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

Dr. Jane Doe
Paul-Ehrlich-Str. 23
72076 TÜBINGEN
GERMANY

Patient	XXX, XX (*DD.MM.YYYY)
Sex	Male
Patient-ID	#
Sample receipt	xxx
Material	DNA
Report date	xxx

Genetic analysis report – XXX, XX (*DD.MM.YYYY)

Indication	Current diagnosis of hereditary spastic paraplegia, childhood onset, scissoring gait, cervical spine lesions present
Order	Panel Diagnostics: Hereditary spastic paraplegia (HSP) (whole exome enrichment)

Dear Dr. Doe,

Thank you for your request for molecular genetic analysis.

RESULTS

- **Detection of a pathogenic variant in gene *ATL1*, which is causative for hereditary spastic paraplegia 3A in your patient.**
- 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
ATL1	**c.650G>A; p.Arg217Gln**	**het.**	**AD**	**-**	**pathogenic**

Information for table interpretation:
AD: Describes a trait or disorder requiring only one copy of a gene variant at a particular locus in order to express an observable phenotype. Single heterozygous gene variants can only be causative for the phenotype if the disorder follows the **autosomal dominant** mode of inheritance.
AR: Describes a trait or disorder requiring the presence of two copies of a gene variant at a particular locus in order to express an observable phenotype. In **autosomal recessive** disorders, a single heterozygous variant in one gene cannot by itself be causative for the observable phenotype.
XL: X-linked mode of inheritance
mitochondrial: gene encoded in the mitochondrial DNA. Information on degree of heteroplasmy (heteropl. or homopl.), frequency and read count (variant/total reads).
MAF: The **minor allele frequency** describes the least frequent allele at a specific locus in a given population. The less frequently a variant occurs, the more likely it is pathogenic; however, the prevalence and mode of inheritance of any particular disease must be considered. For mitochondrial variants, the population frequency (MAF column) is based on the homoplasmic frequency within a reference population (gnomAD).
Classification: Refers to the possible pathogenicity of a variant, but does not necessarily provide clear evidence of clinical significance. Variants are evaluated based upon current data and specific criteria, and are then classified as follows: pathogenic, likely pathogenic, and uncertain significance. **All variants for which clinical relevance cannot be conclusively confirmed or excluded are referred as variants of unknown clinical significance.**

INTERPRETATION

***ATL1*, c.650G>A; p.Arg217Gln (het.), NM_015915.5, rs119476049**

OMIM / Reference	Phenotype	Heredity
613708	Hereditary sensory neuropathy type 1D (HSN1D)	AD
182600	**Spastic paraplegia 3A (SPG3A)**	**AD**

The gene ***ATL1*** (also called *SPG3* or *SPG3A*) encodes the protein atlastin-1, which is a dynamin-dependent GTPase and involved in cellular processes like neuronal growth, intracellular membrane transport, and axon elongation during neuronal development (Zhu et al., 2006, PMID: 16537571, Orso et al., 2009, PMID: 19633650). HSN1D and SPG3A are associated with pathogenic variants in *ATL1* and follow autosomal dominant inheritance. While HSN1D manifests between the 2nd and 5th decade of life, SPG3A usually begins in childhood (Ø 4 years). In patients who develop SPG3A in adulthood, the clinical symptoms show a slower progression. Penetrance of pathogenic *ATL1* variants associated with SPG3A is generally high (about 80-90%), but markedly reduced and sex-dependent for single variants (up to 30%) (Varga et al., 2013, PMID: 23483706; Hedera, updated 2020, PMID: 20862796, GeneReviews).

The variant **c.650G>A; p.Arg217Gln** in gene *ATL1* has been identified in your patient in a heterozygous state. This variant is absent from the gnomAD global population dataset. It has been reported to segregate with spastic paraplegia in a large family from southern Italy with 35 individuals from four generations and has been detected in further SPG3A patients (Muglia et al., 2002, PMID: 12112092; ClinVar Variation ID: 4349). The affected amino acid position is located in the GTP-binding site (UniProt). Experimental studies have shown that this missense variant results in defects in dimerization, loss of GTPase activity and inability to maintain branched endoplasmic reticulum network compared to wild type ATL1 protein (Zhu et al., 2006, PMID: 16537571; Ulengin et al., 2015, PMID: 25761634).

