CeGaT

CeGaT's Exome:
Including
genome-wide
CNV analysis

Exome Diagnostics

Finding the genetic cause of rare diseases

CeGaT's ExomeXtra® includes:

- all protein-coding regions of the genome
- clinically relevant RNA genes
- > 38,000 intergenic and intronic positions associated with genetic disease according to ClinVar, HGMD, and internal databases
- high and uniform coverage of the entire mitochondrial genome to reliably detect different degrees of heteroplasmy
- pharmacogenetically relevant variants in selected genes
- backbone for genome-wide detection of copy number variants (CNVs)

Exome diagnostics

Exome diagnostics is the genetic testing approach of choice for patients with complex, heterogeneous, and unspecific symptoms. It supports physicians in stating a diagnosis, often after their patients have experienced years of uncertainty.

The exome comprises all protein-coding regions (exons) of the approximately 23,000 genes in the human genome. Although the exome accounts for only about 1-2% of the whole genome, about 89% of all known disease-causing variants are located within the exons. Whole exome sequencing allows the simultaneous analysis of genes in any combination. If the exomes of additional family members are also sequenced, as in trio exome diagnostics, an exome-wide segregation analysis can be performed. This approach significantly increases the chances of finding the genetic cause of complex phenotypes in a shorter time compared to genetic testing of small gene sets.

We are committed to identifying the genetic cause of disease for every patient. Therefore, we have developed an innovative diagnostic approach that goes beyond the possibilities of regular exome diagnostics: ExomeFocus® and ExomeXtra® (Single & Trio) offer an efficient solution for every patient scenario and family constellation.


CeGaT

Detailed Side-By-Side Comparison of Our Different Diagnostic Products

Single ExomeFocus®

Focused and cost-efficient singleton analysis for fast results

Single ExomeXtra®

Whole exome diagnostics of all clinically relevant genes

Trio ExomeXtra®

The most effective genetic test for patients with complex, heterogeneous, and unspecific symptoms

Prenatal ExomeXtra®

Understanding genetic conditions prior to birth

	Single ExomeFocus®	Single ExomeXtra®	Trio ExomeXtra®	Prenatal ExomeXtra®
Proprietary exome enrichment	✓	✓	✓	✓
In-house data analysis and variant calling	✓	✓	✓	✓
Reported variants of the ACMG classes	3/4/5	3/4/5	3/4/5	4/5
Variant classification with details on ACMG criteria and scale	✓	✓	✓	✓
Manual gene selection for interpretation	X	✓	✓	✓
IVERP variants*	X	X	✓	✓**
VUS re-evaluation	X	✓	✓	X
Costs	€	€€	€€€	€€€
Solving rate	XXX	XXXX	XXXXX	XXXXX

* *Accounting for variants in genes with known imprinting, variable expressivity and reduced penetrance.*

** *Prenatal diagnostics with conspicuous ultrasound findings.*

CeGaT's Exome Diagnostics

All achievements of genetic testing integrated into our exome diagnostics

With the latest update of our ExomeXtra® enrichment, we offer you the most innovative solution in exome diagnostics. Designed to generate the most comprehensive sequencing data, our exome diagnostics provides an unmatched basis for the best genetic diagnostics with outstanding features.

Extra Smart Clinical Design

CeGaT's exome enrichment is the sum of the many years of experience of CeGaT and our collaborators, combined with clinical knowledge. In contrast to standard whole exome sequencing (WES), CeGaT's proprietary exome design covers not only the coding regions but all known disease-causing regions throughout the genome. With more than 38,000 intronic and intergenic variants, it contains all genomic regions described as disease-related in the Human Gene Mutation Database (HGMD) and ClinVar, the largest public database of genotype-phenotype relationships. In addition, the whole genome is covered with probes at regular intervals to achieve genome-wide array CGH-like CNV calling performance. Our exome enrichment also includes a clinically relevant selection of non-coding RNA genes, flanking intronic sequences, cryptic exons, and the entire mitochondrial genome.

By continuously improving our wet lab process, we have achieved very uniform and complete coverage of all diagnostic targets. To increase sensitivity and to allow us to detect mosaicism, our ExomeXtra® enrichment has an average diagnostic coverage of >100x.

Therefore, CeGaT's ExomeXtra® provides the ideal basis for genetic diagnostics.

Extra Thorough Analysis

CeGaT's data analysis goes beyond normal exome diagnostics and increases solution rates. We address copy number variants (CNVs), including compound heterozygous combinations of sequence variants (single nucleotide variants (SNV), small insertions and deletions (indels)) with CNVs. Sequencing data is routinely screened for repeat expansions related to reported phenotypes.

Different family constellations can be analyzed as we can include various numbers of family members in the analysis, such as duo (2) or trio (3). For Trio ExomeXtra® we account for variants in genes with reduced penetrance, variable expressivity and even account for imprinting effects. In trio analysis, we additionally include the detection of uniparental hetero- and isodisomies (UPD).

Extra Insightful Results

CeGaT combines human know-how with bioinformatic analysis. The in-house developed software generates data that are evaluated by multiple scientific experts – creating the best possible medical report. CeGaT's interdisciplinary team of PhDs uses the most recent literature for data interpretation. Complex constellations are discussed with bioinformaticians, and the final report is revised by medical doctors and geneticists to maximize medical usability.

