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Original scientific article

CLINICAL EXOME SEQUENCING IN SERBIAN PATIENTS WITH MOVEMENT DISORDERS - SINGLE CENTRE EXPERIENCE

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The aim of the study was to analyze the genetic basis of a various range of neurodegenerative disorders manifesting by movement disorders (MD) using next generation sequencing (NGS) clinical exome panel. The study included a total number of 42 cases, 36 unrelated and 3 sibling pairs patients diagnosed with movement disorders, all negative after targeted genetic testing available at Neurology clinic, UCCS, Belgrade, Serbia. In a selection of respondents, preference was given to family cases with the early presentation, patients with a positive family history, or complex MD phenotype. Sequencing of a Clinical exome (CE) panel for 4813 genes with known associated clinical phenotypes was performed on an Illumina MiSeq NGS platform according to the manufacturer's instructions. Sequence variants were analyzed by Illumina's Variant Studio v3 software as well as using previously developed pipeline. Variants analysis and interpretation were based on phenotype gene target approach, literature and databases search, allele frequency, and pathogenicity prediction by *in silico* software. Causative variants were confirmed by Sanger sequencing. Whenever possible, additional family members were studied for segregation analysis. We identified a likely genetic cause of MD in 5 cases. CE panel analysis revealed 7 different missense and one splice site pathogenic/likely pathogenic variants in 5 genes related to rare neurodegenerative disorders. Detected pathogenic/likely pathogenic variants in the *TUBB4A*, *PANK2*, *SETX*, *MFSD8*, and *ARSA* genes have been compatible with the

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clinical phenotype of the patients. Furthermore, in additional three cases variants in the *DCTN1*, *PDGFRB*, and *POLG* genes have been detected as a possible cause of disease. In the rest of the studied cases, genetic diagnosis remains unclear. These results emphasize the significance of CE panel analysis in elucidating the diagnosis of neurodegenerative diseases manifesting by movement disorders and gave us insight into the complexity of the genetic background of this group of disorders.

Keywords: clinical exome sequencing, movement disorders, DNA diagnostics, genetics, gene variant

INTRODUCTION

Next-generation sequencing (NGS) is growingly being applied to clinical testing of neurological disorders (FOO *et al*., 2012). This technology comprises various strategies for massively parallel sequencing that offer ultra-high throughput, scalability, and speed. Given that, NGS is a particularly successful approach in patients with atypical disease presentation, incomplete penetrance, or a complex phenotype that cannot be accurately classified into a particular group of diseases (LEE *et al*., 2014). In such cases, it is sometimes difficult to select specific genes for targeted testing, and usually, many conventional genetic tests would be performed before the diagnosis is established. NGS allows most comprehensive genetic analyses, such as whole genome sequencing (WGS) and whole exome sequencing (WES), but predesigned panels of selected genes could be examined also. The number of genes examined using smaller gene panels is significantly lower compared with the number of genes examined in WES and WGS methods. In practice, due to the high cost, WES/WGS is still not available for small centers with limited resources, so smaller gene panels are used as an alternative approach (REALE *et al*., 2018; MONTAUT *et al*., 2018).

The panel consisting of coding regions of 4813 genes associated with human diseases, enriching for over 62,000 exons and their splice sites is also known as clinical exome (CE) and is becoming widely used in clinical practice lately (OKAZAKI *et al*., 2016; PAJUSALU *et al*., 2018). Using CE panel, single nucleotide variants (SNVs), as well as small indel variants, can be detected in genomic DNA. So far numerous studies using CE panel for analysis of various monogenetic disorders were conducted (OKAZAKI *et al*., 2016; YAMAMOTO *et al*., 2016).

Movement disorders (MD) include a wide range of conditions characterized by: 1) disorders of voluntary movements, with decreased amplitude of movement (or hypokinesia), but the terms bradykinesia (slowness of movement) and akinesia (loss of movement) are used as well, or 2) the existence of inappropriate, involuntary movements (dyskinesia) (FAHN 2011; FAHN *et al*., 2011). MDs have complex etiopathogenesis with the important role of genetic factors. Representatives of the first group are Parkinson disease (PD) and Parkinsonism (FAHN, 2011; FAHN *et al*., 2011), with about 15 genes (PARK loci) responsible for rare Mendelian forms (PUSCHMANN *et al*., 2017), and 111 known gene associations (KÖHLER *et al*., 2021). The second group includes disorders such as dystonia, chorea, and ataxia (FAHN, 2011; FAHN *et al*., 2011) with similar genetic architecture, comprising rare clear monogenic forms (BALINT *et al*., 2018; TERMSARASAB *et al*., 2019; MUNDWILER *et al*., 2018; BEAUDIN *et al*., 2019) and 490, 256, and

886 known gene associations, respectively (KÖHLER *et al*., 2021). Lists of the most prevalent genetic causes of movement disorders are provided in Table1.

