Intelliseq

Whole exome sequencing analysis report

Example-name
Example-surname
Male

Date of birth
02/02/2002

Patient ID
1234567789

Ordering physician
Example-physician

Sample ID
Demo_sample_WES

Source
blood

Sequencing type
whole exome

Received date
03/01/2022

Sequencing date
03/01/2022

Report date
04/02/2022

Summary of results

Variants classified according to ACMG recommendations

2 tested genes

0	1	2
Pathogenic →	Likely pathogenic →	Uncertain Significance →

Scope of analysis
Example description

Patient description (clinical information)
Example description

Limitations of liability
The clinical diagnosis should never be based on the Patient's genomic sequence analysis alone.

Read more

ABCB11

ACMG: Likely pathogenic | Score: 0.95 | Clinvar: Pathogenic | Inheritance Pattern Match: None

Associated diseases
- Cholestasis, benign recurrent intrahepatic, 2 (HPO - OMIM:605479)
- Cholestasis, progressive familial intrahepatic 2 (HPO - OMIM:601847)
- Intrahepatic cholestasis of pregnancy (HPO - ORPHA:69665)

Diseases inheritance
- Autosomal recessive inheritance

Variant
- rs1559183717
- 2-168935398-G-A
- Heterozygote
- ENST00000650372.1/ NM_003742.4
- c.2842C>T
- p.Arg948Cys
- Allele depth (ref/alt): 59/76
- High quality call (QC: 1920.0)

GDF3

ACMG: Uncertain Significance | Score: 0.181 | Clinvar: Conflicting interpretations | Inheritance Pattern Match: Dominant

Associated diseases
- Isolated Klippel-Feil syndrome (HPO - ORPHA:2345)
- Klippel-Feil syndrome 3, autosomal dominant (HPO - OMIM:613702)
- Microphthalmia, isolated 7 (HPO - OMIM:613704)
- Microphthalmia, isolated, with coloboma 6 (HPO - OMIM:613703)

Diseases inheritance
- Autosomal dominant inheritance
- Multifactorial inheritance
- Digenic inheritance

Variant
- rs140926412
- 12-7690177-G-A
- Heterozygote
- ENST00000329913.4/ NM_020634.3
- c.796C>T
- p.Arg266Cys
- Allele depth (ref/alt): 74/67
- High quality call (QC: 1710.0)

GDF3

ACMG Uncertain Significance Score: 0.0938 Clinvar Undefined Inheritance Pattern Match Dominant

Associated diseases
- Isolated Klippel-Feil syndrome (HPO - ORPHA:2345)
- Klippel-Feil syndrome 3, autosomal dominant (HPO - OMIM:613702)
- Microphthalmia, isolated 7 (HPO - OMIM:613704)
- Microphthalmia, isolated, with coloboma 6 (HPO - OMIM:613703)

Diseases inheritance
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- Multifactorial inheritance
- Digenic inheritance

Variant
- rs775383579
- 12-7690086-T-C
- Heterozygote
- ENST00000329913.4/ NM_020634.3
- c.887A>G
- p.Glu296Gly
- Allele depth (ref/alt): 68/60
- High quality call (QC: 1490.0)

Variant Details

ABCB11 ACMG Likely pathogenic Score: 0.95 Clinvar Pathogenic Inheritance Pattern Match None

Associated diseases
- Cholestasis, benign recurrent intrahepatic, 2 (HPO - OMIM:605479)
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- p.Arg948Cys
- Allele depth (ref/alt): 59/76
- High quality call (QC: 1920.0)