Additional Services

ACMG Genes

This additional screening of the ACMG genes[1] allows the detection of relevant pathogenic variants outside the phenotype in a defined group of genes with therapeutic relevance. In all ExomeXtra® services, this option is free of charge for the index patient. For ExomeFocus®, it is available at an additional charge. If selected, we issue a separate report for each person indicating our findings within the ACMG genes.

Upgrade ExomeFocus® to Trio ExomeXtra®

Trio ExomeXtra® is the test that offers the highest chances of identifying the genetic cause of disease. In cases where the treating physician starts with a singleton exome and later wants to expand to Trio ExomeXtra®, this is offered at a reduced price.

HLA Typing

HLA typing can determine tissue compatibility for allogenous transplants, individual drug response (PGx), and differential diagnosis. HLA typing can also be used to judge susceptibility to disease (e.g., HLA-B27: inflammatory and autoimmune disorders). You will receive an additional report stating the HLA alleles.

Pharmacogenetics (PGx)

Pharmacogenetic analysis detects genetic changes that influence the efficacy of drugs. If genetic variants affect proteins responsible for the metabolization of substances, their tolerability and efficacy can be severely altered. These drugs include antidepressants, painkillers, neuroleptics, chemotherapeutics, AIDS drugs, thrombosis drugs, anesthetics, beta-blockers, and statins.

A specific enzyme's reduced activity can lead to increased drug levels at the standard dosage, which is frequently accompanied by undesirable side effects. In the case of pharmaceuticals that are only activated by metabolization, the therapeutic effect may be absent altogether. Similarly, increased enzyme activity, due to the resulting increased drug degradation rate, can lead to insufficient efficacy of the therapy.

The pharmacogenetics option analyzes known variants involved in drug metabolism. If certain gene variants are detected, the treating physician can adjust therapies individually. With the help of pharmacogenetic analysis, serious side effects can be minimized, and treatment failure avoided.

Smarter than Genome

Whole genome sequencing (WGS) is sometimes described as the most comprehensive genetic analysis possible. However, coverage is often too low for reliable variant detection. We sequence our ExomeXtra® with an average diagnostic coverage of >100x, detecting more relevant regions and delivering higher sensitivity than 30x WGS. This increases solution rates and even allows us to detect mosaic variants, which are systematically missed in WGS analyses. At the same time, it avoids thousands of irrelevant variant calls that usually occur in WGS analyses, improving diagnostic speed and accuracy.

Rapid advancement in science is constantly increasing our knowledge about genetic diseases and the potential of genetic analyses. New genomic regions with clinical relevance are identified every year. Our ExomeXtra® enrichment is continuously updated according to the latest clinical findings to always cover all relevant regions. WGS is already a great tool for research.

Until it is ready for diagnostic use, ExomeXtra® offers the best combination of WES, WGS and array CGH (comparative genomic hybridization), providing clinical genomic diagnostics to help patients today.

Table 1: Comparison of standard whole exome sequencing (WGS) and ExomeXtra®. Covered refers to average clinical usable coverage after alignment filtering (uniquely mapping, removal of duplicate and overlapping read ends).

	WGS	ExomeXtra®
Average Diagnostic Coverage	~30x	~110x
Total Gb sequenced	100	15
Coding sequences covered > 20x	93.3%	98.2%
Disease-causing non-coding mutations covered > 20x	96.3%	98.7%
Coding sequences covered > 30x	70.2%	98.1%
Disease-causing non-coding mutations covered > 30x	83.3%	98.3%

[1] According to the current guidelines of the American College of Medical Genetics and Genomics; details on genes and associated diseases can be found at **www.cegat.com/acmg-genes**

The "Xtra" in Our Exome Diagnostics

Copy Number Variants (CNVs)

Beside single nucleotide variants (SNVs) and small insertions and deletions (indels), copy number variants (CNVs) can be causative for a particular phenotype. Genetic testing without screening for deletions and duplications is incomplete and may result in false negative medical reports. All our exome analyses automatically include a deletion/duplication screening - without extra fees. Our CNV analysis in combination with our customized enrichment greatly increases the diagnostic yield.

Our CNV evaluation allows us to detect single exon deletions with a sensitivity of > 81%, larger deletions of three or more exons will be detected with > 96% sensitivity. In addition, we validate our analysis using MLPA or qPCR. The deletion/duplication analysis contributes to CeGaT's high-quality medical reports to provide you with all available analysis options.

Outside of the coding exons, CNV calling covers the whole genome with an array-like resolution of 50 kb (figure 1). This enables accurate characterization and classification of clinically CNVs, allowing the detection of the break points in the intronic region. In order to enable the correct annotation of those structural variants, genome-wide CNV analysis is an essential part of our exome design. Relevant events are included in the medical evaluation. Furthermore, we always determine and describe the quality of the CNV analysis within our medical report. We recommend sending fresh EDTA blood to achieve the highest sensitivity of CNV calling.

Combined Interpretation

CeGaT's analysis strategy enables us to call CNV/SNV combinations to provide even more detailed and important information. SNV/CNV combinations are defined as occurrences of both a SNV and a CNV in the same gene and at least one of them being ACMG class 4 or 5 (likely pathogenic/pathogenic).