Table1. Main categories of movement disorders and number of genes associated with each group according to HPO database (https://hpo.jax.org/app/), as well as list of most prevalent causative genes

Disorder	Number of known gene association according to HPO database	List of diagnostic genes that are most probable genetic cause of presented disorders
Dystonia	490	*TOR1A, THAP1, GNAL, ANO3, TAF1, SGCE, KMT2B, PRKRA, ADCY5, ATP1A3, GCH1, TH, SPR*
Ataxia	886	*SCA1, 2, 3, 6, 7, 12, 17, DRPLA, FXN, ATM, APTX, SETX, SACS, POLG, SYNE1, SPG7, ADCK3, ANO10, TTPA, CYP27A1, SIL1, TWNK*
Parkinsonism	111	*SNCA, LRRK2, VPS35, PRKN, DJ-1, PINK1, GBA, FBXO7, SYNJ1, RAB39B, DNAJC6, VPS13C, PTRHD1, PLA2G6, ATP13A2*
Chorea	256	*HTT, C9ORF72, TBP, JPH3, ATN1, FTL, VPS13A, CP, XK*

MD are frequently presented with a complex phenotype that can make a selection of suitable genetic tests laborious. Besides that, the same phenotype can be caused by pathogenic variants of the same (allelic heterogeneity) or different genes (locus heterogeneity), or the same gene mutation can lead to different phenotypes (KOROS *et al*., 2017). Therefore, there is a need to sometimes perform many different genetic tests on the way to establishing an accurate diagnosis. Currently, emerging proof indicates that routine usage of targeted NGS can improve MD diagnostics, with benefits on patient care and reduced health care expenses (VAN EGMOND *et al*., 2017).

Regarding genetic diagnostics of MD, NGS techniques alleviated identification of causative mutations and proved as a suitable compromise between reliability and throughput (NÉMETH *et al*., 2013; VAN DE WARRENBURG *et al*., 2016).

Thus, in our study, we aimed to analyze the genetic background in a selected group of patients with movement disorders using the CE diagnostic panel in the Serbian population. We

also aimed to determine the distribution and spectrum of pathogenic variants in genes related to neurodegenerative disorders manifesting with movement disorders in our population.

MATERIAL AND METHODS

Study subjects

The study included a total number of 42 cases, 36 unrelated and 3 sibling pairs patients, clinically diagnosed with various movement disorders. This includes 18 cases of dystonia (6 isolated, 10 combined, and 2 complex dystonias), 16 cases of ataxia, 4 cases of PD/parkinsonism, 2 cases of spastic paraplegia, and 2 of chorea. Clinical diagnosis was established based on the clinical presentation of the disease, detailed neurological, imaging, and laboratory investigations. In respondent's selection, preference was given to patients that fulfill some of the following requirements: patients negative after standard (routine) genetic tests available at Neurology clinic, UCCS, Belgrade, Serbia (provided in Table2), family cases, cases with early presentation of symptoms or complex phenotype, suggestive genetic heterogeneity or combination of multiple syndromes. Additionally, for each proband carrying a possible disease causing gene variant, parents and affected relatives were included in the analysis, if available**.** The control group consisted of 200 healthy people from a population of Serbia.

Table2. Standard genetic tests (available at Neurology clinic, UCCS, Belgrade, Serbia) performed for patients with causative variants based on their clinical picture

Gene name	Standard gene testing performed
TUBB4A	*C9ORF72, SOD1, ANG, TOR1A, THAP1, GCH1, PNKD, SLC2A1, SGCE, PRRT2, FXN, ATXN1, ATXN2, ATXN3, CACNA1A, ATXN7*
MFSD8	*MT-ND4* (m.11778G>A), *MT-ND6* (m.14484T>C), *MT-ND1* (m.3460G>A)
PANK2	*PANK2* (exons 5 and 6)
SETX	*ATXN1, ATXN2, ATXN3, CACNA1A, ATXN7*
ARSA	*HTT, TBP, NPC1, NOTCH3, C9ORF72*

Each respondent or designated guardian provided written informed consent for participation in the study and publication of the results. The study was approved by the ethics committee of the Clinical Center of Serbia, Belgrade, Serbia (No.: 402/6, from 01/30/20).

