Patientendaten

Centre for Chronic Immunodeficiency (CCI)
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CCI Genetics and Genomics Unit submission form

Referring physician

Name		Department	
Centre		Institution	
Email		Phone	
Address		City, Country	

Patient information

First name		Last name		Sex	
DOB		Nationality		Consang.	
Address				City	
Main diagnosis				Age onset	
Other diagnosis					

Genetic analysis *(please tick one or more boxes, prices and TAT in appendix)*

- ☐ Sequencing using whole **exome** library
- ☐ (and) bioinformatic analysis: variant calling & annotation
- ☐ (and) variant interpretation from a defined gene panel: _____
- ☐ (and) variant interpretation in the inborn errors of immunity (IEI) exome panel
- ☐ Variant confirmation *via* **Sanger** sequencing

  Gene: Exon: Variant:

☐ Family **Trio** analysis for steps selected above

Microbiome analysis (*prices and TAT in appendix)*

☐ 16sRNA-sequencing and analysis

Declaration

This is to certify the shipments contain samples which are for Research Purpose Only. The samples are derived from humans and they are not hazardous, not infectious, not HIV positive, not toxic, and not radioactive. No import license is required for this shipment.

Bill shall be sent to: Name _____

Address _____

Please be aware that we will proceed with the data production and analysis only after we have received the payment. I hereby confirm that the patient has signed the GGU patient consent form and/or a local genetic consent form, allowing for the genetic study of his/her condition.

_____

Date Signature or stamp of physician

Functional tests

	Gene	Assay	Contact person	Email
☐	*ADA2*	Function	Michele Proietti	Michele.proietti@uniklinik-freiburg.de
☐	*CTLA4*	Expression	Laura Gámez	Laura.gamez@uniklinik-freiburg.de
☐	*CTLA4*	Transendocytosis	Laura Gámez	Laura.gamez@uniklinik-freiburg.de
☐	*LRBA*	FACS	Laura Gámez	Laura.gamez@uniklinik-freiburg.de
☐	*PD1*	FACS	Pavla Mrovecova	Pavla.mrovecova@uniklinik-freiburg.de
☐	*STAT1*	Phosphorylation	Virginia Andreani	Virginia.andreani@uniklinik-freiburg.de
☐	*STAT3*	Phosphorylation	Virginia Andreani	Virginia.andreani@uniklinik-freiburg.de
☐	Th17 cell differentiation		Virginia Andreani	Virginia.andreani@uniklinik-freiburg.de
☐	*NFKB1*	Function	Manfred Fliegauf	Manfred.fliegauf@uniklinik-freiburg.de
☐	*NFKB2*	Function	Manfred Fliegauf	Manfred.fliegauf@uniklinik-freiburg.de

Please contact the corresponding researcher before sending any sample.

General guidelines

- Please write in a legible manner on the spaces allocated for 'Referring physician' and 'Personal information'.
- Please mark with an 'X' on the assigned boxes whenever possible.
- Please fill in one individual form per sample sent.
- In case an additional sample from a healthy travel-control is being sent, please fill in at least the section 'Personal information' on a separate form.
- A signed consent form from both parents is needed if the patient is under 18 years of age**.**

Recommendations for shipment

- Please ensure that fresh blood samples will be kept at room temperature during the travel.
- Please package the EDTA blood tubes in a way that prevents breakage and/or leakage.
- Please take into account that samples should be delivered no later than Friday as our laboratory will not be manned on the weekend.

Specifications for samples sent for genetic testing

- Please send at least 10ml of fresh EDTA blood per sample for any of the genetic tests. Samples of relatives will be appreciated as they can later be used to validate variant segregation.
- In case you prefer to send genomic DNA (gDNA), please send at least 1µg of gDNA in a total volume of at least 30µl.
  Please send samples to **Pavla Mrovecova** (AG Grimbacher) Centrum für chronische Immundefizienz (CCI), Breisacher Str. 115, 79106 Freiburg. Contact in case of questions: ifi.ggu@uniklinik-freiburg.de

Declaration for scientific publication of the generated data

- Please note that with your signature on page 1 you agree to name the Genetics and Genomics Unit (GGU) in the acknowledgements section of any scientific publication that is published based on the data obtained through this genetics and/or functional analysis.