Variant Frequency	WES-AF=None; WGS-AF=None; maxAF=None; maxPOP=None	**Predicted Impact**	SIFT=None; PhastCons=1.0; GERP=5.68; MCAP=0.511
ACMG PVS1 score: 0.0	Not a loss of function variant	**ACMG BA1** score: 0.0	Frequency of this variants is not given in the gnomAD database.
ACMG PS1 score: 1.0	This variant leads to the Arg948Cys substitution in the ABCB11 protein, (transcript: ENST00000650372.1). Variants introducing the same change are classified in the ClinVar database as pathogenic.	**ACMG BS2** score: 0.0	Data about frequency of homozygotes is not provided for this allele by the gnomAD database.
ACMG PS3 score: 0.0	This is a missense variant. Protein mutants with similar amino acid substitutions do not have record in the UniProt database.	**ACMG BP1** score: 0.05	This is a missense variant of the ABCB11 gene. Protein truncating mutations of the ABCB11 gene may lead to disease: ClinVar database describes 45 pathogenic null variants of this gene. Nevertheless, missense mutations in the ABCB11 gene may also lead to disease, as 29 pathogenic missense variants of this gene is also known.
ACMG PM1 score: 0.0	This variant does not change region critical for protein function. Variant is located outside any known mutational hot spot.	**ACMG BP3** score: 0.0	This variant does not lead to any in-frame deletion/insertion.
ACMG PM2 score: 1.0	This variant is absent from the gnomAD database. It is also located in the genomic region having good coverage, (mean coverage: 31.73).	**ACMG BP4** score: 0.0	This variant is not benign according to at least half of the applied coding-sequence predictors(10 out of 10 for which data is available).
ACMG PM4 score: 0.0	This variant does not lead to in-frame length changes of any protein.	**ACMG BP5** score: 0.0	This variant is decribed in the ClinVar database as pathogenic/likely pathogenic or of uncertain impact.
ACMG PM5 score: 0.0	This is not a novel variant.	**ACMG BP7** score: 0.0	This is a coding, protein-changing variant.
ACMG PP2 score: 0.0	This variant leads to amino acid substitution. It affects the ABCB11 gene. Missense variants of the ABCB11 are not a common mechanism of disease. While ClinVar database describes 29 pathogenic missense variants for this gene, they make up only a tiny fraction of all known missense mutations.		

ACMG PP3
score: 0.9

This variant is pathogenic according to the majority of the applied coding-sequence predictors: M-CAP, BayesDel (with maxAF), DANN, FATHMM-MKL, DEOGEN2, LIST-S2, MVP, MutationAssessor, SIFT4G (9 out of 10 for which data is available).

ACMG PP4
score: 0.75

Variant of the ABCB11 gene, likely contributing to the patient phenotype (gene to phenotype match: 75.0).

The post alignment variant visualization for chr2:168935398, using Integrative Genomics Viewer.

The graph presents NGS reads provided by user in bam files. The coverage section presents a cumulative histogram. In alignment section sequencing reads are drawn as grey horizontal bars, with variant bases highlighted in color. Color intensity indicates the quality of the base call. The reference genome sequence is presented above alignment panels. Gene annotation track with marked exons (dark blue rectangles) and introns (dark blue line) is presented below the alignment panels.

GDF3

ACMG Uncertain Significance Score: 0.181 Clinvar Conflicting interpretations Inheritance Pattern Match Dominant