DNA extraction, library preparation, and sequencing

For all participants, genomic DNA was extracted from 5 ml peripheral blood samples using the salting-out method (MILLER *et al*., 1988).

CE library preparation and sequencing

Sequencing of clinical exome (CES), including coding regions and surrounding areas of 4813 genes with known associated clinical phenotypes, was performed using the TruSight One Panel on Illumina MiSeq NGS platform (Illumina, San Diego, CA). DNA libraries were prepared in groups of three patients/respondents according to the manufacture's protocol using Reagent Kit V3 (Illumina, San Diego, CA). Library quantification was performed on the Qubit® 3.0 Fluorimeter with the Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific, MA, USA). The average library size was determined using Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). A paired-end sequencing reaction was conducted on the Illumina MiSeq platform (Illumina, San Diego, CA).

Data analysis and interpretation

NGS data analysis included three sections: 1) primary analysis that involved the conversion of raw instrument signal data into sequence data consisting of nucleotide base calls, creating FASTQ files, 2) secondary analysis that involved the first alignment of the reads against a reference genome (hg19) and then calling of any variants detected, creating BAM and VCF files, respectively and 3) tertiary analysis that implied variant annotation and interpretation. The primary and secondary analysis was done on the Illumina MiSeq platform (Illumina, San Diego, CA). Tertiary analysis was performed using Variant Studio v3.0 software (Illumina, San Diego, CA). In parallel, sequencing data were also processed using an in-house analysis pipeline developed by MAVER *et al*. (2016) and BERGANT *et al*. (2018) based on the bwa-GATK pipeline (DEPRISTO *et al*., 2011). The strategy for data interpretation was primarily based on the combined disease and phenotype gene target approach (MAVER *et al*., 2016). Refseq gene models were used for transcript positioning of variants and annotations from dbSNP v138 were used for single nucleotide polymorphism (SNP) annotation. For each patient, phenotype driven virtual gene subpanels were made using HPO (Human Phenotype Ontology) terminology/system.

For detected variants, assessment of frequency in the general population was based on information for worldwide populations from the GnomAD project (KARCZEWSKI *et al*., 2021).

Variants that we labeled as significant were variants with convincing sequencing quality (≥10 reads for homozygous and ≥20 reads for heterozygous) occurring in genes consistent with the phenotype, variants with frequency ≤1% in our and global population, variants classified as pathogenic and/or likely pathogenic in ClinVar (LANDRUM *et al*., 2018), HGMD (STENSON *et al*., 2003), and LOVD (FOKKEMA *et al*., 2011) databases of genomic variants, or in the published literature, variants with functional studies obtained. Fitting with the known model of inheritance was also required to confirm pathogenicity. For variants that previously were not reported in

databases or literature, we performed predictive *in silico* analysis using CADD (RENTZSCH *et al*., 2021), MutationTaster (SCHWARZ *et al*., 2010), MetaLR, and REVEL software. All considered variants were classified following the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) standards and guidelines as (i) pathogenic, (ii) likely pathogenic, (iii) variants of uncertain significance (VUS), (iv) likely benign, and (v) benign (RICHARDS *et al*., 2015). All significant variants identified in this study have been submitted to the ClinVar database (LANDRUM *et al*., 2018) (submission numbers are provided in Table3).

Sanger sequencing

Confirmation, family members, controls

All detected potentially disease causing variants were validated by direct Sanger sequencing on the Applied Biosystems 3500 Genetic Analyzer (Applied Biosystems, CA, USA). The samples were prepared using BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems, CA, USA), while generated nucleotide sequences were analyzed with Sequencher 4.10.1 Demo software (Gene Codes Corporation, USA).

After confirmation of NGS results in probands direct targeted Sanger sequencing was performed in parental and family members' samples (if available) for segregation analysis, to determine the phase and origin of the identified variants (inherited or *de novo*). Sanger sequencing was also performed in 200 Serbian healthy controls to estimate the frequency of potentially causative variants in this population.