Appendix

Quotation for **sequencing, bioinformatic analysis, interpretation,** and generation of a **research** report

1) **Sequencing**
   This includes sample processing and DNA isolation, quality control, library preparation, and sequencing using Agilent's *SureSelect Human All Exon V6* exome capture kit. Mean depth 100x, ~5Gb of data.

2) **Bioinformatic analysis**
   2.1. Variant Calling
   This includes quality control, preprocessing, read mapping (hg38), deduplication, base quality recalibration, local realignment, variant calling (GATK, samtools, freebayes).
   2.2. Variant Annotation
   This includes annotation of the full set of variants with more than 80 features including consequences, frequencies (from internal and external databases), *in silico* pathogenicity predictions, location, and effect on different transcripts.

   Upon request, we provide fastq-, bam-, and vcf-files for the analyzed samples.

The subsequent steps (3 & 4) include a report in pdf format with a curated detailed description of the variant, the role of the mutated gene in disease, and the gene function. The generation of such a research report strongly benefits from a detailed description of the clinical phenotype. Further, a full list of candidate variants for the requested panel is provided.

3) **A. Interpretation of genetic variants from a panel of genes known to be associated with the given clinical phenotype. ****If you consider that we need to add some gene(s), please inform us and we will include them in the list.***
   This includes variant prioritization, variant filtering, and specific literature searches. This part of the analysis relies on a detailed description of the clinical phenotype of the patient from the referring physician and cannot be guaranteed when the clinical information is missing or incomplete.
   If we fail in identifying genetic alterations that can explain the clinical phenotype in this step (3) then we may move to step 4, upon request.

   B. Interpretation of genetic variants from a custom gene panel requested by referring physician.
   This includes the same tasks as in step 3A.
   If we fail in identifying genetic alterations that can explain the clinical phenotype in this step (3) then we may move to step 4, upon request.

4) **Interpretation of genetic variants of the inborn errors of immunity (IEI) exome panel.**
   Whole exome sequencing data is analyzed looking at all likely disease-causing variants identified in every gene included in the IEI exome panel, to see if the variant and the gene offer an explanation for all, or part, of the patient's phenotype.
   Election of this panel is advisable whenever the results of a smaller panel (step 3) do not fully explain the phenotype of the patient.
   The full list of non-evaluated variants derived from the complete whole exome data is provided upon request.

Table 1. Turnaround times and costs for sequencing and analysis of a **single sample**. Options 3 and 4 include the generation of a research report.

Step	Task	Cost
1.	Sequencing	375 €
2.	Sequencing + bioinformatics	390 €
3.	Sequencing + bioinformatics + variant interpretation of a *defined* or *custom* gene panel (50 genes max.)*	500 €
4.	Sequencing + bioinformatics + variant interpretation of the IEI exome	875 €

* Defined or custom panels containing more than 50 genes will cost 50€ per additional 50 genes analyzed.
Turnaround times are given as working days.

Table 2. Turnaround times and costs for sequencing and analysis of a family **Trio**. Options 3 and 4 include the generation of a research report.

Step	Task	Cost
1.	Sequencing	1000 €
2.	Sequencing + bioinformatics	1125 €
3.	Sequencing + bioinformatics + variant interpretation of a *defined* or *custom* gene panel (50 genes max.)*	1250 €
4.	Sequencing + bioinformatics + variant interpretation of the IEI exome	1750 €

* Defined or custom panels containing more than 50 genes will cost 50€ per additional 50 genes analyzed.
Turnaround times are given as working days.

Table 3. If sequencing is already availabe.

	Task	Cost
	Re-Analysis and report of targeted gene panel	125 €
	Re-Analysis and report of whole exome	500 €

Sanger sequencing costs 50€ per amplicon and turnaround time is 2-4 weeks.

16sRNA sequencing costs 100€ per sample (batch size: <10 samples), 90€ per sample (batch size: 10-50 samples), 80€ per sample (batch size: 51-100 samples) and 75€ (batch size: >100 samples); turnaround time depends on batch size, for more information please contact Mate.krausz@uniklinik-freiburg.de

Functional Testing costs 250€ per assay.
As a contribution to the consumables used for the functional testing of variants in above listed genes, we ask you to contribute 250€ per assay per patient sample. Usually, the testing will only start when the funds have been received in our account.

List of analysis panels (see Point 3)

Panels are shown in alphabetical order. The genes included in the panels may vary over time, depending on the discovery of new genes.

