SHORT COMMUNICATION

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Whole exome sequencing-based testing of adult epilepsy in a Polish population

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ABSTRACT

Aim of the study. Genetic panel testing in paediatric and mixed adult and children populations has demonstrated clinical utility and provided a diagnostic yield of 18–40%. The data on adult epilepsies is limited. We aimed to investigate the diagnostic yield and analyse genetic diagnoses in whole exome sequenced adult patients with epilepsies in Poland.

Material and methods. We recruited 151 patients from 42 clinical centres across Poland. The patients had a diagnosis of epilepsy/seizures, were 18 or older at the time of the genetic testing, and did not have a genetic diagnosis. All patients were tested with whole exome sequencing after an initial testing with a panel of 47 epilepsy-related genes.

Results. We reached a diagnostic yield when considering pathogenic/probably pathogenic variants according to ClinVar of 8.6% (n = 13) and 17% (n = 26) when applying the American College of Medical Genetics (ACMG) criteria. Most patients had a pathogenic/probably pathogenic variant in epilepsy-related genes (54%), followed by potential epilepsy-related genes (19%), and neurodevelopment-associated epilepsy genes (15%).

Conclusions. Our study shows that whole exome sequencing-based testing reaches a slightly higher diagnostic yield that the traditional 300 gene panel. Genes related to childhood onset neurodevelopmental disorders and epilepsy should be considered as well.

Clinical implications/future directions. Patients may have had a diagnosis related to a childhood syndrome, but due to limited diagnostic possibilities, it was not possible to diagnose them in childhood. We would consider testing adult patients with epilepsy with whole exome or genome sequencing (or if not possible with a panel) in cases of a diagnosis of epilepsy with no hints suggesting secondary epilepsy, and especially with clinical features indicating a genetic epilepsy diagnosis, such as neurodevelopmental delay and early onset of seizures.

Keywords: epilepsy, genetics, whole exome sequencing, Polish population

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Introduction

In Poland, there are c.643,000 adults and children with epilepsy [1]. Although epilepsy can develop in people of any age, genetic epilepsy in children is more common than in adults, in which secondary causes such as structural and traumatic epilepsy play an important role. For this reason, children undergo genetic testing more often than adults.

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Currently, there are three main methods for the diagnosis of epilepsy available: next generation-based panel, whole exome sequencing (WES), and whole genome sequencing (WGS). Panel focuses on selected genes and therefore provides quicker and more cost-effective variant interpretation, and reduces the burden of secondary findings and the interpretation of variants of unknown significance. However, panel has also several limitations. Most importantly, it does not permit reanalysis of the gene (unless NGS-based) and the identification of the structural and copy number variants that can be provided with whole genome sequencing, and recently also with whole exome sequencing. The available studies for epilepsy in adults usually use a broad panel of 89–500 genes as a first testing strategy [2–4].

Genetic testing in pediatric populations has demonstrated clinical utility and provided a diagnostic yield of 18-40%, depending on the cohort tested [5–7]. However, the diagnostic yield and data on the potential classification of adult epilepsies are limited. In the largest adult epilepsy study so far, of over 2,000 individuals, as many as 10.9% obtained a genetic diagnosis with a panel testing [4]. Two small studies of adults with epilepsy, primarily those with intellectual disability (ID) or childhood-onset seizures, reported a diagnostic yield of 22-23% [2, 3]. In the Polish population, there has been no study on adults with epilepsy only. A recent paper including mostly children, but also adults, with epilepsy from Poland, reported a monogenic cause of epilepsy in over 20% of patients [8]. Therefore our aim was to investigate the diagnostic yield and analyse genetic diagnoses in adult patients with epilepsies in Poland in a large whole exome sequenced population across the country tested in a single reference genetic centre.

Material and methods

We performed a retrospective analysis. We included 150 patients from 42 clinical centres across Poland. The patients had been referred to the MEDGEN laboratory (Warsaw, Poland) by a treating physician or presented without referral. The patients had a diagnosis of epilepsy/seizures, were 18 or over at the time of the genetic testing, and did not have a genetic diagnosis. Although details regarding family history were not available in all cases, the cohort was enriched with patients with intellectual disability and early seizures onset, suggesting a genetic background of epilepsy. Patients with known epilepsy causes (e.g. stroke, traumatic brain injury, tumour) were excluded. Commercial panel testing of 47 genes followed by whole exome sequencing based on the current literature has been performed commercially since 2017. The list of genes included in the panel is available as Supplementary material. The patients were classified according to the indications for genetic testing according to the International League Against Epilepsy (ILAE) [9], and according to the manifestation of epilepsy in phenotypes into variants of epilepsy-related genes, potential epilepsy genes, neurodevelopment-associated epilepsy genes, and epilepsy genes [10].

This study was conducted in accordance with the Declaration of Helsinki and there were no significant risks to the participants. We ensured the privacy of the participants and their personal information was kept confidential and anonymised for the analysis. Consent for the genetic testing was provided by all patients or their caregivers.