RESULTS

Pathogenic and likely pathogenic variants

Using CES analysis for detection of simple genetic variants in coding regions of the 4813 genes we identified a likely genetic cause in 5 out of 39 unrelated cases of MD, representing a diagnostic yield of 12.82%. All detected variants were confirmed by Sanger sequencing. More specifically, we revealed eight different single nucleotide variants in five different genes related to rare neurodegenerative disorders, seven of which are missense and one is splice site variant. One variant was found in a gene linked to autosomal dominant disorder and was in a heterozygous state (*TUBB4A* c.1174T>C). Seven variants were located in genes linked to autosomal recessive disorders, where six variants were in compound heterozygous (*MFSD8* c.923A>G/c.754+2T>A; *PANK2* c.1583C>T/c.1213T>G; and *ARSA* c.763G>A/ c.542T>G), and one in the homozygous state (*SETX* c.5825T>C). Furthermore, three of them are novel (*TUBB4A* c.1174T>C, *MFSD8* c.923A>G, and *PANK2* c.1213T>G). The rest have been already described in the literature or present in databases (summarized in detail in Table3).

In cases with *PANK2* and *MFSD8* variants, samples from both proband's parents were examined by direct Sanger sequencing confirming variants' presence in a compound heterozygous state. For the rest, the parent samples were not available for analysis. However, in two of these cases (cases with *SETX* and *TUBB4A* variants) relatives were analyzed instead and familiar occurrence of variants was confirmed.

All patients carrying the abovementioned variants had a clinical picture in line with the phenotype described for the corresponding gene. The main clinical characteristics of these

patients are provided in Table 4 alongside the OMIM number (HAMOSH *et al*., 2005) for their final genetic diagnosis established in this study.

According to ACMG standards and guidelines five variants are characterized as pathogenic and the remaining as likely pathogenic. Only *SETX* variant was found in heterozygous state in one Serbian healthy control while other variants from this group were absent in our control samples. Further details on described pathogenic and likely pathogenic variants are provided in Table3.

Table3. Variants that represent a likely genetic cause in Serbian patients with movement disorders

Gene name	HGVS	Variation type, molecular consequence, and variant location	Clinical significance/ Pathogenicity class*	No. of carriers in 200 Serbian healthy controls	GnomADv2.1.1 database MAF	ClinVar accession number #	Theoretical predictions for missense or splice variants	Inheretance type/zygosity	Age, y; Gender	Family history
TUBB4A	NM_001289123.1; c.1174T>C, p.F392L	SNV, missense, exon	Likely pathogenic-4	0	Not reported	SCV001366277.2	MutationTaster: disease causing, CADD: 26.2, MetaLR: damaging, REVEL: pathogenic	AD/het	56; F	Pos
MFSD8	NM_152778.2, c.923A>G,p.Y308C/c.754+2T>A	SNV, missense, exon/SNV, splice site, intron	Likely pathogenic-4/pathogenic-5	0	0.000003980/0.00001064	SCV001367704.2/SCV001368442.2	MutationTaster: disease causing, CADD: 23.2, MetaLR: tolerated, REVEL: benign/MutationTaster: disease causing, CADD: 33, MetaLR: NA, REVEL: NA	AR/comp. het	14; F	Neg
PANK2	NM_153638.3; c.1583C>T, p.T528M/ c.1213T>G,p. Y405D	SNV, missense, exon/SNV, missense, exon	Pathogenic-5/pathogenic-5	0	0.00001414/not reported	SCV001481972.1/SCV001468673.1	MutationTaster: disease causing automatic, CADD: 26.7, MetaLR: damaging, REVEL: benign/MutationTaster: disease causing, CADD: 22, MetaLR: damaging, REVEL: pathogenic	AR/comp. het	32; F	Neg
SETX	NM_015046.6; c.5825T>C,p.I1942T	SNV, missense, exon	Likely pathogenic-4	0.0025	0.00001195	SCV001479302.1	MutationTaster: disease causing, CADD: 26.1, MetaLR: damaging, REVEL: pathogenic	AR/hom	NA; M	Pos
ARSA	NM_000487.6; c.763G>A,p.E255K/c.542T>G,p.I181S	SNV, missense, exon/SNV, missense, exon	Pathogenic-5/pathogenic-5	0	0.000003983/0.00002903	SCV001832551.1/SCV001837608.1	MutationTaster: disease causing automatic, CADD: 26.9, MetaLR: damaging, REVEL: pathogenic/MutationTaster: disease causing automatic, CADD: 25.8, MetaLR: damaging, REVEL: pathogenic	AR/comp. het	46; F	Neg