Associated diseases
- Isolated Klippel-Feil syndrome (HPO - ORPHA:2345)
- Klippel-Feil syndrome 3, autosomal dominant (HPO - OMIM:613702)
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Variant
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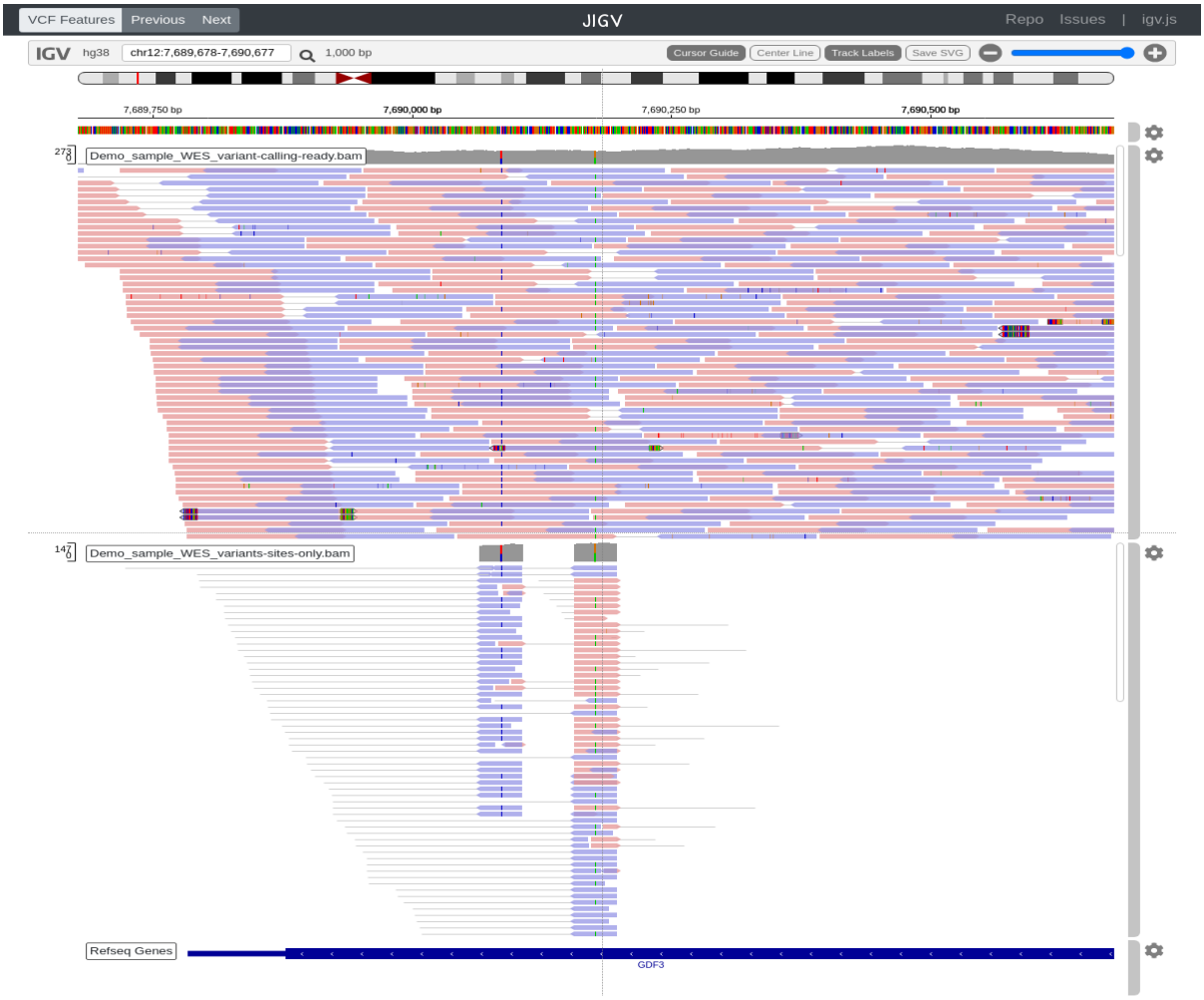
- c.796C>T
- p.Arg266Cys
- Allele depth (ref/alt): 74/67
- High quality call (QC: 1710.0)

Variant Frequency	WES-AF=0.00295; WGS-AF=0.00257; maxAF=0.0064; maxPOP=FIN
ACMG PVS1 score: 0.0	Not a loss of function variant
ACMG PS1 score: 0.0	This is a protein altering variant. Variants introducing the same change are not pathogenic or likely pathogenic, according to the ClinVar database.
ACMG PS3 score: 0.0	This is a missense variant. Protein mutants with similar amino acid substitutions do not have record in the UniProt database.
ACMG PM1 score: 0.0	This variant does not change region critical for protein function. Variant is located outside any known mutational hot spot.
ACMG PM2 score: 0.0	This variant is present in the gnomAD exomes database, and its frequency (0.0030) is not extremely low.
ACMG PM4 score: 0.0	This variant does not lead to in-frame length changes of any protein.
ACMG PM5 score: 0.0	This is not a novel variant.
ACMG PP2 score: 0.0	This variant leads to amino acid substitution. It affects the GDF3 gene. Missense variants of the GDF3 are not a common mechanism of disease. While ClinVar database describes 4 pathogenic missense variants for this gene, they make up only a tiny fraction of all known missense mutations.
ACMG PP3 score: 0.7	This variant is pathogenic according to the majority of the applied coding-sequence predictors: M-CAP, DANN, FATHMM-MKL, DEOGEN2, LIST-S2, MVP, MutationAssessor (7 out of 10 for which data is available).
ACMG PP4 score: 0.75	Variant of the GDF3 gene, likely contributing to the patient phenotype (gene to phenotype match: 75.0).
Predicted Impact	SIFT=0.051; PhastCons=0.449; GERP=4.61; MCAP=0.0257
ACMG BA1 score: 0.0	Frequency of this variant in the gnomAD exomes database is 2.95e-03, which is below the 5% ACMG BA1 threshold.
ACMG BS2 score: 0.0	This variant is not observed in homozygotes in the gnomAD exomes database.
ACMG BP1 score: 0.0	This is a missense variant of the GDF3 gene. Contribution of protein truncating variants to disease is not established for the GDF3 gene, as its null pathogenic mutations are not described in the ClinVar database.
ACMG BP3 score: 0.0	This variant does not lead to any in-frame deletion/insertion.
ACMG BP4 score: 0.0	This variant is not benign according to at least half of the applied coding-sequence predictors(7 out of 10 for which data is available).
ACMG BP5 score: 0.0	This variant is decribed in the ClinVar database as pathogenic/likely pathogenic or of uncertain impact.
ACMG BP7 score: 0.0	This is a coding, protein-changing variant.

