CeGaT GmbH | Paul-Ehrlich-Str. 23 | D-72076 Tübingen | Germany

Dr. Jane Doe
Paul-Ehrlich-Str. 23
D-72076 Tübingen

| | |
|---|---|
| **Patient** | XXX, XX |
| ID # | male (*DD.MM.YYYY) |
| **Patient-ID** | # |
| **Sample receipt** | xxx |
| **Material** | EDTA blood |
| **External ID** | # |
| **Report date** | xxx |
| **Report-ID** | R# |

# Genetic Report – XXX, XX (*DD.MM.YYYY)

**Indication** Suspected hereditary spastic paraplegia; progressive spasticity in both legs

**Order** Panel Diagnostics: Hereditary Spastic Paraplegia (HSP) (whole exome enrichment)

## Result: Report with Significant Findings

- **Detection of two pathogenic variants in gene *SPG7*, which are causative for spastic paraplegia type 7 in your patient under the assumption of a compound heterozygous state.**
- Based upon current scientific knowledge, we did not identify any reportable copy number variants which are likely to be causative for your patient's disease.

| Gene | Variant | Zygosity | Heredity | MAF (%) | Classification |
|---|---|---|---|---|---|
| ***SPG7*** | **c.1053dupC; p.Gly352Arg*fs**44**<br>**chr16:89598370 G>GC (hg19)** | **het.** | **AD, AR, digenic** | **0.05** | **pathogenic** |
| ***SPG7*** | **c.1529C>T; p.Ala510Val**<br>**chr16:89613145 C>T (hg19)** | **het.** | **AD, AR, digenic** | **0.54** | **pathogenic** |

Information for the interpretation of this table can be found in section *Additional Information*.

## Recommendation

We recommend further clinical management according to the current guidelines for spastic paraplegia 7 (Casari and Marconi, updated 2018, PMID: 20301286, GeneReviews).

Carrier testing of both parents regarding the identified variants in gene *SPG7* in your patient may be performed in order to determine their carrier status, as well as to confirm the compound-heterozygous state of these variants.

Testing of adult asymptomatic family members regarding the variants c.1053dupC; p.Gly352Arg*fs*44 and c.1529C>T; p.Ala510Val identified in gene *SPG7* may only be performed following genetic counseling.

## Genetic Relevance

Your patient is heterozygous for pathogenic variants in gene *SPG7*. This may be of relevance for family planning and at-risk family members.

Individual variants have a 50% probability of being passed on to each respective offspring.

In the case of compound-heterozygosity, one altered *SPG7* allele will be passed on to each offspring, who will be heterozygous carriers.

## Clinical Information and Variant Interpretation

### *SPG7*, NM_003119.4

| OMIM / Reference | Phenotype | Heredity |
|---|---|---|
| 607259 | Spastic paraplegia type 7 (SPG7) | AR, AD? |
| PMID: 23065789, PMID: 32548275 | Isolated optic atrophy | AD |
| PMID: 30252181 | Syndromic parkinsonism and optic atrophy | digenic |

The gene ***SPG7*** encodes the protein paraplegin, which is a subunit of an ATP-dependent proteolytic complex at the inner mitochondrial membrane. Paraplegin co-assembles in a high molecular-weight complex with AFG3L2, and inactivation of this complex causes reduced complex I activity in mitochondria (Martinelli et al., 2009, PMID: 19289403). Onset is mostly in adulthood, although symptoms may start in childhood or late adulthood (Casari and Marconi, updated 2018, PMID: 20301286). The clinical phenotype is highly variable, even within families. SPG7 typically follows an autosomal recessive mode of inheritance, however there is evidence for autosomal dominant inheritance for some variants (Sánchez-Ferrero et al., 2013, PMID: 22571692). Magri and colleagues (2018, PMID: 30252181) reported a single patient with concurrent heterozygous pathogenic variants in *AFG3L2* and *SPG7* with a severe complex phenotype characterized by early-onset optic atrophy and L-Dopa responsive syndromic parkinsonism.

### *SPG7*, c.1053dupC; p.Gly352Arg*fs**44 (het.), ClinVar ID: 411675

| ACMG/ACGS Criterion | Points | Description |
|---|---|---|
| **PVS1** | +8 | The variant likely results in a loss (or truncation) of the protein, which is a known pathomechanism for *SPG7* associated disease. |
| **PM2** | +2 | This variant is listed in the gnomAD global population dataset with very low frequency. |
| **PM3** | +2 | The variant has already been detected *in trans* with a pathogenic variant or in a homozygous state.<br>Pfeffer et al., 2015, PMID: 25681447 |
| **ACMG/ACGS Classification:** Pathogenic | +12 | B (≤ -7) · LB (-6 - -1) · VUS (Ice Cold) (0) · VUS (Cold) (1) · VUS (Cool) (2) · VUS (Tepid) (3) · VUS (Warm) (4) · VUS (Hot) (5) · LP (6 - 9) · **P (≥ 10)** |

