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
External ID	#
Report date	xxx

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

Indication	Epileptic generalized tonic-clonic seizures, pathological EEG with right-sided parietotemporal activity with secondary generalization, normal MRI, positive family history (brother of CeGaT ID: #).
Order	Panel Diagnostics: Familial and Idiopathic Epilepsy (whole exome enrichment)

Dear Dr. Doe,

Thank you for your request for molecular genetic analysis.

RESULTS

- **Detection of a pathogenic variant in gene *PRRT2*, which is associated with epilepsy and is causative for your patient's symptoms.**
- 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
PRRT2	**c.649dupC; p.Arg217Pro*fs**8**	**het.**	**AD**	**0.01**	**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

***PRRT2*, c.649dupC; p.Arg217Pro*fs**8 (het.), NM_145239.3, rs587778771**

OMIM / Reference	Phenotype	Heredity
605751	Benign familial infantile seizures type 2 (BFIS2)	AD
128200	Episodic kinesigenic dyskinesia type 1 (EKD1)	AD
602066	Familial infantile convulsions with paroxysmal choreoathetosis (ICCA)	AD
PMID: 23077016	Hemiplegic migraine (HM)	AD

The gene ***PRRT2*** encodes a proline-rich membrane protein. It has been suggested that PRRT2 has an influence on synaptic transmission by regulating synaptic vesicle cycling (Rossi et al., 2016, PMID: 26797119). Haploinsufficiency of PRRT2 causes BFIS2 or EKD1 (see table). About 14% of patients show features of both the seizure phenotype and the paroxysmal movement disorder (in this case referred to as ICCA). BFIS2 usually manifests in the first year of life (mean age of onset: six months) and is self-limited. Characteristically, seizures remit by two years of age (Ebrahimi-Fakhari et al., 2015, PMID: 26598493). Paroxysmal kinesigenic dyskinesia is characterized by attacks of brief, stereotypical episodes of dystonia, chorea and athetosis, often in combination, and precipitated by a kinesigenic trigger. EKD1 manifests in childhood or (early) adulthood. Commonly, the frequency of attacks declines markedly into adulthood. EKD1 is predominantly seen in males (Spacey and Adams, updated 2013, PMID: 20301633). In addition, pathogenic *PRRT2* variants can cause hemiplegic migraine (Riant et al., 2012, PMID: 23077016).

Most *PRRT2*-associated disorders are familial and disease penetrance is reduced, especially in patients with epilepsy, while only a small subset of variants has shown to be *de novo* (about 5%; Ebrahimi-Fakhari et al., 2015, PMID: 26598493). Furthermore, about 95% of patients have truncating variants with c.649dupC being by far the most common variant. Cases of biallelic pathogenic variants and microdeletions, causing a more severe phenotype, have also been reported (Ebrahimi-Fakhari et al., 2015, PMID: 26598493; Miller et al., updated 2015, PMID: 20301775).

The variant **c.649dupC; p.Arg217Pro*fs**8** in gene *PRRT2* has been identified in your patient in a heterozygous state. The variant likely results in the nonsense-mediated decay of the mRNA transcript, which is a known pathomechanism for *PRRT2* associated disease. This duplication has been reported in patients with benign familial infantile epilepsy, infantile convulsions/choreoathetosis, paroxysmal kinesigenic dyskinesia as well as hemiplegic migraine (Chen et al., 2011, PMID: 22101681; Wang et al., 2011, PMID: 22120146; Heron et al., 2012, PMID: 22243967; Schubert et al., 2012, PMID: 22623405; Dale et al., 2012, PMID: 22845787; Steinlein et al., 2012, PMID: 22877996; Riant et al., 2012, PMID: 23077016), and accounts for more than 75% of cases with *PRRT2*-associated disease (Ebrahimi-Fakhari et al., 2015, PMID: 26598493). In patients with epilepsy, this variant is almost always inherited and shows reduced penetrance (Ebrahimi-Fakhari et al., 2015, PMID: 26598493).

The identified heterozygous pathogenic variant in *PRRT2* is causative for the seizures 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 *PRRT2*. This may be of relevance for future family planning and at-risk family members.

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

RECOMMENDATION

Testing of asymptomatic family members regarding the variant identified in gene *PRRT2* may only be performed following genetic counseling.

We recommend further clinical evaluation and management according to the current guidelines for *PRRT2*-associated diseases (Ebrahimi-Fakhari et al., 2018, PMID: 29334453, GeneReviews).

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:

CACNA1A, ***CHRNA2***, ***CHRNA4***, ***CHRNB2***, ***DEPDC5***, ***GABRA1***, ***GABRB3***, ***GABRG2***, ***GRIN2A***, ***HCN1***, ***KCNA1***, ***KCNMA1***, ***KCNQ2***, ***KCNQ3***, ***KCNT1***, ***LGI1***, ***MTOR***, ***NPRL2***, ***NPRL3***, ***PCDH19***, ***PRRT2***, ***RELN***, ***RORB***, ***SCN1A***, ***SCN1B***, ***SCN2A***, ***SCN3A***, ***SCN8A***, ***SLC2A1***, ***STX1B***, ***TBC1D24*** (Familial and Idiopathic Epilepsy)

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.

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, 99.88% 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).

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