The enriched DNA libraries were sequenced by the Illumina NovaSeq 6000 instrument, 2 x 100 bp. All procedures for exome sequencing were conducted by CeGaT (Tübingen, Germany). Raw sequencing reads were mapped to the human reference genome GRCh37 and GrCh38 assembly using BWA MEM (bwa-mem 2.avx2 mem 0.7.17-r1188) [11]. Duplicates were removed using biobambam2 version 2.0.183 [12]. Variants were identified using HaplotypeCaller (GATK v4.2.6.1) [13], FreeBayes v1.3.2, and named using Variant Effect Predictor (VEP109) [14]. The presence of the variant in control populations was checked in the 1,000 Genomes [15] and gnomAD (v.4) (Broad Institute) databases [16]. A filtering criterion of 1% frequency was applied. The in silico splicing analysis was performed using algorithms embedded in Alamut Visual Plus software (Sophia Genetics), i.e. SpliceSiteFinder-like, MaxEntScam, NNSPLICE, GeneSplicer and SpliceAI [17]. The presence of the detected pathogenic/probably pathogenic variants was confirmed by Sanger sequencing.

Results

The median age of the patients was 28 years (18–61). We reached a diagnostic yield when considering pathogenic/probably pathogenic variants according to ClinVar of 8.6% (n = 13) and 17% (n = 26) when applying the American College of Medical Genetics (ACMG) criteria. According to the indications for genetic testing according to the ILAE [9], most adult patients were tested for epilepsy + (n = 100, 66%), 21 patients were tested for drug-resistant epilepsy (14%), 17 for encephalopathy (5%), and six for familial epilepsy (4%). According to the classification of epilepsy in phenotypes [10], most patients had a pathogenic/probably pathogenic variant in epilepsy-related genes (54%), followed by potential epilepsy genes (19%), neurodevelopment-associated epilepsy genes (15%), and epilepsy genes (12%). ID/NDD was the most common comorbidity present in 49% of patients (n = 76). In a cohort with NDD/IDD, the diagnostic yield was 22% (n = 17 patients with a genetic diagnosis). Four patients were diagnosed with Rett syndrome and in the other 22 patients, pathogenic/probably pathogenic variants in 22 different genes were identified. A full list of variants and phenotypes is set out in Table 1.

Table 1. Phenotypic and genetic data of patients with a molecular diagnosis. Variants reported as pathogenic according to American College of Medical Genetics (ACMG) criteria

Patient number	Indications according to ILAE [9]	Additional features	Molecular results
1	epilepsy +	ID/NDD, no speech, obstructed breathing, generalised weakness	Rett syndrome
2	epilepsy +	cerebellar atrophy	Rett syndrome
3	familial epilepsy		Rett syndrome
4	familial epilepsy		Rett syndrome
5	epilepsy +	abnormal muscle tone disorders, dysmorphic features, autism, NDD	*AP4B1*, p.Leu142Arg/p.Arg102Ter
6	drug-resistant epilepsy	atypical autism, NDD	*CHD4*, c.439-2A > C
7	drug-resistant epilepsy	autism, NDD	*TSC2*, p.Val126Phe
8	epilepsy +	neuropathy, epilepsy, binocular cataract, spastic paraparesis	*KIF1A*, p.Ser274Leu
9	epilepsy +	psychogenic epilepsy, muscular hypotonia	*KMT2E*, p.Pro350Ser
10	epilepsy +	childhood autism, anxiety, auditory hypersensitivity	*BBS5*, c.817-1G >T; *BBS10*, p.Ala296Thr
11	drug-resistant epilepsy	cortical dysplasia	*CHRNA4*, p.Met314Thr
12	epilepsy +	cerebellar syndrome, myoclonus-dystonia	*KCNC1*, p.Arg320His
13	epilepsy +	NDD, high iron levels in blood, congenital cataract	*CYP27A1*, p.Arg127Trp/p.Arg127Trp
14	drug-resistant epilepsy	autism spectrum disorder, profound mental retardation, nystagmus, scoliosis, flat-valgus feet, joint laxity, neuropsychiatric disorders	*DYRK1A*, p.His545GlnfsTer18
15	epilepsy +	hereditary epilepsies, dystonia, NDD, hypoglycaemia	*MED12*, c.6268-2A>G
16	epilepsy +	epilepsy, dysmorphia (protruding ears, prominent lips), NDD	*CHD2*, p.Arg1074Trp
17	epilepsy +	atypical autism, NDD	*NBEA*, p.Gly719ValfsTer4
18	epilepsy +	severe mental retardation, dysmorphia, short stature, microcephaly	*KDM3B*, p.Asp377GlyfsTer102
19	epilepsy +	severe mental retardation, hypothyroidism, obesity, neuropsychiatric disorders	*TSC1*, p.Arg420GlyfsTer20
20	epilepsy +	increased muscular tone in all extremities, severe mental retardation, aphasia, agenesis of left kidney, microgyria	*SON*, p.Val629AlafsTer56
21	epilepsy +	NDD, microcephaly, obesity	*ANKRD11*, p.Arg1188Ter
22	epilepsy +	Cornelia de Lange syndrome, moderate mental retardation	*ARID1B*, p.Pro557AlafsTer10
23	epilepsy +	severe mental retardation, wheelchair bound	*NSD1*, p.Lys1938Arg
24	epilepsy +	mild NDD, cerebral palsy	*ATP1A3*, p.Pro775Leu
25	epilepsy +	cerebellar syndrome of unclear aetiology, tics, myotonias, flaccid paraparesis, cerebellar cortex atrophy	*POLG*, p.Trp748Ser/p.Ala143Val
26	drug-resistant epilepsy	cerebral palsy, speech and language disorders, significant ID, spastic quadriparesis, swallowing disorders – PEG-fed	*TCF4*, p.Ala323Val