The post alignment variant visualization for chr12:7690177, using Integrative Genomics Viewer.

The graph presents NGS reads provided by user in bam files. The coverage section presents a cumulative histogram. In alignment section sequencing reads are drawn as grey horizontal bars, with variant bases highlighted in color. Color intensity indicates the quality of the base call. The reference genome sequence is presented above alignment panels. Gene annotation track with marked exons (dark blue rectangles) and introns (dark blue line) is presented below the alignment panels.



GDF3

ACMG Uncertain Significance Score: 0.0938 Clinvar Undefined

Inheritance Pattern Match: Dominant

Associated diseases

- Isolated Klippel-Feil syndrome (HPO - ORPHA:2345)
- Klippel-Feil syndrome 3, autosomal dominant (HPO - OMIM:613702)
- Microphthalmia, isolated 7 (HPO - OMIM:613704)
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- Autosomal dominant inheritance
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Variant

- rs775383579
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- Heterozygote
- ENST00000329913.4/ NM_020634.3
- c.887A>G
- p.Glu296Gly
- Allele depth (ref/alt): 68/60
- High quality call (QC: 1490.0)

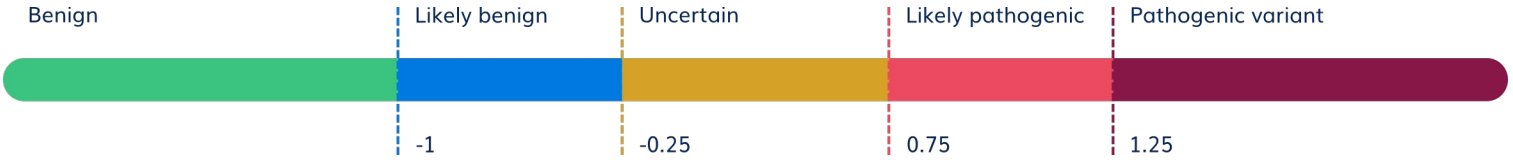
Variant Frequency	WES-AF=0.000114; WGS-AF=0.000201; maxAF=0.000201; maxPOP=NFE
ACMG PVS1 score: 0.0	Not a loss of function variant
ACMG PS1 score: 0.0	This is a protein altering variant. Variants introducing the same change are not described in the ClinVar database.
ACMG PS3 score: 0.0	This is a missense variant. Protein mutants with similar amino acid substitutions do not have record in the UniProt database.
ACMG PM1 score: 0.0	This variant does not change region critical for protein function. Variant is located outside any known mutational hot spot.
ACMG PM2 score: 0.0	This variant is present in the gnomAD exomes database, and its frequency (0.0001) is not extremely low.
ACMG PM4 score: 0.0	This variant does not lead to in-frame length changes of any protein.
ACMG PM5 score: 0.0	This is a protein changing variant. Variants introducing similar, but not identical, changes are not described in the ClinVar database.
ACMG PP2 score: 0.0	This variant leads to amino acid substitution. It affects the GDF3 gene. Missense variants of the GDF3 are not a common mechanism of disease. While ClinVar database describes 4 pathogenic missense variants for this gene, they make up only a tiny fraction of all known missense mutations.
ACMG PP3 score: 0.0	This variant is not pathogenic according to at least half of the applied coding-sequence predictors(5 out of 10 for which data is available).
ACMG PP4 score: 0.75	Variant of the GDF3 gene, likely contributing to the patient phenotype (gene to phenotype match: 75.0).
Predicted Impact	SIFT=0.033; PhastCons=0.989; GERP=3.37; MCAP=0.0198
ACMG BA1 score: 0.0	Frequency of this variant in the gnomAD exomes database is 1.14e-04, which is below the 5% ACMG BA1 threshold.
ACMG BS2 score: 0.0	This variant is not observed in homozygotes in the gnomAD exomes database.
ACMG BP1 score: 0.0	This is a missense variant of the GDF3 gene. Contribution of protein truncating variants to disease is not established for the GDF3 gene, as its null pathogenic mutations are not described in the ClinVar database.
ACMG BP3 score: 0.0	This variant does not lead to any in-frame deletion/insertion.
ACMG BP4 score: 0.0	This variant is not benign according to at least half of the applied coding-sequence predictors(5 out of 10 for which data is available).
ACMG BP5 score: 0.0	This genetic variant is not described in the ClinVar database.
ACMG BP7 score: 0.0	This is a coding, protein-changing variant.