Screening for Repeat Expansions

To gain the maximum possible insights from our exome dataset, we also screen for repeat expansions in several genes: SCA1 (*ATXN1*), SCA2 (*ATXN2*), SCA3 (*ATXN3*), SCA6 (*CACNA1A*), SCA7 (*ATXN7*), SCA17 (*TBP*), *FXN*, *AR*, *CSTB*, *HTT*, *JPH3*, *PABPN1*. If results indicate a possible repeat expansion that fits the patient's phenotype, we offer verification with an independent method.

Analysis of Mitochondrial Genome

Mitochondrial genome is included in our enrichment and it is always sequenced. Variants in the mitochondrial genome are evaluated when the phenotype indicates the possibility of a mitochondrial disease. In the case of prenatal diagnostics, the analysis of mtDNA is always included.

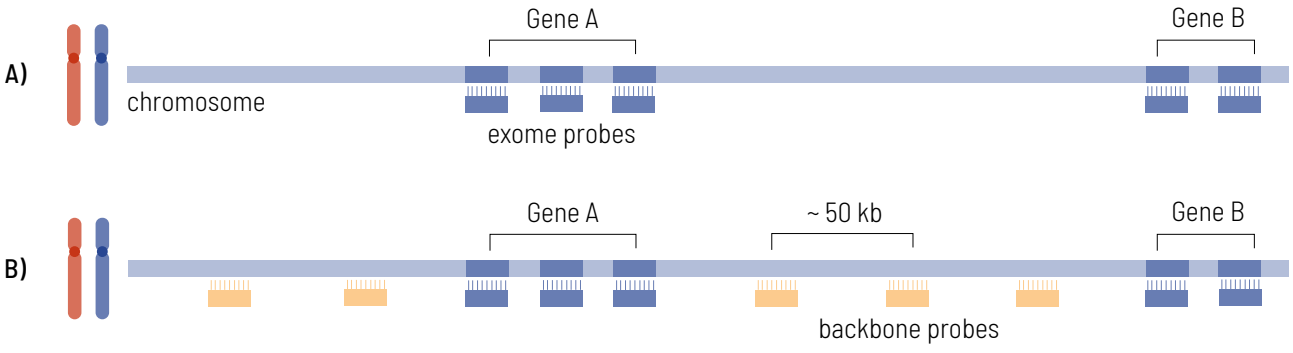


Figure 1: Comparison of CNV calling approaches. **A)** presents the standard approach to CNV calling, where the probes (blue) cover only coding regions. **B)** shows our genome-wide CNV calling, where additonal probes (orange) are included as well to cover the noncoding regions (backbone resolution is ~50 kb).

VUS Re-Evaluation

CeGaT's VUS re-evaluation reassesses previously reported variants of uncertain clinical significance (VUS) as soon as new scientific evidence on the pathogenicity of the variant is available. This leads to an even higher number of diagnoses being made by CeGaT.

Genetic research is making great progress and is constantly improving our knowledge of disease-causing variants. Therefore, it is likely that over time a VUS will be better understood and classified as pathogenic (likely pathogenic/pathogenic) or benign (likely benign/benign).

In the event of such a VUS re-evaluation, CeGaT proactively informs the treating physicians. In addition, the re-evaluated variant is interpreted by our specialists with reference to the patient's clinical picture and a revised medical report will be prepared, **without extra fees (ExomeXtra®).**
The re-evaluation is critical for the care and treatment of patients and family members.

Sample Case

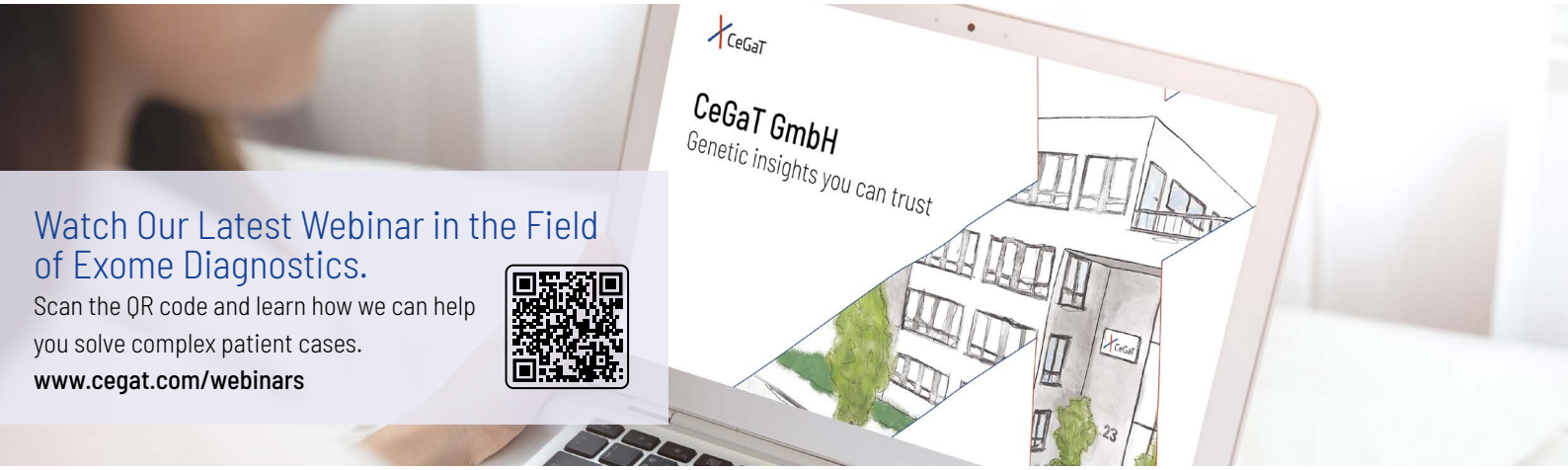
Patient:
boy, 11 months

Clinical Symptoms:
benign infantile epilepsy with tonic-clonic seizures

Primary Report (November 2019):
SCN8A c.802A>T (het.) VUS

VUS Re-Evaluation:
- triggered by HGMD update in January 2021
- based on publication Medlin *et al.*, 2020[2]

→ Variant can now be classified as likely pathogenic. New report has been issued.