Agammaglobulinemia (AGM) [13 genes]

BLNK, BTK, CD79A, CD79B, IGHM, IGKC, IGLL1, LRRC8A, PIK3R1, SLC39A7, SPI1, TCF3, TOP2B

Autoimmune Lymphoproliferative Syndrome (ALPS) [10 genes]

CASP10, CASP8, CD27, CTLA4, FADD, FAS, FASLG, KRAS, NRAS, PRKCD

Autoinflammatory disorder (AID) [42 genes]

ACP5, ADA2, ADAM17, ADAR, ALPI, AP1S3, CARD14, COPA, DNASE1L3, DNASE2, HAVCR2, IFIH1, IL1RN, IL36RN, LPIN2, MEFV, MVK, NLRC4, NLRP1, NLRP12, NLRP3, NOD2, OAS1, OTULIN, PLCG2, POLA1, PSMB8, PSMG2, PSTPIP1, RNASEH2A, RNASEH2B, RNASEH2C, RNF31, SAMHD1, SH3BP2, SLC29A3, TMEM173, TNFAIP3, TNFRSF1A, TREX1, TRIM22, USP18

Combined immunodeficiency (CID) [48 genes]

B2M, BCL10, BCL11B, CARD11, CARMIL2, CASP8, CD27, CD3G, CD40, CD40LG, CD8A, CIITA, CTPS1, DOCK2, DOCK8, FCHO1, ICOS, ICOSLG, IKBKB, IKZF1, IL21, IL21R, ITK, LAT, LCK, LRBA, MAGT1, MALT1, MAP3K14, MSN, POLD1, POLD2, REL, RELA, RELB, RFX5, RFXANK, RFXAP, RHOH, STK4, TAP1, TAP2, TAPBP, TFRC, TNFRSF4, TRAC, UNC119, ZAP70

Common variable immunodeficiency (CVID) [31 genes]

ADA2, ARHGEF1, ATP6AP1, BACH2, BLK, CD19, CD81, CR2, CTLA4, CXCR4, ICOS, IKZF1, IL21, IRF2BP2, ITPKB, LRBA, MOGS, MS4A1, NFKB1, NFKB2, PIK3CD, PIK3R1, PLCG2, PTEN, RAC2, SEC61A1, SH3KBP1, TNFRSF13B, TNFRSF13C, TNFSF12, TRNT1

Chronic mucocutaneous candidiasis disease (CMCD) [11 genes]

AIRE, BCL10, CARD9, EPG5, IL17F, IL17RA, IL17RC, RORC, STAT1, STAT3, TRAF3IP2

Familial Hemophagocytic Lymphohistiocytosis (FHLH) [11 genes]

AP3B1, AP3D1, FAAP24, ITK, LYST, PRF1, RAB27A, SLC7A7, STX11, STXBP2, UNC13D

Hyper-IgE syndromes (HIES) [43 genes]

ADA, ARPC1B, CARD11, CDSN, CHD7, CYBA, CYBB, DOCK2, DOCK8, DSG1, ERBIN, FAS, FASLG, FLG, FOXP3, IL21R, IL2RG, IL6ST, IL6R, IL7R, IRAK4, LIG4, MALT1, MYD88, NCF1, NCF2, NCF4, PGM3, PHF11, RAG1, RAG2, SPINK5, STAT3, STAT5B, STK4, TGFBR1, TGFBR2, TRPV3, TYK2, WAS, WIPF1, ZAP70, ZNF341

Immune dysregulation (IDR) [48 genes]

AIRE, AP3B1, AP3D1, BACH2, CARMIL2, CASP10, CASP8, CD27, CD70, CTLA4, CTPS1, DEF6, FAAP24, FADD, FAS, FASLG, FERMT1, FOXP3, IL10, IL10RA, IL10RB, IL2RA, IL2RB, ITCH, ITK, JAK1, KRAS, LRBA, LYST, MAGT1, NFAT5, NRAS, PEPD, PRF1, PRKCD, RAB27A, RASGRP1, RIPK1, SH2D1A, SLC7A7, STAT3, STX11, STXBP2, TGFB1, TNFRSF9, TPP2, UNC13D, XIAP

Inborn errors of immunity (IEI) exome [529 genes]