The post alignment variant visualization for chr12:7690086, using Integrative Genomics Viewer.

The graph presents NGS reads provided by user in bam files. The coverage section presents a cumulative histogram. In alignment section sequencing reads are drawn as grey horizontal bars, with variant bases highlighted in color. Color intensity indicates the quality of the base call. The reference genome sequence is presented above alignment panels. Gene annotation track with marked exons (dark blue rectangles) and introns (dark blue line) is presented below the alignment panels.

Variants classification according to the ACMG (American College of Medical Genetics and Genomics) criteria.

ACMG Score



Step1: Variant evaluation in terms of fulfilling ACMG minor categories criteria

The minor ACMG categories criteria concern different variant and gene features supporting its pathogenic or benign character [1, 2]. The following characteristics are analyzed:

1) the impact that a given variant has on a particular gene or gene protein product (mutation type, e.g., loss of function, missense);
2) whether the detected kind of mutation is a validated mechanism of disease for the affected gene;
3) evolutionary conservation of the mutated site;
4) whether variant disrupts conserved splicing motifs (dbscSNV);
5) variant and homozygous genotypes frequency in populations (gnomAD and MITOMAP databases);
6) ClinVar database information about the pathogenicity of the same or similar variants;
7) UniProt database information about mutation effect in functional studies;
8) whether variant lies within a region essential for protein function (UniProt database);
9) whether the affected gene is likely to contribute to the patient phenotype (based on the Human Phenotype Ontology database and custom algorithm);

Step2: Variant assignment to one of the main ACMG categories

For fulfilling any of the aforementioned ACMG minor categories criteria, a given variant receives points (positive for pathogenic, negative for benign categories). The final score (a weighted sum of these points) is used to classify variants as pathogenic, likely pathogenic, benign, likely benign, or of uncertain significance. See [3] for detailed information on the scores combining algorithms.

Implemented ACMG minor categories (names as in [1]):
PVS1, PS1, PS3, PM1, PM2, PM4, PM5, PP2, PP3, PP4
(categories indicating pathogenicity of the variant)
BA1, BS2, BP1, BP3, BP4, BP5, BP7
(categories indicating the lack of pathogenicity of the variant).

[1] S. Richards et al., „Standards and Guidelines for the Interpretation of Sequence Variants: A joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology" Genet. Med. Off. J. Am. Coll. Genet., vol 17. no. S, pp. 405-424, May 2015
[2] A. N.Abou Tayoun e. al., „Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion," Hum. Mutat., vol 39, no 11, pp. 1517-1524, 2018, Online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4544753/
[3] S. V. Tavtigian et al., „Modeling the ACMG/AMP Variant Classification Guidelines as a Bayesian Classification Framework," Genet. Med. Off. J. Am. Coll. Med. Genet., vol. 20. no. 9. pp. 1054-1060, Sep. 2018.

Abbreviations used in the tables

MITOMAP	Allele frequency in MITOMAP population
WES-AF	allele frequency in Non-Finnish European population having exomes sequenced in gnomAD project
WGS-AF	allele frequency in Non-Finnish European population having genomes sequenced in gnomAD project
maxAF	maximum allele frequency among populations from gnomAD projects
maxPOP	project and population with maximum allele frequency
SIFT	predicted pathogenicity, high < 0.11
PhastCons	evolutionary conservation, high > 0.93

AFR	African/African
ASJ	Ashkenazi Jewish
AMR	Latino
EAS	East Asian
FIN	Finnish
NFE	Non-Finnish European
SAS	South Asian
OTH	Other
Reference genome:	GRCh38