Watch Our Latest Webinar in the Field of Exome Diagnostics.

Scan the QR code and learn how we can help you solve complex patient cases.
www.cegat.com/webinars

[2] Medlin, L. C., Bello-Espinosa, L. & MacAllister, W. S. Neuropsychological profiles of two patients with differing SCN8A-pathogenic variants. *Applied neuropsychology. Child* **11**, 561–566; 10.1080/21622965.2020.1807983 (2022).

Single Exome Diagnostics

Whole exome diagnostics (WES) test for an individual patient

The exome includes all protein-coding regions (exons) of the approximately 23,000 genes in the human genome. Although the exome accounts for only about 1-2% of the whole genome, approximately 89% of all known disease-causing mutations are identified to be located within the exons. In addition, our exome design also covers more than 38,000 non-coding variants described as disease-relevant in the databases HGMD and ClinVar. Our singleton exome diagnostics is based on our proprietary, high-quality exome enrichment, providing deep and homogeneous coverage of all known disease-causing regions of the genome.

Single ExomeXtra®

Single ExomeXtra® is the best possible whole exome diagnostics (WES) test for an individual patient. It is based on a precise description of the patient's phenotype, an individualized list of candidate genes associated with their symptoms is created. This includes all clinically relevant genes, as well as genes associated with differential diagnoses, based on the Human Phenotype Ontology (HPO) and internal databases.

ExomeFocus®

ExomeFocus® is the result of our many years of experience in genetic diagnostics and bioinformatics, representing the most efficient approach for singleton exome diagnostics. Our in-house developed software uses our extensive in-house database of genetic variants as well as all publicly available databases to data-mine the patient's exome for high-impact variants. Our scientific team then evaluates these high-impact variants to ensure clinical relevance for the patients' phenotype.

In case of unresolved cases, there is always an option to expand the analysis to Trio ExomeXtra®.

Service Details

Medical Report With:

- variants with clinical relevance (ACMG class 3, 4, 5)
- interpretation & discussion of these variants and the affected genes
- interpretation of genetic relevance for family planning
- recommendations for clinical disease management & further tests
- genome-wide detection of copy number variants (CNV)

Additional Services:

- ACMG gene panel
- pharmacogenetics
- HLA typing

Our Standard Sample Requirements:

- 1-2 ml EDTA blood (recommended sample type)
- genomic DNA (1-2 µg)
- DBS cards, buccal swabs, or saliva are also possible

Other samples are possible – please consult with us at diagnostic-support@cegat.com.

We gladly answer your questions and assist you in choosing the optimal sample for your patient.

Trio ExomeXtra®

The most effective genetic test for patients with complex, heterogeneous, and unspecific symptoms

Trio ExomeXtra® is used to diagnose an affected patient with unaffected parents. Including both unaffected parents in the analysis significantly increases the chances of a successful diagnosis. For Trio ExomeXtra®, the exomes of the parents and the index patient are sequenced. This allows an exome-wide segregation analysis and, thus the highest solution rates. Comparative exome diagnostics is particularly effective because the number of variants to be evaluated is minimized, and single variants' numerous and cost-intensive segregation analyses are avoided. In addition to SNVs and CNVs, we also check for uniparental disomies (UPD), covering both isodisomies as well as heterodisomies affecting whole or partial chromosomes.

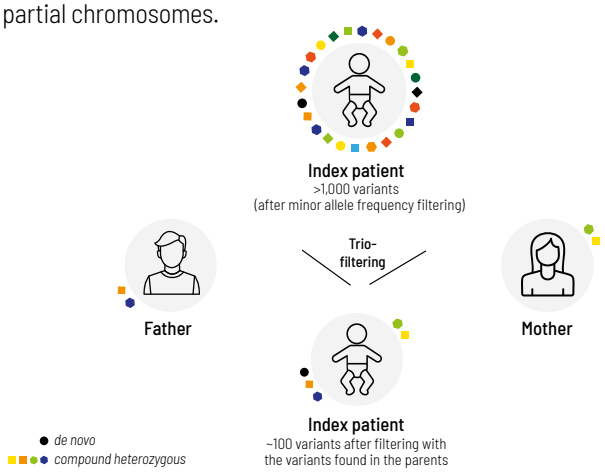


Figure 2: Schematic representation of trio exome filtering, which also considers mildly affected parents. Filtering with the parental variants enables, for example, the detection of compound heterozygous variants.

In the evaluation process, our experienced team investigates all variants according to the latest scientific knowledge. For variants in genes that are not clearly associated with the patient's suspected phenotype, we predict the possible contribution through extensive additional literature research. If we find evidence that a variant not yet described contributes to the patient's phenotype, this variant is described in our medical report.

