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

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

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

Indication	Complement deficiency; absent classical complement pathway (absent CH50 value), alternative complement pathway normal (normal AP50 value), invasive pneumococcal disease
Order	Panel Diagnostics: Defects of the complement system (whole exome enrichment)

Dear Dr. Doe,

Thank you for your request for molecular genetic analysis.

RESULTS

- **Detection of a homozygous pathogenic variant in gene *C2*, which confirms your clinical diagnosis of complement deficiency.**
- 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
C2	c.841_849+19del; p.?	homo.	AR	0.71	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

_C2_, c.841_849+19del; p.? (homo.), NM_001282459.2, rs9332736

OMIM / Reference	Phenotype	Heredity
217000	C2 deficiency	AR

The gene ***C2*** encodes the second component of the complement system, which is involved in the generation of complement cleavage products with opsonizing, chemotactic and bacteriolytic activities ("Complement Deficiencies", Sullivan, 2016, Pediatric Allergy: Principles and Practice). The clinical expressivity of C2 deficiency is variable. C2-deficient patients may be asymptomatic, may develop signs of autoimmunity such as systemic lupus erythematosus, Henoch-Schönlein purpura and Sjögren's syndrome, or may show increased susceptibility to encapsulated bacteria (Jönsson et al., 2005, PMID: 15643297; Macedo et Isaac, 2016, PMID: 26941740; OMIM: 217000). Immune complex-mediated hemolytic activity (CH50 test) is absent in C2-deficient patients and C2 protein levels are below 1%, whereas the alternative complement pathway (AH50 test) is unaffected (Wen et al., 2004, PMID: 15100659).

The homozygous variant **c.841_849+19del; p.?** in gene *C2* has been identified in your patient. This alteration is a known pathogenic variant that has been detected in a homozygous state in many patients showing biochemical and clinical signs of C2 deficiency, but also in clinically asymptomatic probands (amongst others Johnson et al., 1992, PMID: 1577763; Blazina et al., 2018, PMID: 29619023; El Sissy et al., 2019, PMID: 31440263; ClinVar Variation ID: 50634). The variant is a deletion of 28 bp which spans an exon-intron boundary and is therefore expected to have a deleterious effect on RNA splicing. Functional studies have confirmed that the variant leads to skipping of exon 6 and a premature stop-codon which results in a null allele in gene *C2* for which loss-of-function is a known pathomechanism (Johnson et al., 1992, PMID: 1577763). With an allele frequency of 0.71% in the non-Finnish european population dataset (gnomAD) the variant occurs quite frequent, which is in accordance with the rather high prevalence, the variable disease manifestation and reduced penetrance of C2 deficiency (Cole et al., 1985, PMID: 2582254; Macedo and Isaac, 2016, PMID: 26941740).

A heterozygous deletion of the alternative allele was not detected in the exome data, therefore the variant is likely present in a homozygous state in your patient.

Based upon the available data, we conclude that the detection of the pathogenic *C2* variant confirms your clinical diagnosis of C2 deficiency.

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 homozygous for a pathogenic variant in gene *C2*. This may be of relevance for at-risk family members.

One altered *C2* allele will be passed on to each of your patient's children, who will be heterozygous carriers.

RECOMMENDATION

Carrier testing of both parents, regarding the identified variant in gene *C2* in your patient, may be performed in order to determine their carrier status, as well as to confirm the homozygous variant state in your patient, and to determine the risk of reoccurrence for further offspring of the parents of your patient.

Testing of asymptomatic adult family members regarding the variant c.841_849+19del; p.? identified in gene *C2* may only be performed following genetic counseling.

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:

C1QA, ***C1QB***, ***C1QC***, ***C1R***, ***C1S***, ***C2***, ***C3***, ***C5***, ***C6***, ***C7***, ***C8A***, ***C8B***, ***CFB***, ***CFD***, ***CFH***, ***CFI***, ***CFP***, ***FCN3***, ***MASP1***, ***MASP2***, ***MBL2*** (Defects of the complement system)

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, 99.02% 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.