SNV-single nucleotide variant, AD: autosomal dominant, AR: autosomal recessive; Pos-positive, Neg-negative; NA-not available; het-heterozygous, hom-homozygous, comp. het-compound heterozygous; y-years; M-male, F-female; *classification by ACMG/AMP guidelines (10); #our submission to ClinVar; CADDv1.6-suggested cutoff on deleteriousness is between 10 and 20

Table 4. Clinical characteristics of patients with movement disorders included in this study

Gene name	Clinical picture	Final diagnosis	Citation	Novel variant
TUBB4A	Dystonia, cognitive and behavioural changes	Dystonia type 4 (OMIM:128101)	ClinVar930298	Yes
MFSD8	Ataxia, epilepsia, vertigo, proteunuria	Ceroid lipofuscinosis, neuronal, type 7 (OMIM:610951)	ClinVar930986 /LOVD:MFSD8_000002 HGMD:CS073501 ClinVar65897 PMID:16199547, 17564970, 19177532, 19201763, 21990111, 20301601, 25439737, 33546218	Yes/No
PANK2	hypomimia, dysphagia, dystonia, decreased visual acuity, ("eye-of-the-tiger" MRI finding)	Neurodegeneration with brain iron accumulation 1 (OMIM:234200)	ClinVar4556; HGMD:CM014249 PMID:11479594, 12510040, 15565311, 15659606, 16272150, 16437574, 23968566, 25802776, 26087139, 27185474, 28781879 /ClinVar995568	No/No
SETX	Ataxia, oculomotor apraxia	Spinocerebellar ataxia, autosomal recessive, with axonal neuropathy 2 (OMIM:606002)	LOVD:SETX_000103; ClinVar807687; HGMD:CM169967; PMID: 30363866, 27549087, 27528516	No
ARSA	Chorea, involuntary head movement, cognitive impairment, leukodystrophia	Metachromatic leukodystrophy (OMIM:250100)	LOVD:ARSA_000021; ClinVar3091; HGMD:CM024340 PMID:11941485, 18693274, 18786133 /LOVD:ARSA_000115; ClinVar3057; HGMD:CM910051 PMID:1684088, 9096767, 9600244, 12081727, 12116203, 15952986, 16966551, 18693274, 18786133, 19606494, 23701968, 24001781, 25741868, 26462614, 26890752, 28492532, 31186049, 20301309, 31980526, 32632536	No/No

VUS variants and sibling pairs analysis

Additionally, we detected three different variants of uncertain significance (VUS) in three unrelated patients, all as heterozygous and in genes linked to autosomal dominant inheritance (*DCTN1* c.1732G>A, *PDGFB* c.716T>C, and *POLG* c.3151G>C). The implications of detected VUS in particular MD remain doubtful and must be further studied.

Furthermore, no common disease causing genotype was identified in any of the three sibling pairs included in the study.

Only a single pathogenic heterozygous frameshift duplication c.12009dupA was detected in one sibling pair with ataxia, in a clinically relevant *SYNE1* gene. However *SYNE1* gene mutations are responsible for an autosomal recessive spinocerebellar ataxia type 8, and the second disease causing variant could not be identified in our study.

The second disease causing variant in the deep intronic or promoter region or any quantitative or complex structural changes cannot be ruled out in these cases.

In the remaining cases included in the study, no variants survived stringent filtering criteria, thus, in these cases, genetic diagnosis remains unclear.