Comparison of the affected patient's data with those of the unaffected parents allows us to identify the following SNV/CNV combinations:

- *de novo* – new in the index, not present in parents
- homozygous – homozygous in the index, heterozygous in both parents
- compound heterozygous – two or more variants in the same gene in the index on different alleles; each parent is a heterozygous carrier of one variant
- x-linked – male patient is hemizygous, the mother is heterozygous
- loss of heterozygosity – index is homozygous, one parent heterozygous, other parent wildtype
- parental mosaicism – index is heterozygous, one parent has a genetic mosaicism

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We gladly answer your questions and assist you in choosing the optimal sample for your patient.

Benefit from Our Comparative ExomeXtra®

Our unique analysis strategy considers often overlooked issues, such as mildly affected parents

In comparative family exome diagnostics, not only the affected patient but also other relatives are sequenced. The most commonly performed analysis is the trio exome: The inclusion of both unaffected parents of the index patient highly increases the chances of diagnostic success to be approximately twice as high compared to single exome diagnostics.[3]

In family constellations where it is not possible to obtain a sample from both genetic parents, or one parent might be affected as well, the adaptive ExomeXtra® bioinformatic pipeline allows us to analyze all kinds of family combinations, like duos. This goes beyond commonly performed analyses of private and shared variants between two persons.

Our additional analyses are the best approach for solving cases with only one parent (affected or unaffected) or any other index-relative constellations. A detailed phenotypic description of all affected individuals is the basis for a precise and successful data interpretation. Standard trio exome diagnostics rely on the assumption that both parents are not affected. This filtering ignores that some parents are only mildly affected, for example, which would result in negative findings. We compensate for these situations with a unique analysis strategy that allows us to solve cases where the phenotype is caused by variants with

- reduced penetrance
- variable expressivity
- imprinting effects

Detection of Uniparental Disomies (UPDs)

We also detect and report uniparental disomies (UPDs). UPDs can cause diseases due to imprinting effects, underlying homozygous pathogenic variants, or low-level mosaic aneuploidies.

We are able to report on all four possible UPD constellations: maternal heterodisomies, maternal isodisomies, paternal heterodisomies, and paternal isodisomies (fig. 3).

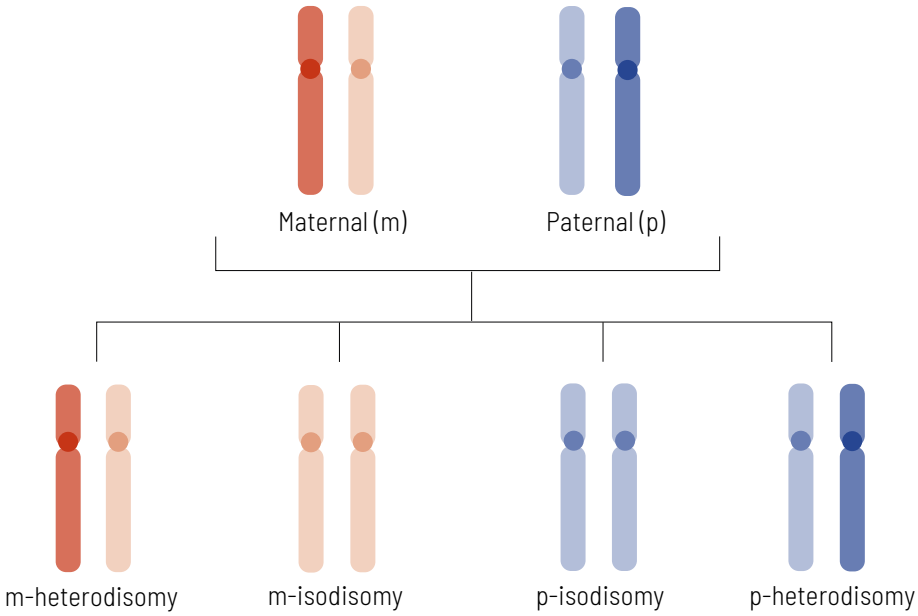


Figure 3: Schematic illustration of the inheritance pattern of uniparental disomy (UPD). UPD occurs when both pairs of chromosomes are inherited from only one parent, which means the other parent's chromosome for that pair is missing. The figure shows the possible types of UPDs that can occur, which may be inherited maternally (both red) or paternally (both blue). UPDs are also further sub-categorized depending on the constellation; if one chromosome is inherited in a duplicated fashion (isodisomy) or if each chromosomes in the pair is inherited (heterodisomy).

[3] Farwell, K. D. *et al.* Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unselected families with undiagnosed genetic conditions. *Genetics in medicine : official journal of the American College of Medical Genetics* **17**, 578–586; 10.1038/gim.2014.154 (2015).

Case Report – Saving a Little Girl's Life

An exact diagnosis is the basis for optimal treatment. Here is a case from our daily work where exome diagnostics saved a little girl's life.

Our patient had shown signs of severe illness at an early age. Her parents sought medical assistance when she was about six months old. Examining the baby, the physicians identified a bone marrow developmental defect. But the reasons for the deficiency remained unclear. However, the physicians knew that time was precious since the little girl's older brother had already died at the age of seven months, displaying similar symptoms. Facing this, the only option the physicians had was to prepare for bone marrow transplantation. The surgery posed a high risk for the girl's life because survival rates for this type of procedure for a baby are far from good, at around 50%. But the physicians also knew that if two siblings show such similar symptoms, there had to be a genetic cause.