Methods

Variant annotation and annotation-based filtering

Information that can be used for variant pathogenicity evaluation was added to the vcf file. Databases used for annotation included: gnomAD v2.1 and v3 (frequencies, coverage, constraint), 1000Genomes (frequencies), MITOMAP (frequencies, contributed diseases), ClinVar (contributed diseases, pathogenicity), HPO (inheritance mode, contributed phenotypes and diseases), UCSC (repeats, PHAST conservation scores), SIFT4G (constraint), SnpEff (predicted impact on gene product), dbSNP (rsID), Ensembl (gene and transcript information), COSMIC (somatic mutations data). Variants with QUAL < 200.0 were removed. Common and low impact variants (with max frequency threshold of 0.05 and minimal SnpEff predicted impact on gene product set as MODERATE), as well as low quality multiallelic variants (QUAL < 300) were then removed.
Annotated variants, for genes that are likely to contribute to the patient phenotype and/or for genes from the user-defined gene list/panels, were then classified and prioritized according to the ACMG criteria (see Variants classification according to the ACMG criteria for details). Possible compound heterozygote variants are presented together (one by one) and prioritized based on the highest scoring variant. A maximum number of 50 variants are shown in the main report (together with a possible second variant from compound heterozygote). These include all variants classified in the ClinVar database as pathogenic or likely pathogenic, along with variants that gained the highest pathogenicity score in the ACMG classification.

Diseases names and inheritance pattern

Diseases names are assigned based on the association with a particular gene symbol in the Human Phenotype Ontology database or the Genetics Home Reference database. The names should be inspected and approved by a laboratory analyst or physician based on other sources of information.
The inheritance pattern is assigned based on the Human Phenotype Ontology. This information should also be confirmed by a laboratory analyst or physician.

Inheritance Pattern Match

The value in this field indicates whether the patient's genotype is a probable cause of the disease, given the inheritance pattern of the damaged gene:

- Dominant: given to heterozygous and homozygous genotypes within the gene of dominant inheritance;
- Possibly dominant: given to heterozygous variants within the gene for which both modes of inheritance (dominant and recessive) are assigned;
- Recessive: given to homozygous variants of the gene with a recessive mode of inheritance;
- Likely compound het: assigned to variants within the gene of recessive inheritance pattern if we found more than one probably damaging variant of the gene. Of note, we do not know the phasing of the variants, and compound heterozygote occurs only when variants are on different chromosomes (and damage both copies of the gene);
- Mitochondrial: is given to all mitochondrial variants. Of note, such variants can be damaging even if present only in some fraction of mitochondria;
- None: assigned to variants with genotype insufficient to cause disease (for example, heterozygous variants within the gene having recessive inheritance pattern).

Versions of tools and databases

The analysis was performed using WES hereditary disorders ACMG report (input: vcf) workflow version 1.7.20. Detailed information on databases and tool versions are available in the bco.json file in the analysis results folder.

Limitations of liability

The clinical diagnosis should never be based on the Patient’s genomic sequence analysis alone.

Factors such as errors due to sample contamination, rare events of technical errors, genetic events affecting the patient’s condition impossible to detect using currently available knowledge as well as other technical limitations should always be considered. This report should be one of many aspects used by the healthcare provider to help with the diagnosis and treatment plan, but it is not the diagnosis itself.

Data protection

According to Article 24(1) of the Law of 29 August 1997 on Personal Data Protection, the patient's Personal data will be used solely for the purpose of conducting the DNA analysis and will not be shared with any third party. Intelliseq SA declares that it applies all necessary measures to protect the patient's personal data, and in particular will refrain from sharing the data with any unauthorized party as well as prevent the loss of data or data corruption.

Limitations of the method

The employed method is based on the DNA isolated from blood samples and consequently might not reflect the changes in other parts of the body or detect mosaic mutations. Regions characterized with high homology to other parts of genome may not be represented accurately. Some parts of exome have limited coverage which impacts the variant detection accuracy. The ability to detect structural variants is limited. The method is largely based on utilizing the information from external databases, hence the ability to detect variants relevant to patient's condition is limited by the scope of information present in those databases.

List of genes from gene panel

The custom gene panel was created based on the inputs selected or/and entered by the user:
• disease names: *N/A*
• gene panel names: *N/A*
• gene names: GDF3, ABCB11
• patient phenotypes: *N/A*
• HPO terms: *N/A*

Gene name	ABCB11	GDF3
phenotype match%	75.00%	75.00%
type	Genes names	Genes names

Type - the source of the gene list included in the extended panel. Possible sources are: gene list provided by a physician, diseases provided by a physician, HPO terms, panels from Genomics England Panel App or epicrysis converted to HPO terms.

Phenotype match - percent of a maximum possible score that is attributed to a gene. For each HPO term and/or phenotype description provided by a physician, the algorithm traverses the phenotype ontology tree searching for a match with each gene and assigning a score based on the distance between the original term and the term that matches the gene. For specific genes provided by a physician, the phenotype match is assigned to be 75%. For specific diseases provided by a physician, the phenotype match is assigned to be 50%. For genes from the gene panel, phenotype match is assigned to be 30%.