CeGaT

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

Patient	XXX, XX (*DD.MM.YYYY)
Sex	Female
Patient-ID	xxx
Sample receipt	xxx
Material	EDTA blood
Report date	xx.xx.2021

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

Order Prevention-Panel (Module 01 - Module 08)

Dear Dr. Doe,

Thank you for your request for molecular genetic analysis.

RESULTS OVERVIEW

Tumor diseases (Module 01)	**Variant found in gene *RAD51D***
Cardiovascular diseases (Module 02)	Without pathological findings
Thrombosis and coagulation disorders (Module 03)	Without pathological findings
Iron and copper storage disorders (Module 04)	Without pathological findings
Hypercholesterolemia (Module 05)	Without pathological findings
Glaucoma (Module 06)	Without pathological findings
Malignant hyperthermia / anesthesia intolerance (Module 07)	Without pathological findings
Pharmacogenetics (Module 08)	**Individual recommendations**

Apart from the variant listed below, we did not detect any variant associated with an increased disease risk in modules 01-07 with the used methods.

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RESULTS

- **Detection of a likely pathogenic variant in gene *RAD51D*, which is associated with an increased tumor risk.**

Gene	Variant	Zygosity	Heredity	MAF (%)	*in silico* Prediction	Classification
RAD51D	**c.738+1G>T; p.?**	**het.**	**AD**	**-**	**splice effect**	**likely 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.
***in silico* Prediction:** The ACMG (American College of Medical Genetics) guidelines recommend using prediction programs to assess the possible pathogenicity of a variant. Each program calculates its predictions based upon different criteria, and the correspondence between a prediction and the actual functional effect of a variant is variable. **These predictions may therefore not serve as the sole basis for the evaluation of pathogenicity.**
Classification: Variants are evaluated based upon current scientific information. Only pathogenic or likely pathogenic variants are listed. **This analysis will detect variants which are not clearly associated with any disease. A reevaluation of the results can be requested at a later time point.**

INTERPRETATION

RAD51D, c.738+1G>T; p.? (het.), NM_002878.4:

OMIM / Reference	Phenotype	Heredity
614291	Breast-ovarian cancer, familial, susceptibility to, 4	AD

RAD51D encodes a tumor suppressor involved in DNA repair. Various pathogenic *RAD51D* variants have been associated with breast cancer (OMIM, 602954). Moreover, carriers of pathogenic variants in *RAD51D* have a moderately increased risk to develop ovarian cancer (Loveday et al., 2011, PMID: 21822267; Song et al., 2015, PMID: 26261251; Suszynska et al., 2020, PMID: 32359370).

In your proband we identified the intronic variant**, c.738+1G>T; p.?** in gene *RAD51D* in a heterozygous state, which has no known allele frequency in control populations (gnomAD). The detected substitution is located in an essential splice site and is therefore expected to have a deleterious effect on splicing. A substitution affecting the same nucleotide position has been previously identified in a family with two patients with ovarian cancer. It has been demonstrated that this described substitution results in aberrant splicing and creates a shift in the reading frame (c.738+1G>A; p.Val246fs*92, Konstanta et al., 2018, PMID: 30111881), which will result in loss of function of the protein. As a presumed null variant, the variant detected in your proband is in line with the established pathomechanism for *RAD51D*-associated cancer susceptibility (Wickramanayake et al., 2012, PMID: 22986143; Yang et al., 2020, PMID: 32107557).

The identified likely pathogenic variant in *RAD51D* increases the lifetime risk of cancer.

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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 (especially *PMS2*, *TTN*). 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. Further the degree of heteroplasmy of mitochondrial variants can vary remarkably between different tissues (Wallace & Chalkia 2013; PMID: 24186072). Therefore, it is possible that disease causing variants are not detectable in the mtDNA from leucocytes, but present in other tissues.

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 proband is heterozygous for a likely pathogenic variant in gene *RAD51D*. This may be of relevance for family members.

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

RECOMMENDATION

We advise that your proband should discuss, in detail, the consequences of the results for herself and family members with an approved genetic counsellor.

Predictive testing concerning the *RAD51D* variant can be offered to family members in the framework of appropriate genetic counseling.

We recommend further clinical evaluation and regular surveillance according to the current guidelines for hereditary breast and ovarian cancer (Daly et al., 2020, PMID: 32259785).