Finding the needle in the haystack

To get results as fast as possible and retain a chance to spare the girl the risky bone marrow transplantation, the treating physician approached CeGaT directly. At CeGaT, the DNA from the little girl and both parents were analyzed (so-called "trio exome diagnostics").

Every human has thousands of genetic alterations, and nearly all of them do not cause disease. Finding the one genetic variant that causes a patient's disease is the well-known "needle in a haystack" problem. This procedure requires a profound understanding of sequencing technology, molecular biology, and human genetics.

Gene analysis reveals a hidden condition

CeGaT was able to identify two variants in our patient, one inherited from each parent, in a gene called *TCN2*. The protein encoded by this gene binds and transports vitamin B12 into cells, such as bone marrow cells. This is essential for bone marrow development. As the girl's blood values for vitamin B12 were normal, the physicians had no indication of this condition. In order to compensate for the reduced transfer of vitamin B12 into the bone marrow, the physicians decided to provide her with B12 supplements through medication and postponed the bone marrow transplantation. The treatment worked – the little girl recovered; bone marrow transplantation was not needed. She is developing well: Today, she is a healthy, happy four-year-old girl.

Sample Case

Patient:

girl, 6 months

Familial History:

brother with same symptoms passed away at the age of seven months.

Initial Planned Therapy Strategy:

bone marrow transplantation which features a chance of survival only approx. 50%.

Genetic Testing:

trio exome diagnostics at CeGaT

Symptoms:

congenital bone marrow failure, recurrent infections, anemia, dystrophy, reduced neutrophil and thrombocyte count

Results:

Homozygous mutation in *TCN2* (encodes for a vitamin B12 carrier protein) leading to a defect in vitamin B12 metabolism (despite normal blood values)

Read Our Latest Publication in the Field of Prenatal Diagnostics.

We have conducted studies with a cohort of over 1,000 prenatal trios.
Visit our website for more information:
www.cegat.com/prenatal-publications

Prenatal ExomeXtra®

Understanding genetic conditions prior to birth

Fetal structural anomalies are found in up to 3% of all pregnancies following routine prenatal care ultrasound screening.[4] A diagnosis is difficult due to limited phenotypic information, but it is crucial to gain information about disease prognosis. The Prenatal ExomeXtra® analysis assists in such cases by identifying the genetic cause of the disease. The human exome contains all protein-coding regions (exons) of about 23,000 genes in the genome. The exome makes up only about 1-2% of the whole genome, but close to 89% of all known disease-causing mutations are located within the exons. In addition, our proprietary design also covers more than 38,000 non-coding variants described as disease-relevant in the databases HGMD and ClinVar.

Diagnostics - with conspicuous ultrasound findings:
The Prenatal ExomeXtra® is used to determine the genetic cause of a disease in a fetus with abnormal ultrasound findings. In addition, we investigate the risk of serious health complications in the early stages of a child's life. The analysis of the exomes of the fetus and both parents (trio exome diagnostics) enables a comparative analysis and increases the probability of identifying disease-causing variants.

Prenatal ExomeXtra® diagnostics enable the detection of variants that affect metabolism, for example and offer the possibility of initiating treatment immediately after birth. Our unique analysis approach also allows the detection of variants with imprinting effects, variable expressivity, and reduced penetrance (see page 14 for more details).

Diagnostics - with inconspicuous ultrasound findings:
The Prenatal ExomeXtra® can also be performed if prenatal ultrasound examinations are inconspicuous. A study by Sukenik-Halevy *et al.* shows that more than 50% of cases with postnatal neurocognitive disorders did not show prenatal ultrasound abnormalities.[5]

We have compiled a comprehensive panel of over 2,000 genes associated with severe early-onset diseases in cooperation with leading experts in prenatal clinical human genetics. After trio exome analysis and filtering, we screen all genes in the panel for pathogenic and likely pathogenic (ACMG class 4, 5) variants associated with severe, early-onset disease.
Our experts also report all pathogenic and likely pathogenic variants outside this gene panel and discuss their clinical relevance if they lead to severe childhood disorders. In this way, we always consider the latest scientific findings.

Service Details

Medical Report With:

- variants with clinical relevance (ACMG class 4, 5)
- interpretation & discussion of these variants and the affected genes
- interpretation of genetic relevance for family planning
- recommendations for clinical disease management & further tests
- detection of copy number variants (CNV)

Additional Services:

For the parents:

- ACMG gene panel
- pharmacogenetics
- HLA typing

Our Standard Sample Requirements:

For the fetus:

- amniotic fluid (native or cultured)
- chorionic villi (native or cultured)
- extracted fetal DNA
- abortion material

For the parents:

- 1-2 ml EDTA blood (recommended sample type)
- genomic DNA (1-2 µg)
- DBS cards, buccal swabs, or saliva are also possible

Other samples are possible – please consult with us at diagnostic-support@cegat.com and we will gladly answer your questions.

For prenatal analyses, we always require a sample from the mother to test for maternal cell contamination (MCC).