DISCUSSION

Movement disorders are a clinically and genetically diverse group of neurological disorders which represent a significant cause of illness and death. Due to the complexity of the clinical presentations and genetic causes across various MDs, establishing an accurate diagnosis in the patients promptly is still a challenge (FEDERICO, 2013). NGS technology has enabled large-scale, rapid, and low-cost genome analyses and it has been vastly applied to MDs (FOO *et al*., 2012). Various NGS gene panels exist and the main difference between them is the number of genes examined (XUE *et al*., 2015). The use of smaller gene panels is more suitable for a small genetic center with limited resources. Also, the volume of data obtained with smaller gene panels is less than with WES/WGS which makes data interpretation easier and faster (REALE *et al*., 2018; MONTAUT *et al*., 2018). Targeted NGS has thus far shown similar diagnostic rates to exome sequencing for various disorders, including intellectual disability (25%) (REDIN *et al*., 2014), mitochondrial disorders (22%) (LIEBER *et al*., 2013), cerebellar ataxias (18%) (NÉMETH *et al*., 2013), and spastic paraplegia (17%) (VAN DE WARRENBURG *et al*., 2016). Collectively, these studies show that targeted resequencing using NGS panels of known disease causing genes is a suitable compromise between reliability and throughput (NÉMETH *et al*., 2013; VAN DE WARRENBURG *et al*., 2016; SIKKEMA-RADDATZ *et al*., 2013).

Studies that included patients with different MDs, similar to our study design, using different small gene panels, reported diagnostic yields of 11.3% (REALE *et al*., 2018) and 22% (MONTAUT *et al*., 2018). Further, numerous studies using CE panel for analysis of various monogenetic disorders were conducted as well, with encouraging results (OKAZAKI *et al*., 2016; YAMAMOTO *et al*., 2016).

In our study, the diagnostic power of the clinical exome panel comprising 4813 genes was analyzed in patients with MD. We have investigated 36 unrelated and three sibling pairs patients diagnosed with various movement disorders. Disease causing genotypes were detected in five unrelated probands. The study analyzed *in silico* created gene subpanels that are made according to each patient's clinical phenotype using HPO terminology and we observed a diagnostic yield of 12.82%.

In our cases with pathogenic or likely pathogenic variants in the TUBB4A**,** MFSD8**,** PANK2**,** SETX**,** and ARSA genes genetic findings are in line with the clinical phenotypes of the patients. However, in patients with VUS further scientific and clinical evidence is necessary to establish variant's association to the patient's diagnosis. In the patient clinically diagnosed with ataxia heterozygous variant in the POLG gene has been detected. His family history suggests dominant inheritance of disease and his symptomatic mother has the same heterozygous pathogenic POLG variant. However POLG related disorders are inherited in an autosomal recessive manner (except for autosomal dominant progressive external ophthalmoplegia, type 1, adPED) and so far described patients have a more severe phenotype than our one. We speculate that our case could be explained as a rare case of phenotypic expression of a recessive mutation in heterozygous carriers. On the other hand, there is no literature data about the association of such clinical presentation and detected POLG genotype. Considering all the above mentioned, currently, there is not enough evidence supporting detected POLG variant as disease cause in this family. For that reason, we classify this variant as VUS.

Considering patient with DCTN1 heterozygous VUS variant, this variant is *in silico* predicted as pathogenic by most software, it is absent from all control populations and resides in a conserved region of a gene. However, there is not enough functional evidence about the variant's pathogenicity. While all variants in DCTN1 associated with the Perry syndrome so far are located in exon 2 (FARRER *et al*., 2009), our variant is located in exon 16. Further, the variant was also found in the patient's asymptomatic mother. Taking all of the above into account, it is likely that the variant is not disease causing, and the real (if any) genetic cause of the disease in this proband is still undetermined. Considering patient with heterozygous VUS in the PDGFB gene, mutations in this gene are associated with idiopathic basal ganglia calcification, type 5 (IBGC5), commonly presented with progressive neurologic symptoms that are associated with brain calcification on neuroimaging (KELLER *et al*., 2013). Although the variant detected in our study is novel, rare, predicted to be pathogenic by *in silico* tools, and resides in a conserved region of the gene which all speak in favor of variant pathogenicity, a presented patient does not show neuroimaging changes pathognomonic for IBGC5. Currently, the evidence is too limited to determine whether this variant is disease causing or benign gene polymorphism.

In sibling pair patients, only in one pair with ataxia pathogenic common variant was detected in clinically relevant SYNE1 gene. However, in both patients, only a single-allele variant is observed as a putative cause of autosomal recessive disease. Interestingly enough, their sister also shows signs of disease, but she was not analysed in this study. Such pedigree with asymptomatic parents suggests an autosomal recessive inheritance pattern. Therefore, the possibility of the second disease causing variant in the deep intronic or promoter region or any quantitative or complex structural variants cannot be ruled out in these cases.