ABCB1, ACD, ACP5, ACTB, ADA, ADA2, ADAM17, ADAR, ADGRE2, AICDA, AIRE, AK2, ALPI, AP1S3, AP3B1, AP3D1, APOL1, ARHGEF1, ARPC1B, ARPC5, ATG16L1, ATG5, ATM, ATP6AP1, B2M, BACH2, BANK1, BCL10, BCL11B, BCL6, BLK, BLM, BLNK, BRCA1, BRCA2, BRIP1, BTK, BTNL2, C1QA, C1QB, C1QC, C1R, C1S, C2, C3, C4A, C4B, C5, C6, C7, C8A, C8B, C8G, C9, CALCOCO2, CARD11, CARD14, CARD9, CARMIL2, CASP10, CASP8, CCBE1, CCDC88B, CCL2, CCR2, CCR5, CD19, CD209, CD247, CD27, CD36, CD3D, CD3E, CD3G, CD4, CD40, CD40LG, CD46, CD55, CD59, CD70, CD79A, CD79B, CD81, CD8A, CDC42, CDCA7, CDH17, CDSN, CEBPE, CFB, CFD, CFH, CFHR1, CFHR2, CFHR3, CFHR4, CFHR5, CFI, CFP, CFTR, CHD7, CIB1, CIITA, CLCN7, CLEC7A, CLPB, COG6, COL7A1, COPA, CORO1A, CPT2, CR2, CSF2RA, CSF2RB, CSF3R, CTC1, CTLA4, CTNNBL1, CTPS1, CTSC, CXCR2, CXCR4, CYBA, CYBB, CYBC1, DBF4, DBR1, DCLRE1C, DDX58, DEF6, DGAT1, DGKE, DIAPH1, DKC1, DNAJC21, DNASE1, DNASE1L3, DNASE2, DNMT3A, DNMT3B, DOCK2, DOCK8, DSG1, EFL1, EGFR, ELANE, EPCAM, EPG5, ERBIN, ERCC4, ERCC6L2, EXTL3, F12, FAAP24, FADD, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCI, FANCL, FANCM, FAS, FASLG, FAT4, FCGR1A, FCGR2A, FCGR2B, FCGR3A, FCGR3B, FCHO1, FCN3, FERMT1, FERMT3, FLG, FOXN1, FOXP3, FPR1, FUT2, FYB1, G6PC3, G6PD, GATA1, GATA2, GFI1, GINS1, GUCY2C, HAVCR2, HAX1, HELLS, HLA-DQB1, HLA-DRB1, HMOX1, HYOU1, ICAM1, ICOS, ICOSLG, IFIH1, IFNAR1, IFNAR2, IFNG, IFNGR1, IFNGR2, IGHM, IGKC, IGLL1, IKBKB, IKBKG, IKZF1, IL10, IL10RA, IL10RB, IL12B, IL12RB1, IL12RB2, IL17F, IL17RA, IL17RC, IL18BP, IL1RN, IL21, IL21R, IL23R, IL2RA, IL2RB, IL2RG, IL36RN, IL6, IL6R, IL6ST, IL7, IL7R, INAVA, INO80, IRAK1, IRAK4, IRF1, IRF2BP2, IRF3, IRF4, IRF5, IRF7, IRF8, IRF9, IRGM, ISG15, ITCH, ITGB2, ITK, ITPKB, JAGN1, JAK1, JAK2, JAK3, KDM6A, KMT2A, KMT2D, KPNA2, KRAS, LAMTOR2, LAT, LCK, LIG1, LIG4, LPIN2, LRBA, LRRC8A, LYST, MAD2L2, MAGT1, MALT1, MAN2B1, MAN2B2, MAP3K14, MASP1, MASP2, MBL2, MC2R, MC3R, MCM2, MCM4, MECP2, MEFV, MMACHC, MME, MPO, MRAP, MRTFA, MS4A1, MSH6, MSN, MST1, MTHFD1, MTHFR, MVK, MYD88, MYH9, MYO5A, MYO5B, MYSM1, NBAS, NBN, NCF1, NCF2, NCF4, NCKAP1L, NCSTN, NEUROG3, NFAT5, NFE2L2, NFKB1, NFKB2, NFKBIA, NHEJ1, NHP2, NLRC4, NLRP1, NLRP12, NLRP3, NOD2, NOP10, NOS2, NPHS1, NR0B1, NRAS, NSMCE3, NUP214, OAS1, ORAI1, OSTM1, OTULIN, P2RX7, PADI4, PALB2, PARN, PAX1, PBX1, PDCD1, PDGFRA, PEPD, PGM3, PHF11, PIK3CD, PIK3CG, PIK3R1, PLCG2, PLEKHM1, PMS2, PNP, POLA1, POLD1, POLD2, POLE, POLE2, POLR3A, POLR3C, POLR3F, POMP, POU2AF1, PRF1, PRKCD, PRKDC, PRKG1, PRPS1, PSEN1, PSENEN, PSMB10, PSMB4, PSMB8, PSMG2, PSTPIP1, PTEN, PTPN2, PTPN22, PTPRC, RAB27A, RAC2, RAD51, RAD51C, RAG1, RAG2, RANBP2, RASGRP1, RBCK1, RECQL4, REL, RELA, RELB, RFWD3, RFX5, RFXANK, RFXAP, RHOH, RIPK1, RMRP, RNASEH2A, RNASEH2B, RNASEH2C, RNF168, RNF31, RNU4ATAC, RORC, RPSA, RTEL1, SAMD9, SAMD9L, SAMHD1, SASH3, SBDS, SEC61A1, SEMA3E, SERPING1, SH2D1A, SH3BP2, SH3KBP1, SIAE, SKIV2L, SLC11A1, SLC26A3, SLC29A3, SLC35C1, SLC37A4, SLC39A7, SLC46A1, SLC7A7, SLC9A3, SLX4, SMARCAL1, SMARCD2, SNORA31, SNX10, SOCS1, SP110, SPATA5, SPINK5, SPINT2, SPPL2A, SREBF1, SRP54, SRP72, STAT1, STAT2, STAT3, STAT4, STAT5B, STIM1, STK4, STN1, STX11, STXBP2, TAP1, TAP2, TAPBP, TAZ, TBK1, TBX1, TBX21, TCF3, TCIRG1, TCN2, TERC, TERT, TET2, TFRC, TGFB1, TGFBR1, TGFBR2, THBD, TICAM1, TINF2, TIRAP, TLR1, TLR2, TLR3, TLR5, TLR8, TMC6, TMC8, TMEM173, TNF, TNFAIP3, TNFRSF11A, TNFRSF13B, TNFRSF13C, TNFRSF1A, TNFRSF4, TNFRSF9, TNFSF11, TNFSF12, TNFSF13, TNFSF15, TNFSF4, TOP2B, TP53, TPP2, TRAC, TRAF3, TRAF3IP2, TREX1, TRIM22, TRNT1, TRPV3, TTC37, TTC7A, TYK2, UBE2T, UNC119, UNC13D, UNC93B1, UNG, USB1, USP18, VAV1, VPS13B, VPS45, VTN, WAS, WDR1, WIPF1, WRAP53, XIAP, XRCC2, ZAP70, ZBTB24, ZCCHC8, ZNF341