The identified missense variant in *ATL1* is assessed as pathogenic and is causative for hereditary spastic paraplegia 3A in your patient.

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. Although unlikely, it is also possible that the classification of variants may change in the future due to improvements in scientific understanding.

GENETIC RELEVANCE

Your patient is heterozygous for a pathogenic variant in gene *ATL1*. 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.

RECOMMENDATION

To determine whether the detected variant in gene *ATL1* is *de novo* in your patient or was inherited, testing of both parents regarding this variant is recommended.

According to § 10 of the German Genetic Diagnostics Legislation, appropriate genetic counseling should be offered with all diagnostic genetic testing. Following the identification of the molecular cause of a genetic disease, genetic counseling has to be offered.

Medical report written by: Scientist 1

Proofread by: Scientist 2

With kind regards,

Dr. med. Dr. rer. nat. Saskia Biskup
Dr. med. Friedmar Kreuz, M.A.

Consultant for Human Genetics

Dr. rer. nat. Heinz-Dieter Gabriel
PD Dr. biol. hum. Christiane Maier
Dr. rer. nat. Christian Wilhelm
Dr. rer. nat. Martin Ritthaler

Diagnostics

ADDITIONAL INFORMATION

Requested Regions

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

ABCD1, ***AFG3L2***, ***AIMP1***, ***ALDH18A1***, ***ALS2***, ***AP4B1***, ***AP4E1***, ***AP4M1***, ***AP4S1***, ***AP5Z1***, ***ARL6IP1***, ***ATL1***, ***B4GALNT1***, ***BSCL2***, ***CAPN1***, ***CPT1C***, ***CYP2U1***, ***CYP7B1***, ***DDHD1***, ***DDHD2***, ***DSTYK***, ***ERLIN2***, ***FA2H***, ***FARS2***, ***GALC***, ***GBA2***, ***HSPD1***, ***KIDINS220***, ***KIF1A***, ***KIF1C***, ***KIF5A***, ***L1CAM***, ***MAG***, ***MTRFR***, ***NIPA1***, ***NKX6-2***, ***NT5C2***, ***PLA2G6***, ***PLP1***, ***PNPLA6***, ***REEP1***, ***REEP2***, ***RTN2***, ***SACS***, ***SLC16A2***, ***SOD1***, ***SPART***, ***SPAST***, ***SPG11***, ***SPG21***, ***SPG7***, ***TECPR2***, ***TFG***, ***TUBB4A***, ***UBAP1***, ***UCHL1***, ***WASHC5***, ***ZFYVE26*** (Hereditary spastic paraplegia (HSP))

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 NovaSeq6000 system. In order to provide a second, independent validation, one or more rare variant(s) identified within the NGS data were additionally sequenced using conventional Sanger sequencing.

NGS based CNV-Calling: (only applicable for nuclear encoded genes) Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high-quality reads using an internally developed method based on sequencing coverage depth. 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 can not 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, 90.64% 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).

Communication, dissemination and usage of this report for scientific purposes is only permitted in accordance with the German Genetic Diagnostics Legislation.

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APPENDIX: ADDITIONAL VARIANTS IN ANALYZED GENES

Every individual carries many rare variants which are not necessarily causative for genetic disease. The requested analysis yielded variants of uncertain significance that, according to current scientific knowledge, are not likely to be causative for the phenotype of your patient. They have therefore not been included in the medical report. However, they are provided in the following table for transparency.

Based on the currently available scientific data, we do not encourage further investigation of these variants, should the clinical features of your patient remain unchanged.

Please note, that meaningful reevaluation of a rare variant requires additional phenotypic information or new scientific data.

Gene	Position Ref/Alt	Variant	Transcript	Zygosity	MAF (%)	rs-Number
ALDH18A1	chr10:97373557 C/T	c.1865G>A; p.Arg622Gln	NM_001323413.2 NM_001323414.2 NM_002860.4	het.	< 0.01	rs774274057

Please note that manual curation of the variants listed above has been performed without finding evidence for reliable disease relevance.

MAF: The minor allele frequency describes the least frequent allele at a specific locus in a given population (gnomAD). Het = Heterozygous, Homo = Homozygous, Hemi = Hemizygous, Heteropl = Heteroplasmic, Homopl = Homoplasmic. Positions refer to the hg19 genome build. For mitochondrial variants, the population frequency (MAF column) is based on the homoplasmic frequency within a reference population (gnomAD).