As this analysis is of predictive nature, we recommend confirming the result by a second independent blood sample.

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PHARMACOGENETICS

Pharmacogenetics is the analysis of common variants in genes that code for drug metabolizing enzymes, drug transporters, drug targets, or proteins involved in immune response. These variants with pharmacogenetic relevance are associated with a variable response or tolerance to a variety of medications. The knowledge of these variants in a patient (PGx profile) facilitates individualization of the patient's treatment. The individual PGx profile applies to the drugs listed below. This table is based on current knowledge. However, recommendations can change in the future and/or new drugs can be added or removed.

Currently, two large consortia publish guidelines for pharmacogenetically relevant variants, DPWG (Dutch Pharmacogenetics Working Group) and CPIC (Clinical Pharmacogenetics Implementation Consortium). If the consortia have differing opinions regarding your proband's genotype, we will list both.

PGx profile – Variants with pharmacogenetic relevance

Genotype	Consortium	Effect on drug metabolism
CACNA1S WT/WT	SONOGEN	normal risk of adverse events
*CYP2B6*1/*5*	SONOGEN	normal metabolism
*CYP2C19*1/*1*	CPIC	normal metabolism
*CYP2C9*1/*1*	SONOGEN	normal metabolism
*CYP2D6*1/*5*	SONOGEN	slow metabolism
*CYP3A4*1/*1*	SONOGEN	normal metabolism
*CYP3A5*3/*3*	SONOGEN	normal metabolism
*CYP4F2*1/*3*	SONOGEN	slow metabolism
*DPYD*1/*1*	SONOGEN	normal metabolism
*HLA-A*03:01/*03:01*	SONOGEN	normal risk of adverse events
*HLA-B*07:02/*15:01*	SONOGEN	normal risk of adverse events
IFNL3 rs12979860-CT	CPIC	low drug-dependent response rate
MT-RNR1 WT	SONOGEN	normal risk of adverse events
*NUDT15*1/*1*	CPIC	normal risk of adverse events
*POR*1/*28*	SONOGEN	fast metabolism
RYR1 WT/WT	SONOGEN	normal risk of adverse events
*SLCO1B1*1a/*5*	CPIC	drug-dependent altered efficacy
*TPMT*1/*1*	CPIC	normal metabolism
*UGT1A1*1/*1*	CPIC	normal metabolism
VKORC1-1639GA	SONOGEN	increased drug efficacy

Information for interpretation of the table: A genetically changed activity of liver enzymes can lead to faster or slower metabolism of medical compounds. An increase in the rate of metabolism can result in insufficient response to treatment with standard doses. If „prodrugs“ are prescribed, which require the compound to be activated by metabolizing enzymes, the risk of adverse side effects can be increased due to higher levels of the activated compound. A slower metabolism can lead to a higher level of a medical compound due to delayed degradation resulting in side effects or intoxication. For prodrugs dependent on activation by metabolizing enzymes, therapy may have no effect.

Your proband's genetic factors can influence the efficacy or strength of side effects of medications. We list all guidelines with clinical relevance which are based on solid evidence:

