarily see patients with refractory epilepsy, and even though **KCNQ2**, **SCN2A** and **SCN8A** usually cause severe encephalopathies, we speculate that these patients may achieve seizure-freedom or have a more stable epilepsy in adulthood, and are therefore no longer followed at our center. However, reports on adults harboring these variants are extremely limited. The genes causing self-limiting epilepsy, such as **PPRT2**, will not be seen in our center at all. Furthermore, the **TSC1** and **TSC2** genes are not seen in our cohort, primarily because these patients have been diagnosed in childhood (as tuberous sclerosis is a relatively recognizable diagnosis) and are not referred for genetic testing as adults.
SCN1A was the most prevalent gene found in our cohort. Patients with pathogenic variants in SCN1A causing significant protein dysfunction most often result in Dravet syndrome, which is a recognizable clinical syndrome, consisting of prolonged febrile hemiclonic seizures with onset around six months of age, and a disease course characterized by progressive developmental delay and gait disturbances. The features of Dravet Syndrome and the fact that more than 85% of Dravet Syndrome cases are caused by pathogenic variants in SCN1A, makes it an obvious candidate gene also in adults. However, early clinical characteristics may have faded or been forgotten and the epilepsy in adults may be indistinguishable from other DEEs, which makes it more difficult to make the clinical diagnosis of Dravet syndrome. This might have been the case for the majority of the patients with SCN1A variants in this study, where only two patients had a clinical diagnosis of Dravet syndrome prior to genetic testing. Additionally, the initial diagnosis may have simply been wrong, as illustrated by the three Dravet Syndrome patients in the study by Borlot et al., where the patients were initially diagnosed with alleged vaccine encephalitis.

For eight patients, the genetic diagnosis led to changes in treatment, which improved seizure frequency in all of them. Seven of the patients carried SCN1A variants, where it is well-known that sodium channel blockers are contraindicated, as SCN1A variants typically lead to loss-of-function of Nav1.1. The present study confirms this, as these patients improved both in relation to seizures, cognitive function, language skills and general well-being, when they were tapered off SCBs. Improvement in seizures frequency and also cognitive performance after tapering off contraindicated drugs in Dravet patients has also previously been shown by Catarino et al., and our data adds to this notion. Of note, several of the patients had an initial worsening in seizure frequency when they began tapering off SCBs, after which they then improved. Additionally, none of the patients became seizure free, indicating a clear need for relevant targeted treatment options in patients with Dravet Syndrome. Three SCN1A patients had a positive response to treatment with CBD (cannabidiol). Recently, fenfluramine has shown positive aspects in treating patients with SCN1A variants. The drug is currently awaiting approval in children, even though it could possibly have a positive impact in adults as well. Two of the SCN1A patients in this study remained on treatment with
SCBs, despite their genetic diagnosis. This paradox might be due to clinical effects of SCBs that persist despite a pathogenic SCN1A variant. This may lead to a hesitation to taper off drugs that a patient has been on for several years, in the fear of seizure worsening / relapse (patient #25), or actual seizure worsening when trying to taper off the drug (patient #26).

The last patient carried a SLC2A1 variant (GLUT1 deficiency), and improved significantly, as soon as ketogenic diet was initiated. SLC2A1 encodes the most prominent glucose transporter of the human brain, and it is well known that the ketogenic diet is an effective treatment, as it bypasses the glucose metabolism\textsuperscript{10}. This patient was 28 years old at the time of genetic diagnosis, and even though some of the damage caused by the genetic variant was not ameliorated by the treatment, she still became seizure free, and as such, the treatment has led to an improved quality of life.

Recently, Oates et al. investigated the costs of waiting with genetic testing vs. first line genetic testing\textsuperscript{23}. In their neonatal cohort, they found that costs could be (theoretically) reduced with up to 70\%\textsuperscript{23}. These numbers may or may not also be applicable in the adult cohort. In adults, a genetic diagnosis might aid decision-making when contemplating certain procedures, such as epilepsy surgery, which poses a major intervention for the patient, as well as constitute a definite economic burden\textsuperscript{24}. However, these are solely speculations, as we have not investigated the socioeconomic aspects of receiving a genetic diagnosis as an adult.

Even though the diagnostic yield in this study was high, therapeutic consequences and ultimate improvement of the patient’s clinical state was still limited. However, for the patients who benefitted from a change in treatment the impact of the genetic finding was important. Currently, precision medicine approaches only exist for a few specific genes. In the future, precision medicine will hopefully expand to several other genes, making genetic testing in adults even more relevant. Furthermore, genetic findings in adults provides insight into the natural history of these rare genetic epilepsies, which are of high value in the genetic counselling of children and families with the same genetic epilepsy.

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Conflicts of interest:
None of the other authors have any conflict of interest to disclose.

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.

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Key bullets:
- NGS approaches should be considered in adults with epilepsy
- Gene panel screening in an adult epilepsy cohort had a diagnostic yield of 23%
- In 8/46 patients a precision medicine approach led to improvement in the patient’s seizure burden and overall well-being
- Genetic findings in adults provide information on the natural history of the disease

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**Figure legends:**

Figure 1: Distribution of genetic findings across genes