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	Female
Patient-ID	#
Sample receipt	xxx
Material	EDTA blood
Report date	xxx

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

Indication	Isolated microscopic hematuria; positive family history of isolated hematuria
Order	Panel Diagnostics: Alport Syndrome and Disorders of Glomerular Basement Membrane (GBM) (whole exome enrichment)

Dear Dr. Doe,

Thank you for your request for molecular genetic analysis.

RESULTS

- **Detection of one heterozygous pathogenic variant in gene *COL4A5*, which is associated with Alport syndrome and consistent with 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
COL4A5	**c.1226G>T; p.Gly409Val**	**het.**	**XL**	**-**	**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

***COL4A5*, c.1226G>T; p.Gly409Val (het.), NM_000495.5**

OMIM / Reference	Phenotype	Heredity
301050	**Alport syndrome (AS)**	**XL**

The ***COL4A5*** gene encodes the α5 chain of type IV collagen (IV). Together with two other types of alpha (IV) chains (the α3 and α4 chains) this protein forms a complete type IV collagen molecule, which has an important role in the basement membranes of the kidney, inner ear and eye. Pathogenic variants in *COL4A5* are associated with Alport syndrome (AS), with most of the pathogenic alterations being glycine substitutions within the triple helical (TH) domain containing Gly-X-Y repeats. Patients with AS usually present with continuous microscopic hematuria and episodes of macroscopic hematuria in childhood, often associated with pyrexia from upper respiratory tract infections (URTI). 100% of male patients, and more than 90% of females with pathogenic *COL4A5* variants have microhematuria. Proteinuria, hypertension, and renal insufficiency develop in all males. Bilateral high-frequency sensorineural hearing loss typically becomes apparent in males in late childhood in 80-90% of cases. Ocular lesions occur in 15-20% of affected males. Heterozygous females have widely variable disease outcomes and are at risk of developing nephrotic syndrome, decreased kidney function, and can progress to end-stage renal disease (Kashtan et al., 2019, PMID: 20301386).

The variant **c.1226G>T; p.Gly409Val** in gene *COL4A5* has been identified in your patient in a heterozygous state. This glycine substitution disrupts a Gly-X-Y motif of the highly conserved TH domain. This alteration has not been reported in literature in association with Alport syndrome nor is it listed in the reference database ClinVar. However, this missense alteration affects an amino acid residue where different missense changes determined to be pathogenic and have already been described in the scientific literature in association with Alport syndrome (p.Gly409Ser, p.Gly409Asp see HGMD Professional). The variant is absent from the gnomAD global population dataset.

The detected *COL4A5* variant is classified as pathogenic and therefore causative for Alport syndrome 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

The patient is heterozygous for a pathogenic variant in gene *COL4A5*. This may be of relevance for future family planning and at-risk family members.

This variant in gene *COL4A5* has a 50% probability of being passed on to each respective offspring. The risk of disease occurrence in male offspring is 50%. Female offspring have a 50% probability of being heterozygous for the identified variant and may show clinical features of disease with variable severity.

RECOMMENDATION

We recommend further clinical management according to the current guidelines for *COL4A5*-associated Alport syndrome (Kashtan, updated 2021, PMID: 20301386, GeneReviews).

It is possible to investigate further affected family members regarding the variant identified in gene *COL4A5*.

Testing of asymptomatic family members regarding the variant c.1226G>T; p.Gly409Val identified in gene *COL4A5* 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.	Dr. rer. nat. Heinz-Dieter Gabriel PD Dr. biol. hum. Christiane Maier Dr. rer. nat. Christian Wilhelm Dr. rer. nat. Martin Ritthaler
Consultant for Human Genetics	Diagnostics

ADDITIONAL INFORMATION

Requested Regions

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

CD151, ***COL4A3***, ***COL4A4***, ***COL4A5***, ***FN1***, ***LMX1B***, ***MYH9***, ***PXDN*** (Alport Syndrome and Disorders of Glomerular Basement Membrane (GBM))

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, 99.8% 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.