[4] Edwards, L. & Hui, L. First and second trimester screening for fetal structural anomalies. *Seminars in fetal & neonatal medicine* **23**, 102–111; 10.1016/j.siny.2017.11.005 (2018).

[5] Sukenik-Halevy, R. *et al.* The prevalence of prenatal sonographic findings in postnatal diagnostic exome sequencing performed for neurocognitive phenotypes: A cohort study. *Prenatal diagnosis vol.* 42,**6**, 717-724. doi:10.1002/pd.6095 (2022).

Sample Requirements and Order Form

Sample Requirements

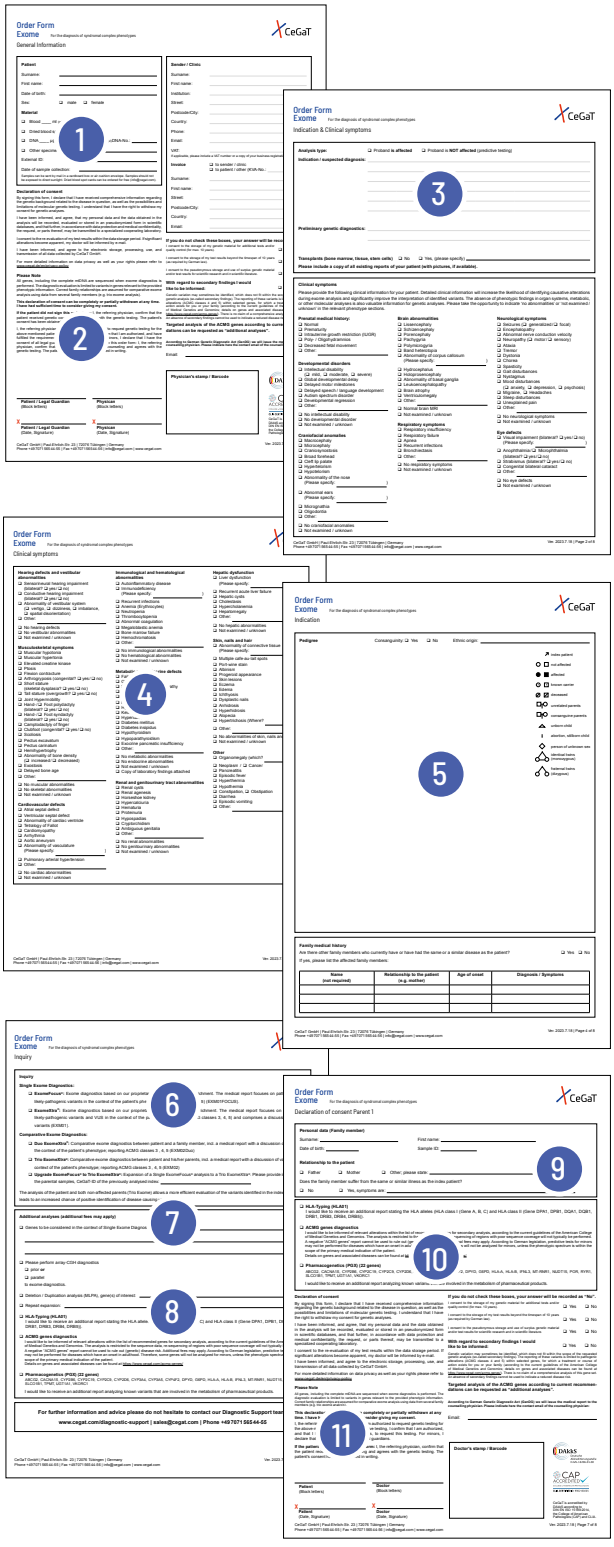
Our standard sample requirements are 1-2 ml EDTA blood (recommended sample type). We also accept a variety of different materials such as DBS cards, buccal swabs, saliva, or isolated DNA.

Order Form

1. **Patient details**
   (Name, date of birth, sample material, ...)
2. **Declaration of consent**
   For genetic testing, the consent of the patient tested is mandatory. We understand that patients might not want to sign a consent in a foreign language. In such cases, we can accept a confirmation that the ordering physician has obtained the patient's consent.
3. **Clinical symptoms**
   Please describe the patient's phenotype. Please
4. choose the appropriate symptoms using the pre-defined set of HPO terms and/or include additional information regarding the patient's phenotype. The more detailed the clinical description, the more valid is our diagnostics.
5. **Family history**
   The inclusion of pedigree information helps to clarify the family history and indicate if there have been similar symptoms in the family. CeGaT provides a software to draw pedigree charts, the Pedigree Chart Designer.
6. **Inquiry**
   Single exome, trio exome, clinical trio exome, or duo exome. Please select the genetic diagnostic you wish to perform.
7. **Additional analyses**
   Please indicate whether you would like further analyses (array CGH, MLPA, ...).
8. **ACMG genes screening**
   If desired, ACMG genes screening can be ordered here. This analysis is offered free of charge for the index patient.

If trio requested:

9. **Parent's details**
10. **ACMG genes screening for parents and additional analysis**
11. **Parent's consent**
    See point 2 above.



Also Possible via myCeGaT Portal

The process described above can also be completed digitally via our portal. To do so, scan the QR code or visit **my.cegat.com**

Medical Report

The test results are described and explained in a medical report delivered to the referring physician. A total of four scientific experts contributes to every medical report – generating the best possible results. CeGaT's interdisciplinary team of PhDs uses the most recent literature for data interpretation. Complex constellations are discussed with bioinformaticians. The final report is revised by medical doctors and geneticists to maximize medical usability.