Further, in undiagnosed cases, mutations may be located in non-coding regions, such as regulatory or deep intronic regions as well. The presence of copy number variations that were not detected by the present gene panel test cannot be ruled out in undiagnosed cases, too. In such cases, WES and WGS analysis could be suggested, but complex multifactor etiology of a particular disease is possible also.

Making a genetic diagnosis of MDs using traditional genetic tools is often difficult to even for experienced clinicians and neurogeneticists and requires recognition of characteristic patterns of signs or symptoms to guide targeted genetic testing for the confirmation of diagnoses. Recent research suggests that in patients with strongly suggested genetic diseases comprehensive WES/WGS analysis should be performed in the first step (CLARK *et al*., 2018). However, such analyses are still expensive for routine implementation, and the interpretation of results is demanding. Hence, targeted NGS gene panels (for NGS) with high sequencing fidelity and deep attainable coverage of included genes, significantly reduce expenses and make interpretation of results easier (LIN *et al*., 2012). Considering the CE panel of 4813 genes, specifically, the main advantages would be a reasonable price and provided commercial kits and bioinformatics support. The main disadvantage is that it cannot detect new disease causing genes but is limited to those known.

In conclusion, for each patient with MD, the use of a CE panel focusing on virtual smaller gene subpanels should be considered as a tailored strategy and should be carefully considered by geneticists and clinicians with expertise in movement disorders. All to define a

priori the genes of major interest within the panel. Even though the use of gene panels analysis by NGS in the MD subgroup allows a definite genetic diagnosis in a still limited percentage of cases, these figures are likely to increase in the future with the discovery of new genes related to MD.

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REZULTATI ANALIZE SEKVENCIRANJA KLINIČKOG EGZOMA KOD POREMEĆAJA POKRETA U POPULACIJI SRBIJE – ISKUSTVO JEDNOG CENTRA

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Izvod

Cilj studije bio je analizirati genetičku osnovu poremećaja pokreta sekvenciranjem kliničkog egzoma. Studija je obuhvatila ukupno 42 pacijenta, 36 nesrodnih i 3 para srodnika sa dijagnozom različitih poremećaja pokreta, a koji su bili negativni nakon ciljanih genetičkih testiranja dostupnih na Neurološkoj klinici, UKCS, Beograd, Srbija. U selekciji ispitanika prednost su imali porodični slučajevi sa ranom prezentacijom, pacijenti sa pozitivnom porodičnom anamnezom ili složenim fenotipom poremećaja pokreta. Sekvenciranje panela kliničkog egzoma koji obuhvata 4813 gena povezanih sa poznatim kliničkim fenotipima izvedeno je na Illumina MiSeq NGS platformi prema uputstvima proizvođača. Rezulati sekvenciranja su analizirani pomoću Variant Studio v3 softvera kao i internog pipelina. Analiza i interpretacija varijanti zasnovane su na analizi virtualnih genskih panela odabranih prema fenotipu pacijenta, pretraživanju literature i baza podataka, učestalosti alela i *in silico* analizama. Uzročne varijante su potvrđene Sangerovim sekvenciranjem. Kod pojedinih članova porodice rađene su segregacione analize. Utvrđen je verovatni genetički uzrok poremećaja pokreta kod 5 slučajeva. Analizom panela kliničkog egzoma otkriveno je 7 različitih missens i jedna splice site patogena/verovatno patogena varijanta u 5 gena povezanih sa poremećajem pokreta kod 5 pacijenata. Otkrivene patogene/verovatno patogene varijante u genima *TUBB4A*, *PANK2*, *SETX*, *MFSD8* i *ARSA* kompatibilne su sa kliničkim fenotipom pacijenata. Pored toga, detektovane varijante u genima *DCTN1*, *PDGFRB* i *POLG* predstavljaju mogući uzrok bolesti kod tri dodatna slučaja. Kod ostalih ispitanika genetička dijagnoza je za sada nerazjašnjena. Ovi rezultati naglašavaju značaj analize panela kliničkog egzoma u rasvetljavanju dijagnoze motornih poremećaja i daju nam uvid u složenost genetičke pozadine ove grupe poremećaja.

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