### *SPG7*, c.1529C>T; p.Ala510Val (het.), ClinVar ID: 42016

| ACMG/ACGS Criterion | Points | Description |
|---|---|---|
| **PS3** (supporting) | +1 | Functional studies support the pathogenicity of this variant.<br>Bonn et al., 2010, PMID: 20186691 |
| **PM2** | +2 | This variant is listed in the gnomAD global population dataset with very low frequency. |
| **PM3** (very strong) | +8 | The variant has already been detected *in trans* with a pathogenic variant or in a homozygous state.<br>Mancini et al., 2019, PMID: 30098094 |
| **PP1** | +1 | Cosegregation of the variant with disease in multiple affected family members.<br>Choquet et al., 2016, PMID: 26626314 |
| **ACMG/ACGS Classification:** Pathogenic | +12 | B (≤ -7) · LB (-6 - -1) · VUS (Ice Cold) (0) · VUS (Cold) (1) · VUS (Cool) (2) · VUS (Tepid) (3) · VUS (Warm) (4) · VUS (Hot) (5) · LP (6 - 9) · **P (≥ 10)** |

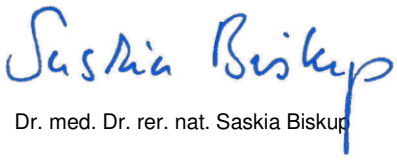
Genetic counseling should be offered with all diagnostic genetic testing, especially following the identification of the molecular cause of a genetic disease.

Medical report written by: XXX

Proofread by: XXX

Validated by: XXX

With kind regards,



Dr. med. Dr. rer. nat. Saskia Biskup

Consultant for Human Genetics

## Additional Information

**Requested Regions**

The whole exome of the individual above was targeted and sequenced. Analysis was restricted to the following gene regions:

***ABCD1***, ***ABHD16A***, ***AFG3L2***, ***AIMP1***, ***ALDH18A1***, ***ALS2***, ***AP4B1***, ***AP4E1***, ***AP4M1***, ***AP4S1***, ***AP5Z1***, ***ARG1***, ***ATL1***, ***ATP13A2***, ***B4GALNT1***, ***BSCL2***, ***CAPN1***, ***CYP2U1***, ***CYP7B1***, ***DARS1***, ***DDHD1***, ***DDHD2***, ***DSTYK***, ***ENTPD1***, ***ERLIN1***, ***ERLIN2***, ***FA2H***, ***FARS2***, ***GALC***, ***GBA2***, ***GCH1***, ***HACE1***, ***HPDL***, ***HSPD1***, ***KCNA2***, ***KDM5C***, ***KIDINS220***, ***KIF1A***, ***KIF1C***, ***KIF5A***, ***KLC2***, ***L1CAM***, ***MAG***, ***MTRFR***, ***NIPA1***, ***NKX6-2***, ***NT5C2***, ***PCYT2***, ***PLP1***, ***PNPLA6***, ***REEP1***, ***RNF170***, ***RTN2***, ***SACS***, ***SELENOI***, ***SLC16A2***, ***SLC33A1***, ***SPART***, ***SPAST***, ***SPG11***, ***SPG21***, ***SPG7***, ***TECPR2***, ***TFG***, ***TNR***, ***TUBB4A***, ***UBAP1***, ***UCHL1***, ***WASHC5***, ***WDR45B***, ***ZFYVE26*** (Hereditary Spastic Paraplegia (HSP))

**General Remarks**

Additional variants may be present within regions which were not analyzed (e.g. introns, promoter and enhancer regions and long repeats). A lower specificity enrichment and/or inaccurate variant calling cannot be ruled out for homologous regions with multiple genomic copies. The occurrence of low frequency somatic mosaicism cannot be reliably assessed using a pipeline optimized for germline variant detection, and may therefore remain undetected. Moreover, detection of large deletions and duplications is not guaranteed by next-generation high-throughput sequencing. The classification of variants may change in the future due to new evidence or improvements in scientific understanding.

**Information for the interpretation of the tables**

**Heredity**: AD – autosomal dominant, AR – autosomal recessive, XL – X-linked, mito – mitochondrial

**MAF:** The ***minor allele frequency*** describes the least frequent allele at a specific locus in a given population. For mitochondrial variants, the population frequency (MAF column) is based on the homoplasmic frequency within a reference population (gnomAD).

