CeGaT GmbH | Paul-Ehrlich-Str. 23 | D-72076 Tübingen | Germany

Dr. Jane Doe
Paul-Ehrlich-Str. 23
D-72076 Tübingen

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

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

Order Prevention-Panel (Module 01 - Module 09)

Results Overview

Tumor diseases (Module 01)	**Variant found in gene *ATM***
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
Hypercholesterolaemia (Module 05)	Without pathological findings
Glaucoma (Module 06)	Without pathological findings
Malignant hyperthermia (Module 07)	Without pathological findings
Pharmacogenetics (Module 08)	**Individual recommendations**
Familial diabetes (Module 09)	Without pathological findings

Within the genes listed in module 01-07 and 09, we did not detect any further variants, aside from those listed below, which are associated with an increased disease risk.

Results

- **Detection of a pathogenic variant in gene *ATM*, which is associated with an increased cancer risk.**

Gene	Variant	Zygosity	Heredity	MAF (%)	Classification
ATM	**c.4683_4689del; p.Asp1563Phe*fs**36** **chr11:108164109-108164116 CTTTTAGA>C (hg19)**	**het.**	**AD, AR**	**-**	**pathogenic**

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

Recommendation

We advise that the individuals requesting genetic services should discuss, in detail, the consequences of the results for themselves and family members with an approved genetic counsellor.
As this analysis is of predictive nature, we recommend confirming the result by a second independent blood sample.

Genetic Relevance

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

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

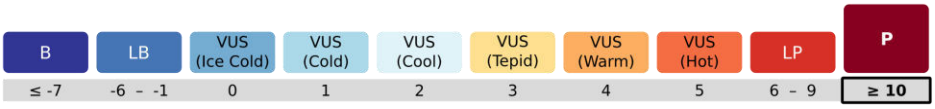
Clinical Information and Variant Interpretation

***ATM*, NM_000051.4**

OMIM / Reference	Phenotype	Heredity
208900	Ataxia-telangiectasia (AT)	AR
114480	Breast cancer, susceptibility to	AD

The ***ATM*** gene encodes a member of the phosphatidylinositol 3-kinase family of proteins that responds to DNA damage by phosphorylating key substrates involved in DNA repair and / or cell cycle control. Biallelic pathogenic variants in the *ATM* gene, which lead to chromosomal instability (Iourov et al., 2009, PMID: 19414482), can cause the recessive disorder Ataxia-telangiectasia (AT), which begins in early childhood and is characterized by cerebellar ataxia, telangiectasias, immune defects, and an increased risk for cancer. Nonclassical forms of AT include a less progressive course of the disease, adult onset, and often the development of dystonia (Gatti and Perlman, updated 2016, PMID: 20301790, GeneReviews). The cancer risk of individuals heterozygous for a pathogenic variant in the *ATM* gene has been reported to be moderately elevated, primarily because of the increased risk for breast cancer (Rosenthal et al., 2017, PMID: 28011157; Jerzak et al., 2018, PMID: 29719442; Moslemi et al., 2021, PMID: 33402103). A large-scale study also demonstrated a moderately to severely increased risk of pancreatic, prostate, and gastric cancers, among others. Slightly to moderately increased risk was shown for ovarian cancer, male breast cancer, colorectal cancer, and melanoma, among others (Hall et al., 2021, PMID: 33509806).

***ATM*, c.4683_4689del; p.Asp1563Phe*fs**36 (het.), ClinVar ID: 265382**

ACMG/ACGS Criterion	Points	Description
PVS1	+8	The variant likely results in a loss (or truncation) of the protein, which is a known pathomechanism for *ATM* associated disease.
PM2	+2	This variant is absent from the gnomAD global population dataset.
ACMG/ACGS Classification: pathogenic	+10	

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). When 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
ABCG2 421CC	SONOGEN	normal drug efficacy
CACNA1S WT/WT	SONOGEN	normal risk of adverse events
*CYP2B6*2/*6A*	SONOGEN	slow metabolism
*CYP2C19*1/*1*	CPIC	normal metabolism
*CYP2C9*1/*1*	SONOGEN	normal metabolism
*CYP2D6*1/*6*	SONOGEN	slow metabolism
*CYP3A4*1/*1*	SONOGEN	normal metabolism
*CYP3A5*3/*3*	SONOGEN	normal metabolism
*CYP4F2*1/*1*	SONOGEN	normal metabolism
*DPYD*1/HapB3*	SONOGEN	slow metabolism
G6PD B/B	CPIC	normal metabolism
*HLA-A*02:01/*24:02*	SONOGEN	normal risk of adverse events
*HLA-B*07:02/*51:01*	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/*3A*	CPIC	slow metabolism