1 Patient information

In the header, we summarize patient information:

- name, sex, external ID
- sample source and date of receipt
- CeGaT specified ID
- suspected diagnosis or indication for molecular genetic testing
- requested test

2 Results

This part summarizes the identified genetic changes according to ACMG guidelines, tabulated and sorted by their disease relevance. You will find a table that addresses the most likely causative variant(s) and provides information on zygosity, inheritance, allele frequency in the population, and our classification. We also report likely pathogenic variants and variants of uncertain significance (VUS) potentially causative for the patient's phenotype. CNV findings and the quality of the CNV analysis are explicitly described.

3 Recommendation

Clinical recommendations for the referring physician are given. These are, for example, additional diagnostic options for the patient (in case of a negative report), potential therapeutic approaches based on the pathogenic genetic variant(s) of the patient, or further molecular genetic tests for both affected and unaffected family members (e.g., segregation analysis).

4 Genetic relevance

We explain the inheritance pattern of the patient's disease; the risk of disease recurrence in the family, and the extent to which other family members may be unaffected carriers.

5 Clinical information and variant interpretation

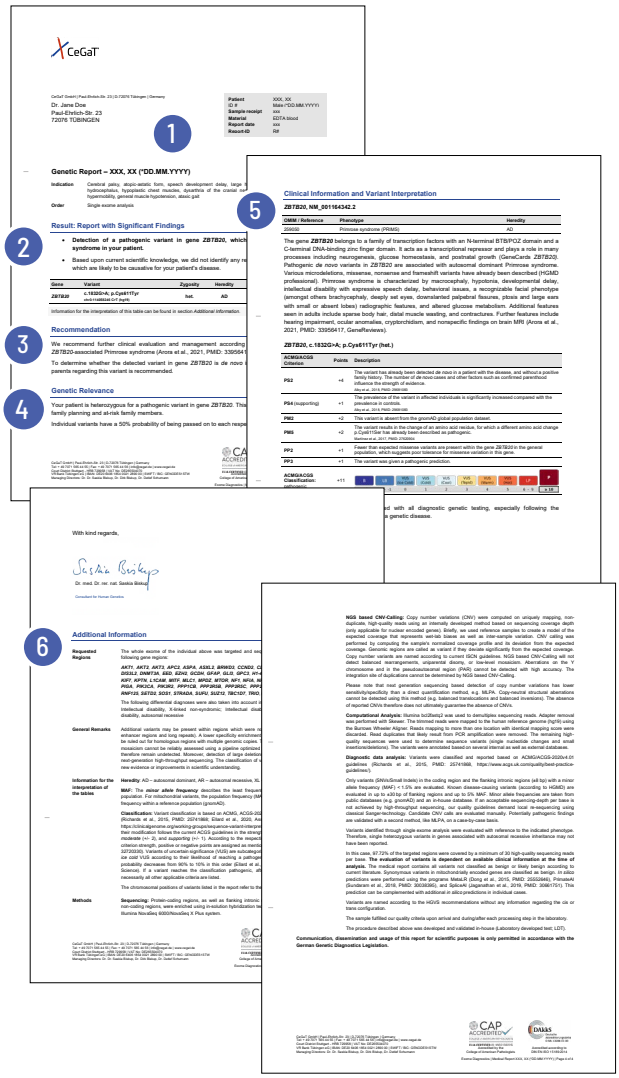
We summarize the current state of the scientific literature for the variants found. We explain and describe the detected variant(s) and affected genes in detail and how they contribute to the patient's phenotype. The more clinical information is provided by the referring physician, the more accurate and precise our evaluation can be.

Variants of uncertain significance

We summarize the current state of the scientific literature for the variants found. We explain and describe the detected variant(s) and affected genes in detail and how they contribute to the patient's phenotype. In addition, the ACMG criteria used for the variant(s) are clearly presented in a table and the variant classification is visualized using an ACMG classification scale. The more clinical information is provided by the referring physician, the more accurate and precise our evaluation.

6 Additional Information

We present detailed technical information on the methods used.



About Us

CeGaT is a global provider of genetic analyses for a wide range of medical, research, and pharmaceutical applications.

Founded in 2009 in Tübingen, Germany, the company combines state-of-the-art sequencing technology with medical expertise – with the aim of identifying the genetic causes of diseases and supporting patient care. For researchers and pharmaceutical companies, CeGaT offers a broad portfolio of sequencing services and tumor analyses. CeGaT generates the data basis for clinical studies and medical innovations and drives science forward with its own insights.

The owner-managed company stands for independence, comprehensive personal customer service, and outstanding quality. CeGaT's laboratory is accredited according to CAP/CLIA, DIN EN ISO 15189, DIN EN ISO/IEC 17025, and thus meets the highest international standards. To obtain first-class results, all processes are carried out in-house under scientific supervision. We would be pleased to provide you with our award-winning service.

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Accredited by DAkkS according to DIN EN ISO 15189:2014

CAP ACCREDITED COLLEGE of AMERICAN PATHOLOGISTS

CLIA CERTIFIED ID: 99D2130225

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

Phone: +49 7071 56544-55
Fax: +49 7071 56544-56
Email: info@cegat.com

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