Discussion

We present the first study in a Polish adult population investigating the genetic background of adult epilepsies only. We showed a diagnostic yield of 8.6% when applying ClinVar criteria, and of 17% when applying ACMG criteria. We also applied the ILAE classification criteria to an adult only population in Poland.

Polish patients tend to obtain access to genetic testing later than in the US and Western Europe, so it should be expected that the diagnostic rate will be higher. However, the diagnostic yield reported in our study is lower than in some studies where the diagnostic yield reached 22–23% [2, 3], while it was higher than in the biggest study on adults available so far [4].

It must be taken into consideration that the patients were tested with a panel of 47 genes only, followed by the WES, whereas many laboratories apply an epilepsy panel of around 300 genes. Currently, there is no consensus regarding how many genes should be included in a diagnostic panel. In other studies, panels have ranged from 89 to 580 genes [2–4]. The highest diagnostic yield has been reported for panels consisting of 100–299 genes, and the inclusion of additional genes did not increase the diagnostic yield.

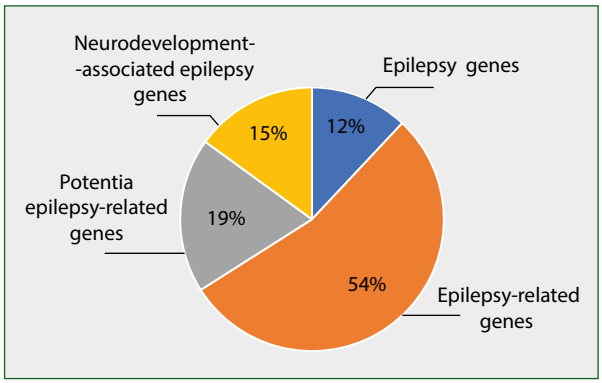

Figure 1. Categories according to manifestation of epilepsy in phenotypes, as in Wang et al. [10]

Our patients obtained diagnoses of differing origins. Only four patients were diagnosed with the same molecular diagnosis. This was of Rett syndrome, which is characterised by a high prevalence of epilepsy, of up to 67% [18], and may still pose a diagnostic challenge in some cases. Most importantly in some patients, a clinically actionable genetic finding was identified, such as pathogenic/probably pathogenic variants in *TSC1* and *TSC2*. For the diseases associated with these genes, there exists a recently approved therapy with mechanistic target of rapamycin complex 1 (mTORC1) [19].

Our study has some limitations. Firstly, it may be biased by the fact that we induced the commercial testing, so that patients with a suspected genetic diagnosis and comorbidities such as NDD/ID or a positive family history may have been mainly those who were tested. For the ID/IDD cohort, we also reached a diagnostic yield higher than in other cohorts i.e. 22% diagnostic yield vs 16% diagnostic yield as described in McKnight et al. [5]. Furthermore, the methodology did not permit the detection of CNVs and structural variants. Nevertheless, our study is a valuable contribution to the discussion on the testing of adult patients with epilepsy.

Ours is the first study on an adult Polish population applying whole exome sequencing. Taking into account that currently more than 900 genes are involved in epilepsy, broad testing with whole genome or exome sequencing is recommended as the first choice testing in ILAE guidelines [20]. Panels should be performed only in particular situations, e.g. if exome or genome sequencing are not available or not covered by the insurance [20]. In cases of epilepsy with neurodevelopmental delay, an indicative phenotype and no diagnosis after panel, structural variants should be considered. Also, in cases of a clear hereditary component in the pedigree without a genetic diagnosis after the panel, new genes and deep splicing variants should be sought. CNVs and structural variants as well as splicing variants may reliably be detected with WGS.

Conclusions

Our study shows that whole exome sequencing-based testing reaches a slightly higher diagnostic yield that the traditional 300 gene panel. Adults in Poland may be affected by childhood onset disease with epilepsy, so that genes related to childhood onset neurodevelopmental disorders and epilepsy should be considered as well.

Clinical implications/future directions

Patients may have had a diagnosis related to childhood syndrome, but due to limited diagnostic possibilities, it was not possible to diagnose them in childhood. We suggest testing adult patients with epilepsy with whole exome or genome sequencing (or if not possible with panel) in cases of a diagnosis of epilepsy, and especially in patients with clinical features indicative of a genetic epilepsy diagnosis, such as neurodevelopmental delay and early onset of seizures.

Article information

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