Drug	relevant genes	Recommendation
Acenocoumarol	*CYP2C9* *CYP4F2* *VKORC1*	• Patient may need reduced acenocoumarol dose. Consider maintenance dose of 1.4-3 mg/day (10-20.5 mg/week). • Check INR more frequently. • Be aware that other factors, such as clinical/demographic factors, drug interactions or other genes may influence the acenocoumarol dose requirement.
Amitriptyline	*CYP2C19* *CYP2D6*	• High dose (e.g. depression): Consider 25% reduction of recommended starting dose. Utilize TDM to guide dose adjustment. • Low dose (e.g. neuropathic pain): Initiate therapy with recommended starting dose, but monitor closely for side effects.
Aripiprazole	*CYP2D6*	• Follow drug label dosing recommendation. • Be alert to increased plasma concentrations of aripiprazole and increased risk of ADRs.
Atomoxetine	*CYP2D6*	• Start with 40 mg/day. If no clinical response and in the absence of adverse events after 2 weeks increase dose to 80 mg/day to approach 400 ng/ml peak plasma concentration.
Atorvastatin	*SLCO1B1*	• alert to symptoms of myopathy. • If the patient has additional risk factors for statin-induced myopathy, choose an alternative drug.
Clomipramine	*CYP2C19* *CYP2D6*	• High dose (e.g. depression): Consider 25-30% reduction of recommended starting dose. Utilize TDM to guide dose adjustment. • Low dose (e.g. neuropathic pain): Initiate therapy with recommended starting dose, but monitor closely for side effects.
Codeine	*CYP2D6*	• Use label recommended age- or weight-specific dosing. • Be careful with concomitant use of CYP2D6 inhibitors, CYP3A4 inhibitors and CYP3A4 inducers. Accordingly, a dose increase or reduction may be necessary. • If no response, consider alternative analgesics such as morphine or a non-opioid.
Desipramine	*CYP2D6*	• High dose (e.g. depression): Consider 25% reduction of recommended starting dose. Utilize TDM to guide dose adjustments. • Low dose (e.g. neuropathic pain): Initiate therapy with recommended starting dose, but monitor closely for side effects.
Doxepin	*CYP2C19* *CYP2D6*	• High dose (e.g. depression): Consider 20-25% reduction of recommended starting dose. Utilize TDM to guide dose adjustments and monitor the effect and side effects. • Low dose (e.g. neuropathic pain): Initiate therapy with recommended starting dose, but monitor closely for side effects.
Flecainide	*CYP2D6*	• Reduce dose by 25%, record ECG, monitor plasma concentration.
Imipramine	*CYP2C19* *CYP2D6*	• High dose (e.g. depression): Consider 25-30% reduction of recommended starting dose. Utilize TDM to guide dose adjustment and monitor the effect and side effects.

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		• Low dose (e.g. neuropathic pain): Initiate therapy with recommended starting dose, but monitor closely for side effects.
Lansoprazole	*CYP2C19*	• Initiate standard starting daily dose. • Consider increasing dose by 50-100% for the treatment of H. pylori infection and erosive esophagitis. Daily dose may be given in divided doses. • Monitor for efficacy.
Metoprolol	*CYP2D6*	• Heart failure: select alternative drug (e.g., bisoprolol, carvedilol) or reduce dose by 50%. • Other indications: be alert to ADEs (e.g., bradycardia, cold extremities) or select alternative drug (e.g., atenolol, bisoprolol).
Nortriptyline	*CYP2D6*	• High dose (e.g. depression): Consider 25-40% reduction of recommended starting dose. Utilize TDM to guide dose adjustments. • Low dose (e.g. neuropathic pain): Initiate therapy with recommended starting dose, but monitor closely for side effects.
Omeprazole	*CYP2C19*	• Initiate standard starting daily dose. • Consider increasing dose by 50-100% for the treatment of H. pylori infection and erosive esophagitis. Daily dose may be given in divided doses. • Monitor for efficacy.
Pantoprazole	*CYP2C19*	• Initiate standard starting daily dose. • Consider increasing dose by 50-100% for the treatment of H. pylori infection and erosive esophagitis. Daily dose may be given in divided doses. • Monitor for efficacy.
Peginterferon Alfa-2a	*IFNL3*	• Low response rates in treatment naïve patients. • Approximately 60% chance for SVR after 24–48 weeks of treatment. Consider implications before initiating PEG-interferon-alfa and ribavirin -containing regimens.
Peginterferon Alfa-2b	*IFNL3*	• Low response rates in treatment naïve patients. • Approximately 60% chance for SVR after 24–48 weeks of treatment. Consider implications before initiating PEG-interferon-alfa and ribavirin -containing regimens.
Pimozide	*CYP2D6*	• Use 80% of the standard maximal dose of pimozide and do not exceed 16 mg/day.
Propafenone	*CYP2D6*	• Adjust dose in response to plasma concentration and record ECG or • Select alternative drug (e.g., sotalol, disopyramide, quinidine, amiodarone).
Ribavirin	*IFNL3*	• Low response rates in treatment naïve patients. • Approximately 30-60% chance for SVR after 24–48 weeks of treatment. Consider implications before initiating PEG-interferon-alfa and ribavirin-containing regimens.
Simvastatin	*SLCO1B1*	• Consider an alternative statin (e.g. pravastatin or rosuvastatin) or • Prescribe a lower dose and consider routine CK surveillance.
Tak-390mr	*CYP2C19*	• Initiate standard starting daily dose. • Consider increasing dose by 50-100% for the treatment of H. pylori infection and erosive esophagitis. Daily dose may be given in divided doses. • Monitor for efficacy.
Tamoxifen	*CYP2D6*	• Consider alternative hormonal therapy such as aromatase inhibitor for postmenopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women. • If aromatase inhibitor use is contraindicated, consider use of a higher tamoxifen dose (40 mg/day). • Avoid concomitant use of CYP2D6 inhibitors (strong to weak).