*TPMT*3B/*3C*	CPIC	very slow metabolism
*UGT1A1*1/*28*	CPIC	slow metabolism
VKORC1-1639AA	SONOGEN	high 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	*VKORC1*	• Use 50% of the standard initial dose and check INR more frequently.
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.
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*	• Use not more than 40 mg as a starting dose and adjust doses based on disease-specific guidelines. • Be alert to symptoms of myopathy, especially with 40 mg atorvastatin. • If the patient has additional risk factors for statin-induced myopathy, choose an alternative drug. • If dose over 40 mg is needed, consider combination therapy.
Azathioprine	*NUDT15* *TPMT*	• Select alternative drug. • If azathioprine is warranted, phenotyping of TPMT is recommended. Adapt dosing according to phenotype.
Capecitabine	*DPYD*	• Reduce starting dose by 50% followed by titration of dose based on toxicity or TDM.
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*	• Be alert to reduced effectiveness. • If no response, try a dose increase or consider alternative analgesics such as morphine or a non-tramadol.

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.
Efavirenz	*CYP2B6*	• Consider initiating with decreased dose of 400 mg/day. • Use TDM to ensure plasma concentrations in therapeutic range.
Flecainide	*CYP2D6*	• Reduce to 75% of the standard dose and record an ECG, monitor plasma concentration.
Flucytosine	*DPYD*	• Be alert to the occurrence of severe side effects. • Flucytosine should be stopped if severe side effects occur.
Fluorouracil	*DPYD*	• Reduce starting dose by 50% followed by titration of dose based on toxicity or TDM.
Iloperidone	*CYP2D6*	• Be alert to higher exposure of iloperidone.
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. • 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.
Lovastatin	*SLCO1B1*	• Prescribe an alternative statin depending on the desired potency. • If lovastatin therapy is warranted, limit dose to 20 mg/day or less.
Mercaptopurine	*NUDT15* *TPMT*	• Select alternative drug. • If mercaptopurine is warranted, phenotyping of TPMT is recommended. Adapt dosing according to phenotype.
Metoprolol	*CYP2D6*	• If a gradual reduction in heart rate is desired, or in the event of symptomatic bradycardia: Increase the dose in smaller steps and/or prescribe no more than 50% of the standard dose. • Other cases: Use standard dose.
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.
Phenprocoumon	*VKORC1*	• Use 50% of the standard initial dose and check INR more frequently. • For patients younger than 75 years, the initial dose and the maintenance dose can be calculated using an algorithm as found in EU-PACT
Pimozide	*CYP2D6*	• Use 80% of the standard maximal dose and do not exceed 16 mg/day.
Pitavastatin	*SLCO1B1*	• Prescribe 2mg or less as a starting dose and adjust doses based on disease-specific guidelines. • If dose >2mg needed for desired efficacy, consider an alternative statin or combination therapy. • Be aware of possible increased risk for myopathy especially for doses >1mg.
Pravastatin	*SLCO1B1*	• Use desired starting dose and adjust doses based on disease-specific guidelines. • Be aware of possible increased risk for myopathy especially with doses >40mg per day.
Propafenone	*CYP2D6*	• Guide the dose by therapeutic drug monitoring, perform an ECG and be alert to side effects or • Choose an alternative (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.
Rosuvastatin	*ABCG2* *SLCO1B1*	• Use desired starting dose and adjust doses based on disease-specific and specific population guidelines.

		• Be aware of possible increased risk for myopathy especially for doses over 20mg.
Simvastatin	*SLCO1B1*	• Use an alternative statin. • If simvastatin is warranted, limit dose to 20 mg/day 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*	• Select an alternative or • If aromatase inhibitor use is contraindicated, measure the endoxifen concentration and increase the dose if necessary by a factor of 1.5-2. Avoid co-medication with CYP2D6 inhibitors such as paroxetine and fluoxetine.
Thioridazine	*CYP2D6*	• Select alternative drug, as thioridazine is contraindicated.
Tioguanine	*NUDT15* *TPMT*	• Select alternative drug. • If thioguanine is warranted, phenotyping of TPMT is recommended. Adapt dosing according to phenotype.
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 a warfarin dose algorithm (e.g. http://www.warfarindosing.org).
Zuclopenthixol	*CYP2D6*	• Use 75% of standard dose.

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.

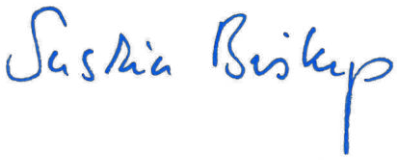
Genetic counseling should be offered with all diagnostic genetic testing. For predictive tests genetic counseling has to be offered.

