CeGaT

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Dr. Mary Public
Model Company
Model Street
12345 Model City
Model Country

Patient	Doe, Jane
ID #	Female (*DD.MM.YYYY)
Sample receipt	DD.MM.YYYY
Material	DNA
External ID	#
Report date	DD.MM.YYYY
Report-ID	R#

Genetic Report – Doe, Jane (*DD.MM.YYYY)

Indication Microhematuria

Order Panel Diagnostics: Alport Syndrome (ExomeXtra® enrichment)

Result: Report with Significant Findings

- **Detection of one likely pathogenic variant in gene *COL4A5*, which is associated with X-linked Alport syndrome 1 (ATS1) and is consistent with your patient's microhematuria.**
- 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.412G>C; p.Gly138Arg** **chrX:107814670 G>C (hg19)**	**het.**	**XL**	**-**	**likely pathogenic**

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

Recommendation

We recommend further clinical evaluation and management according to the current guidelines for Alport syndrome (Nozu et al., updated 2025, PMID: 20301386, GeneReviews).

To determine whether the detected variant in gene *COL4A5* is *de novo* in your patient or inherited, testing of both parents regarding this variant is recommended.

Testing of asymptomatic family members regarding the variant c.412G>C; p.Gly138Arg identified in gene *COL4A5* may only be performed following genetic counseling.

Genetic Relevance

The patient is heterozygous for a likely 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.

Clinical Information and Variant Interpretation

COL4A5, NM_000495.5

OMIM / Reference	Phenotype	Heredity
301050	X-linked Alport syndrome 1 (ATS1)	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 X-linked Alport syndrome 1, the most pathogenic alterations being glycine substitutions within the triple helical (TH) domain containing Gly-X-Y repeats. Furthermore, loss-of-function is a known pathomechanism for ATS1. Patients with Alport syndrome usually present with continuous microscopic hematuria and episodes of macroscopic hematuria in childhood, often associated with pyrexia from upper respiratory tract infections (URTI). All 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 and in 45% of females. 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, but also asymptomatic carriers are described (e.g. Raju et al., 2013, PMID: 24286007; Nozu et al., updated 2025, PMID: 20301386, GeneReviews).

COL4A5, c.412G>C; p.Gly138Arg (het.)

ACMG/ACGS Criterion	Points	Description
PM1 (strong)	+4	The variant is located within a critical region of the gene *COL4A5*. Triple-helical domain (amino acid position 42-1456, UniProt)
PM2	+2	This variant is absent from the gnomAD global population dataset.
PM5	+2	The variant results in the substitution of an amino acid residue, for which different amino acid substitutions p.Gly138Ser/p.Gly138Asp have already been described as pathogenic. Fallerini et al., 2014, PMID: 24033287; Yamamura et al., 2017, PMID: 29270492
PP3	+1	The variant was given a pathogenic prediction by *in silico* tools.
ACMG/ACGS Classification: likely pathogenic	+9	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. Berit Kerner

Consultant for Human Genetics

Additional Information

CeGaT ExomeXtra® based diagnostics

The genetic analysis was performed using CeGaT's in-house designed ExomeXtra® enrichment (version 6), which includes:

- all exons of the approximately 20,000 protein-coding genes
- more than 46,000 intergenic and intronic positions associated with genetic diseases
- the entire mitochondrial genome at high coverage
- selected clinically relevant RNA genes, including all snRNAs of the spliceosome
- genome wide CNV calling with an array-like resolution of approximately 50 kb in intergenic regions and higher resolution of CNVs in coding regions
- high evidence pharmacogenetic variants
- genomic regions associated with repeat expansion disorders
- screening for infections of human papillomavirus (HPV), Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Herpes simplex virus (HSV) 1 and 2, Toxoplasma gondii (Toxoplasmosis), Varicella Zoster virus, Parvovirus B19 (Fifth disease), and Treponema pallidum (Syphilis)

Please note that data evaluation is based on the provided phenotype. Therefore, not all of the above-mentioned characteristics are relevant for every case and reporting is restricted to phenotypically relevant variants. Further methodological details on the specific diagnostics for this patient are provided below.

Requested Regions

The analysis focused on the following gene regions:

CD151, ***COL4A3***, ***COL4A4***, ***COL4A5***, ***COQ6***, ***MYH9*** (Alport Syndrome)

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-2024v1.2, and ClinGen SVI WG guidelines (Richards et al., 2015, PMID: 25741868; Durkie et al., 2024, Association for Clinical Genomic Science; https://clinicalgenome.org/working-groups/sequence-variant-interpretation/). If applicable, the following approach is used. 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 (Durkie et al., 2024, 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 copy number neutral structural variants such as balanced translocations, inversions, uniparental heterodisomy or low-level mosaicism. Aberrations within 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-2024v1.2 guidelines (Richards et al., 2015, PMID: 25741868, Durkie et al., 2024, Association for Clinical Genomic Science).

Only variants (single-nucleotide 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. Possible exceptions include risk factors and hypomorphic alleles. 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, 97.81% 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.