**Classification:** Variant classification is based on ACMG, ACGS-2020v4.01, and ClinGen SVI WG guidelines (Richards et al., 2015, PMID: 25741868; Ellard et al., 2020, Association for Clinical Genomic Science; https://clinicalgenome.org/working-groups/sequence-variant-interpretation/). The weighting of criteria and their modification follows the current ACGS guidelines in the strength levels *very strong* (+ 8), *strong* (+/- 4), *moderate* (+/- 2), and *supporting* (+/- 1). According to the respective category (pathogenic or benign) and criterion strength, positive or negative points are assigned as mentioned above (Tavtigian et al., 2020, PMID: 32720330). Variants of uncertain significance (VUS) are subcategorized into *hot*, *warm*, *tepid*, *cool*, *cold*, and *ice cold* VUS according to their likelihood of reaching a pathogenic classification in the future. Posterior

probability decreases from 90% to 10% in this order (Ellard et al., 2020, Association for Clinical Genomic Science). If a variant reaches the classification pathogenic, after checking of all benign criteria, not necessarily all other applicable criteria are listed.

The chromosomal positions of variants listed in the report refer to the human reference genome hg19.

**Methods**

**Sequencing:** Protein-coding regions, as well as flanking intronic regions and additional disease-relevant non-coding regions, were enriched using in-solution hybridization technology, and were sequenced using the Illumina NovaSeq 6000/NovaSeq X Plus system.

**NGS based CNV-Calling:** Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high-quality reads using an internally developed method based on sequencing coverage depth (only applicable for nuclear encoded genes). Briefly, we used reference samples to create a model of the expected coverage that represents wet-lab biases as well as inter-sample variation. CNV calling was performed by computing the sample's normalized coverage profile and its deviation from the expected coverage. Genomic regions are called as variant if they deviate significantly from the expected coverage. Copy number variants are named according to current ISCN guidelines. NGS based CNV-Calling will not detect balanced rearrangements, uniparental disomy, or low-level mosaicism. Aberrations on the Y chromosome and in the pseudoautosomal region (PAR) cannot be detected with high accuracy. The integration site of duplications cannot be determined by NGS based CNV-Calling.

Please note that next generation sequencing based detection of copy number variations has lower sensitivity/specificity than a direct quantification method, e.g. MLPA. Copy-neutral structural aberrations cannot be detected using this method (e.g. balanced translocations and balanced inversions). The absence of reported CNVs therefore does not ultimately guarantee the absence of CNVs.

**Computational Analysis:** Illumina bcl2fastq2 was used to demultiplex sequencing reads. Adapter removal was performed with Skewer. The trimmed reads were mapped to the human reference genome (hg19) using the Burrows Wheeler Aligner. Reads mapping to more than one location with identical mapping score were discarded. Read duplicates that likely result from PCR amplification were removed. The remaining high-quality sequences were used to determine sequence variants (single nucleotide changes and small insertions/deletions). The variants were annotated based on several internal as well as external databases.

**Diagnostic data analysis:** Variants were classified and reported based on ACMG/ACGS-2020v4.01 guidelines (Richards et al., 2015, PMID: 25741868, https://www.acgs.uk.com/quality/best-practice-guidelines/).

Only variants (SNVs/Small Indels) in the coding region and the flanking intronic regions (±8 bp) with a minor allele frequency (MAF) < 1.5% are evaluated. Known disease-causing variants (according to HGMD) are evaluated in up to ±30 bp of flanking regions and up to 5% MAF. Minor allele frequencies are taken from public databases (e.g. gnomAD) and an in-house database. If an acceptable sequencing-depth per base is not achieved by high-throughput sequencing, our quality guidelines demand local re-sequencing using classical Sanger-technology. Candidate CNV calls are evaluated manually. Potentially pathogenic findings are validated with a second method, like MLPA, on a case-by-case basis.

In this case, 97.76% of the targeted regions were covered by a minimum of 30 high-quality sequencing reads per base. **The evaluation of variants is dependent on available clinical information at the time of analysis.** The medical report contains all variants not classified as benign or likely benign according to current literature. Synonymous variants in mitochondrially encoded genes are classified as benign. In silico predictions were performed using the programs MetaLR (Dong et al., 2015, PMID: 25552646), PrimateAI (Sundaram et al., 2018, PMID: 30038395), and SpliceAI (Jaganathan et al., 2019, PMID: 30661751). This prediction can be complemented with additional *in silico* predictions in individual cases.

Variants are named according to the HGVS recommendations without any information regarding the cis or trans configuration.

The sample fulfilled our quality criteria upon arrival and during/after each processing step in the laboratory.

The procedure described above was developed and validated in-house (Laboratory developed test; LDT).



CeGaT GmbH | Paul-Ehrlich-Str. 23 | D-72076 Tübingen | Germany
Tel: + 49 7071 565 44 55 | Fax: + 49 7071 565 44 56 | info@cegat.de | www.cegat.de
Court District Stuttgart - HRB 729958 | VAT No: DE265504070
VR Bank Tübingen eG | IBAN: DE20 6406 1854 0021 2890 00 | SWIFT / BIC: GENODES1STW
Managing Directors: Dr. Dr. Saskia Biskup, Dr. Dirk Biskup, Dr. Detlef Schumann