Medical report written by: XXX

Proofread by: XXX

Validated by: XXX

With kind regards,



Dr. med. Dr. rer. nat. Saskia Biskup

Consultant for Human Genetics

Additional Information

Requested Genes

Modules 01-09, which have been requested in the context of this investigation, contain the following genes:

Module 01: ***APC***, ***ATM***, ***AXIN2***, ***BAP1***, ***BARD1***, ***BMPR1A***, ***BRCA1***, ***BRCA2***, ***BRIP1***, ***CDC73***, ***CDH1***, ***CDKN2A***, ***CHEK2***, ***DICER1***, ***EPCAM***, ***FH***, ***FLCN***, ***KIT***, ***MEN1***, ***MET***, ***MLH1***, ***MSH2***, ***MSH6***, ***NF1***, ***NF2***, ***PALB2***, ***PDGFRA***, ***PMS2***, ***POLD1***, ***POLE***, ***PTCH1***, ***PTEN***, ***RAD51C***, ***RAD51D***, ***RB1***, ***RET***, ***SDHA***, ***SDHAF2***, ***SDHB***, ***SDHC***, ***SDHD***, ***SMAD4***, ***SMARCA4***, ***SMARCB1***, ***STK11***, ***TMEM127***, ***TP53***, ***TSC1***, ***TSC2***, ***VHL***, ***WT1*** (Tumor diseases)

Module 02: ***ACTA2***, ***ACTC1***, ***ACVRL1***, ***ALPK3***, ***BAG3***, ***BMPR2***, ***CALM1***, ***CALM2***, ***CALM3***, ***CASQ2***, ***COL3A1***, ***DES***, ***DSC2***, ***DSG2***, ***DSP***, ***EMD***, ***ENG***, ***FBN1***, ***FHL1***, ***FLNC***, ***GDF2***, ***JUP***, ***KCNH2***, ***KCNK3***, ***KCNQ1***, ***LAMP2***, ***LMNA***, ***LOX***, ***MYBPC3***, ***MYH11***, ***MYH7***, ***MYL2***, ***MYL3***, ***MYLK***, ***PKP2***, ***PLN***, ***PRKAG2***, ***PRKG1***, ***RBM20***, ***RYR2***, ***SCN5A***, ***SMAD3***, ***SMAD9***, ***TBX4***, ***TECRL***, ***TGFB2***, ***TGFBR1***, ***TGFBR2***, ***TMEM43***, ***TNNC1***, ***TNNI3***, ***TNNT2***, ***TPM1***, ***TTN***, ***TTR*** (Cardiovascular diseases)

Module 03: ***ADAMTS13***, ***F10***, ***F11***, ***F12***, ***F13A1***, ***F13B***, ***F2***, ***F5***, ***F7***, ***F8*** (complex intronic rearrangements not included), ***F9***, ***GFI1B***, ***GP1BA***, ***GP1BB***, ***GP6***, ***GP9***, ***HRG***, ***ITGA2B***, ***ITGB3***, ***LMAN1***, ***MCFD2***, ***NBEAL2***, ***PROC***, ***PROS1***, ***SERPINC1***, ***SERPIND1***, ***SERPINF2***, ***VWF*** (Thrombosis and coagulation disorders)

Module 04: ***ATP7B***, ***CP***, ***GLRX5***, ***HAMP***, ***HFE***, ***HJV***, ***SLC40A1***, ***TFR2*** (Iron and copper storage disorders)

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

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

Module 07: ***CACNA1S***, ***RYR1*** (Malignant hyperthermia)

Module 08: ***ABCG2***, ***CACNA1S***, ***CYP2B6***, ***CYP2C19***, ***CYP2C9***, ***CYP2D6***, ***CYP3A4***, ***CYP3A5***, ***CYP4F2***, ***DPYD***, ***G6PD***, ***HLA-A***, ***HLA-B***, ***IFNL3***, ***MT-RNR1***, ***NUDT15***, ***POR***, ***RYR1***, ***SLCO1B1***, ***TPMT***, ***UGT1A1***, ***VKORC1*** (Pharmacogenetics)

Module 09: ***GCK***, ***HNF1A***, ***HNF1B***, ***HNF4A***, ***PDX1*** (Familial diabetes)

General remarks

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.
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, deletions and duplications are not detectable in the mtDNA from leukocytes, but are present in other tissues.

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-2020v4.01, and ClinGen SVI WG guidelines (Richards et al., 2015, PMID: 25741868; Ellard et al., 2020, Association for Clinical Genomic Science; https://clinicalgenome.org/working-groups/sequence-variant-interpretation/). 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 (Ellard et al., 2020, 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, flanking intronic regions and additional disease-relevant non-coding 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 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 balanced rearrangements, uniparental disomy, or low-level mosaicism. Aberrations on the Y chromosome and in 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.

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.

If pathogenic alterations are present within a gene which are not the result of copy number changes (e.g. SNVs), these cannot be detected via MLPA unless covered by variant-specific probes, and therefore cannot be ruled out.

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 AG (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, 97.67% 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 uncertain 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).