Inflammatory bowel disease [31 genes]

ABCB1, ADAM17, ARPC1B, ATG16L1, CALCOCO2, CCDC88B, EGFR, EPCAM, GUCY2C, IL10, IL10RA, IL10RB, IL23R, INAVA, IRF5, IRGM, LRBA, MST1, NFAT5, NOD2, OTULIN, PTPN2, PTPN22, RIPK1, SLC9A3, SPINT2, STAT3, TGFB1, TNFSF15, TTC7A, XIAP

Mendelian susceptibility to mycobacterial disease (MSMD) [14 genes]

CYBB, IFNGR1, IFNGR2, IL12B, IL12RB1, IL12RB2, IL23R, IRF8, ISG15, JAK1, RORC, SPPL2A, STAT1, TYK2

Severe combined immunodeficiency (SCID) [20 genes]

ADA, AK2, CARD11, CD247, CD3D, CD3E, CD3G, CORO1A, DCLRE1C, IL2RG, IL7R, JAK3, LAT, LIG4, NHEJ1, PRKDC, PTPRC, RAC2, RAG1, RAG2

Type I Interferonopathies (T1IFN) [15 genes]

ACP5, ADA2, ADAR, DNASE1L3, DNASE2, IFIH1, OAS1, POLA1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, TMEM173, TREX1, USP18