Tramadol	*CYP2D6*	• Be alert to decreased efficacy (symptoms of insufficient pain relief). • Consider dose increase. • If response is still inadequate, select alternative drug- not oxycodone or codeine-
Trimipramine	*CYP2C19* *CYP2D6*	• High dose (e.g. depression): Consider 25% reduction of recommended starting dose. Utilize TDM to guide dose adjustment. • Low dose (e.g. neuropathic pain): Initiate therapy with recommended starting dose, but monitor closely for side effects.
Venlafaxine	*CYP2D6*	• Avoid venlafaxine or • If side effects occur reduce the dose and monitor the effect and side effects or check the plasma concentrations of venlafaxine and O-desmethylvenlafaxine.
Warfarin	*CYP2C9* *CYP4F2* *VKORC1*	• Calculate dose with warfarin dose algorithm (e.g. http://www.warfarindosing.org) or • Use recommended warfarin maintenance dose: 4.6-6.8 mg/day (32-47.5 mg/week). • Consider higher starting dose (up to 9 mg at day 1 and 2).
Zuclopenthixol	*CYP2D6*	• Reduce dose by 25% or • Select alternative drug.

PHARMACOGENETIC RECOMMENDATION

If one of the drugs listed above is prescribed, your proband should confer with the attending clinician. An individual medical approach should be considered, taking into account the genetic predisposition, age, diet, physical condition, environmental influences, comorbidities and drug-drug-interactions. **Drug dosing adjustments should exclusively be performed following consultation with the attending clinician.**

According to § 10 of the German Genetic Diagnostics Legislation, appropriate genetic counseling should be offered with all diagnostic genetic testing. For predictive tests genetic counseling has to be offered.

With kind regards,

Dr. med. Dr. rer. nat. Saskia Biskup Dr. med. Friedmar Kreuz, M.A. Consultant for Human Genetics	xxx Diagnostics	xxx Diagnostics	xxx Diagnostics

ADDITIONAL INFORMATION

Requested Genes

Modules 01-08, which have been requested in the context of this investigation, contain the following genes:

Module 01: ***APC***, ***ATM***, ***BAP1***, ***BMPR1A***, ***BRCA1***, ***BRCA2***, ***CDC73***, ***CDH1***, ***CDKN2A***, ***CHEK2***, ***EPCAM***, ***FH***, ***FLCN***, ***MEN1***, ***MLH1***, ***MSH2***, ***MSH6***, ***MUTYH***, ***NBN***, ***NF1***, ***NF2***, ***PALB2***, ***PMS2***, ***POLD1***, ***POLE***, ***PTEN***, ***RAD51C***, ***RAD51D***, ***RET***, ***SDHA***, ***SDHAF2***, ***SDHB***, ***SDHC***, ***SDHD***, ***SMAD4***, ***SMARCA4***, ***STK11***, ***TMEM127***, ***TP53***, ***TSC1***, ***TSC2***, ***VHL*** (Tumor diseases)

Module 02: ***ACTA2***, ***ACTC1***, ***ACTN2***, ***ACVRL1***, ***BAG3***, ***BMPR2***, ***CACNA1C***, ***CALM1***, ***CALM2***, ***CAV1***, ***COL3A1***, ***CSRP3***, ***DES***, ***DMD***, ***DSC2***, ***DSG2***, ***DSP***, ***EMD***, ***ENG***, ***FBN1***, ***FHL1***, ***FLNC***, ***GJA5***, ***GLA***, ***HCN4***, ***JUP***, ***KCNA5***, ***KCND3***, ***KCNE1***, ***KCNE2***, ***KCNH2***, ***KCNJ2***, ***KCNQ1***, ***LAMP2***, ***LDB3***, ***LMNA***, ***LOX***, ***MYBPC3***, ***MYH11***, ***MYH6***, ***MYH7***, ***MYL2***, ***MYL3***, ***MYPN***, ***NKX2-5***, ***PKP2***, ***PLN***, ***PRKAG2***, ***RBM20***, ***RYR2***, ***SCN1B***, ***SCN5A***, ***SMAD3***, ***SMAD9***, ***TBX4***, ***TGFB2***, ***TGFB3***, ***TGFBR1***, ***TGFBR2***, ***TMEM43***, ***TNNC1***, ***TNNI3***, ***TNNT2***, ***TPM1***, ***TRPM4***, ***TTN***, ***TTR***, ***VCL*** (Cardiovascular diseases)

