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Dr. Mary Public
Model Company
Model Street
Model City
United Kingdom

Patient	Doe, Jane
ID 999999	Female (*##.##.19##)
Sample receipt	DD.MM.YYYY
Material	EDTA blood
Report date	DD.MM.YYYY
Report-ID	R9999999999

Genetic Report – Doe, Jane (*##.##.19##)

Order Parkinson (predictive set), Dementia (predictive set) and *APOE* (genotyping) (ExomeXtra® enrichment)

Result: Report with Significant Findings

- **Detection of one pathogenic variant in gene *NOTCH3*, which is associated with cerebral arteriopathy with subcortical infarcts and leukoencephalopathy type 1 (CADASIL1).**
  The variant c.1774C>T leads to an additional cysteine and is located in EGFr domain 15 of the protein. Pathogenic variants in EGFr domains 7-34 are associated with a milder course and reduced penetrance.
- Detection of the E3/E3 genotype in the *APOE* gene. The neutral E3 allele is referred to as the normal allele and has no influence on the likelihood of developing Alzheimer's disease (AD) (Genin et al., 2011, PMID: 21556001; Bird et al., updated 2018, PMID: 20301340, GeneReviews; Li et al., 2020, PMID: 33148290).
- No evidence of reportable copy number variants in the analyzed regions, which are currently known to be predisposing factors for Parkinson's disease or dementia.

Gene	Variant	Zygosity	Heredity	MAF (%)	Classification
NOTCH3	**c.1774C>T; p.Arg592Cys** **chr19:15297982 G>A (hg19)**	**het.**	**AD, AR**	**< 0.01**	**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 CADASIL (Hack et al., updated 2019, PMID: 20301673, GeneReviews; Cramer et al., updated 2024, PMID: 29261860, StatPearls).

Testing of adult asymptomatic family members regarding the variant c.1774C>T; p.Arg592Cys identified in gene *NOTCH3* may only be performed following genetic counseling.

As this is a predictive test, these results can be confirmed with a second independent sample.

Genetic Relevance

Your proband is heterozygous for a pathogenic variant in gene *NOTCH3*. This may be of relevance for at-risk family members.

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

Clinical Information and Variant Interpretation

NOTCH3, NM_000435.3

OMIM / Reference	Phenotype	Heredity
125310	**Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy type 1 (CADASIL1)**	**AD**
130720	Lateral meningocele syndrome (LMNS)	AD
615293	?Infantile myofibromatosis type 2 (IMF2)	AD

The gene ***NOTCH3*** encodes a transmembrane protein belonging to the NOTCH receptor family. It plays an important role in cellular differentiation, proliferation, and apoptosis. Heterozygous pathogenic variants in *NOTCH3* lead to the development of various diseases. CADASIL1 is a progressive adult-onset disorder. It is mostly caused by pathogenic missense variants in exons 2-24 of *NOTCH3* that lead to the loss or gain of a cysteine residue in one of the epidermal growth factor-like repeat (EGFr) domains. CADASIL is characterized by mid-adult onset of recurrent ischemic stroke, cognitive decline progressing to dementia, a history of migraine with aura, mood disturbance, apathy, and diffuse white matter lesions and subcortical infarcts on neuroimaging. Ischemic episodes are often recurrent, leading to severe disability with gait disturbance, urinary incontinence, and pseudobulbar palsy. There is a high degree of penetrance, although its severity varies in terms of age of onset, severity of clinical symptoms, and course of the disease. While pathogenic variants in EGFr domains 1-6 appear to be fully penetrant with the classic CADASIL phenotype, pathogenic variants in EGFr domains 7-34 are likely to lead to milder disease with reduced penetrance (Rutten et al., 2016, PMID: 27844030; Rutten et al., 2019, PMID: 30032161; Hack et al., updated 2019, PMID: 20301673, GeneReviews). Pathogenic truncating variants in the last exon of *NOTCH3* (exon 33), which mainly arise *de novo*, are associated with LMNS (Ejaz et al., updated 2022, PMID: 27336130, GeneReview). According to current data, only one family with a pathogenic *NOTCH3* variant has been described with IMF2. In this case, the disease started in childhood (Martignetti et al., 2013, PMID: 23731542). To date, only a few splice variants, insertions, and deletions in *NOTCH3* have been associated with disease (Rutten et al., 2014, PMID: 24844136).

NOTCH3, c.1774C>T; p.Arg592Cys (het.), ClinVar ID: 808495

ACMG/ACGS Criterion	Points	Description
PS4	+4	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. Ni et al., 2022, PMID: 35822697; Wang et al., 2022, PMID: 35401403; internal data
PM1 (strong)	+4	The variant is located within a critical region of the gene *NOTCH3*.
PM2	+2	This variant is listed in the gnomAD global population dataset with a very low frequency.
ACMG/ACGS Classification: pathogenic	+10	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. For predictive tests genetic counseling has to be offered.

Medical report written by: Ph.D. Writer Doe

Proofread by: Ph.D. Proofreader Doe

Validated by: Ph.D. Validator Doe

With kind regards,

Dr. med. Friedmar Kreuz

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 proband are provided below.

Requested Regions

The analysis focused on the following gene regions:

APP, ***CSF1R***, ***GRN***, ***ITM2B***, ***MAPT***, ***NOTCH3***, ***PRNP***, ***PSEN1***, ***PSEN2*** (Dementia (predictive set))

ATP1A3, ***C19orf12***, ***DNAJC6***, ***FBXO7***, ***GRN***, ***LRRK2***, ***MAPT***, ***PANK2***, ***PARK7***, ***PINK1***, ***PLA2G6***, ***PRKN***, ***SLC30A10***, ***SLC39A14***, ***SNCA***, ***SPR***, ***VPS35*** (Parkinson (predictive set))

APOE (genotyping, NM_000041.4)

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 variant classification guidelines (Richards et al., 2015, PMID: 25741868; Durkie et al., 2024, Association for Clinical Genomic Science). 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. 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 and ClinVar) 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, 97.67% 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 uncertain, 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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