Module 03: ***F10***, ***F11***, ***F13A1***, ***F2***, ***F5***, ***F7***, ***F8*** (complex intronic rearrangements not included), ***F9***, ***HRG***, ***PROC***, ***PROS1***, ***SERPINC1***, ***SERPIND1***, ***SERPINE1***, ***SERPINF2***, ***THBD***, ***VWF*** (Thrombosis and coagulation disorders)

Module 04: ***ATP7B***, ***HAMP***, ***HFE***, ***HJV***, ***SLC40A1***, ***TFR2*** (Iron and copper storage disorders)

Module 05: ***APOB***, ***LDLR***, ***LDLRAP1***, ***PCSK9*** (Hypercholesterolemia)

Module 06: ***CYP1B1***, ***MYOC***, ***OPTN*** (Glaucoma)

Module 07: ***CACNA1S***, ***RYR1*** (Malignant hyperthermia / anesthesia intolerance)

Module 08: ***CACNA1S***, ***CYP2B6***, ***CYP2C19***, ***CYP2C9***, ***CYP2D6***, ***CYP3A4***, ***CYP3A5***, ***CYP4F2***, ***DPYD***, ***HLA-A***, ***HLA-B***, ***IFNL3***, ***MT-RNR1***, ***NUDT15***, ***POR***, ***RYR1***, ***SLCO1B1***, ***TPMT***, ***UGT1A1***, ***VKORC1*** (Pharmacogenetics)

Due to the existence of pseudogenes, variants detected in the homologous regions of the genes *PMS2* (NM_000535.7) and *TTN* (NM_133378.4) cannot be further evaluated, as it is not possible to distinguish these regions.

Methods

Sequencing: The coding and flanking intronic regions of the nuclear encoded genes as well as the mitochondrial DNA were enriched using in solution hybridization technology and were sequenced using the Illumina HiSeq/NovaSeq system. At least one rare variant is resequenced using conventional Sanger sequencing, providing a second, independent confirmation.

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. 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. 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.

Additional Analyses: Deletion and duplication analysis of the genes *BRCA1* and *BRCA2* was performed using MLPA (*multiplex ligation-dependent probe amplification*, MRC Holland). Quantification was performed in comparison to reference sample DNA.

MLPA analysis cannot determine the allele configuration of copy number variants. In rare cases, the presence of an unexpected copy number distribution, e.g. a gene duplication on one allele and a deletion on the other allele, may lead to false negative results.

The data for the pharmacogenetics module were evaluated by SONOGEN XP (Zurich). The table shown in the medical report lists abstracts of the external report, which is available upon request.

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) of the nuclear encoded genes and in the mitochondrial DNA with a minor allele frequency (MAF) < 1.5% and known disease-causing variants (according to HGMD® and MITOMAP) are evaluated. Minor allele frequencies are taken from public databases (e.g. gnomAD, MITOMAP) 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.

In this case, > 99.9% of the targeted regions were covered by a minimum of 30 high-quality sequencing reads per base. The medical report contains only SNVs, small indels and larger deletions/duplications, which are, based upon

the available data, evaluated to be clearly pathogenic or likely pathogenic. Single heterozygous variants in genes, which are exclusively associated with recessive diseases, are not reported.

The pharmacogenetic report (module 08) does not include all known variants of a gene, it considers only variants with therapeutic relevance indicated by drug dosing guidelines.

Variants are named according to the HGVS recommendations without any information regarding *cis* or *trans* configuration.

The results do not rule out the possibility of an increased disease risk in the addressed disease modules.

This analysis will detect variants of unknown significance which are not clearly associated with disease. If your proband has a conspicuous family history, a genetic consultation could be extended to include the evaluation of unclear variants. A reevaluation of the results can be requested at a later time point.

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 by CeGaT GmbH (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.