Patientendaten

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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		City	
Address				Age	
Main diagnosis				Consang.	
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 whole exome
- ☐ Variant confirmation *via* **Sanger** sequencing

  Gene: Exon: Variant:

☐ **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*	Funtion	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
☐	*STAT1*	Phosphorylation	Stefanie Frey-Jakobs	stefanie.frey-jakobs@uniklinik-freiburg.de
☐	*STAT3*	Phosphorylation	Stefanie Frey-Jakobs	stefanie.frey-jakobs@uniklinik-freiburg.de
☐	Th17 cell differentiation		Stefanie Frey-Jakobs	stefanie.frey-jakobs@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 **Hanna Haberstroh** (AG Grimbacher) Centrum für chronische Immundefizienz (CC), Breisacher Str. 115, 79106 Freiburg. Contact in case of questions hanna.haberstroh@uniklinik-freiburg.de

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, 5-6Gb 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 89 features including consequences, frequencies (from internal and external databases), *in silico* pathogenicity predictions, location, and effect on different transcripts.

The subsequent steps include a report 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.

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 (4) then we may move to step 5, upon request.

   B. Interpretation of genetic variants from a custom gene panel requested by referring physician.
   This includes the same tasks as in step 4A.
   If we fail in identifying genetic alterations that can explain the clinical phenotype in this step (4) then we may move to step 5, upon request.

4) **Interpretation of genetic variants of the complete exome.**
   WES data is analyzed looking at all likely disease-causing variants identified in every gene included in the exome design, to see if the variant and the gene offer an explanation for all, or part, of the patient's phenotype.
   This step of the analysis can be performed, upon request of the referring scientist, in case that the variants identified in step 4 do not fully explain the phenotype of the patient.

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	TAT	Cost
1.	Sequencing	10 days	300 €
2.	Sequencing + bioinformatics	14 days	350 €
3.	Sequencing + bioinformatics + variant interpretation of a *defined* or *custom* gene panel (50 genes max.)*	30 days	400 €
4.	Sequencing + bioinformatics + variant interpretation of the whole exome	50 days	700 €

* 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 **Trio**. Options 3 and 4 include the generation of a research report.

Step	Task	TAT	Cost
1.	Sequencing	10 days	800 €
2.	Sequencing + bioinformatics	16 days	900 €
3.	Sequencing + bioinformatics + variant interpretation of a *defined* or *custom* gene panel (50 genes max.)*	33 days	1000 €
4.	Sequencing + bioinformatics + variant interpretation of the whole exome	55 days	1400 €

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

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

16sRNA sequencing costs 75€ per sample and turnaround time is 4-6 weeks.

List of analysis panels (see Step 4)

Panels shown in alphabetical order.

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) [500 genes]

ABCB1, ACD, ACP5, ACTB, ADAM17, ADA, ADA2, ADAR, ADGRE2, AICDA, AIRE, AK2, ALPI, AP1S3, AP3B1, AP3D1, APOL1, ARHGEF1, ARPC1B, ARPC5, ATG16L1, ATG5, ATM, ATP6AP1, B2M, BACH2, 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, CARD11, CARD14, CARD9, CARMIL2, CASP10, CASP8, CCBE1, 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, CEBPE, CFB, CFD, CFH, CFHR1, CFHR3, CFHR5, CFI, CFP, CFTR, CHD7, CIB1, CIITA, CLCN7, CLEC7A, CLPB, COG6, COPA, CORO1A, CR2, CSF2RA, CSF2RB, CSF3R, CTC1, CTLA4, CTPS1, CTSC, CXCR4, CYBA, CYBB, CYBC1, DBR1, DCLRE1C, DDX58, DEF6, DGAT1, DGKE, DKC1, DNAJC21, DNASE1, DNASE1L3, DNASE2, DNMT3A, DNMT3B, DOCK2, DOCK8, EFL1, EGFR, ELANE, EPCAM, EPG5, 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, HNF1A, 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, MME, MOGS, MPO, MRAP, MRTFA, MS4A1, MSH6, MSN, 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, OAS1, ORAI1, OSTM1, OTULIN, PADI4, PALB2, PARN, PAX1, PBX1, PDCD1, PDGFRA, PEPD, PGM3, PIK3CD, PIK3CG, PIK3R1, PLCG2, PLEKHM1, PMS2, PNP, POLA1, POLD1, POLD2, POLE, POLE2, POLR3A, POLR3C, POLR3F, PRF1, PRKCD, PRKDC, PRKG1, PRPS1, PSEN1, PSENEN, PSMB10, PSMB8, PSMG2, PSTPIP1, PTEN, 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, SBDS, SEC61A1, SEMA3E, SERPING1, SH2D1A, SH3BP2, SH3KBP1, SKIV2L, SLC11A1, SLC26A3, SLC29A3, SLC35C1, SLC37A4, SLC39A7, SLC46A1, SLC7A7, SLC9A3, SLX4, SMARCAL1, SMARCD2, SNORA31, SNX10, SOCS1, SP110, SPATA5, SPINK5, SPINT2, SPPL2A, SRP54, SRP72, STAT1, STAT2, STAT3, STAT4, STAT5B, STIM1, STK4, STN1, STX11, STXBP2, TAP1, TAP2, TAPBP, TAZ, TBK1, TBX1, TBX21, TCF3, TCIRG1, TCN2, TERC, TERT, TFRC, TGFB1, TGFBR1, TGFBR2, THBD, TICAM1, TINF2, TIRAP, TLR1, TLR2, TLR3, TLR5, TMC6, TMC8, TMEM173, TNF, TNFAIP3, TNFRSF11A, TNFRSF13B, TNFRSF13C, TNFRSF1A, TNFRSF4, TNFRSF9, TNFSF11, TNFSF12, TNFSF13, TNFSF4, TOP2B, TP53, TPP2, TRAC, TRAF3, TRAF3IP2, TREX1, TRIM22, TRNT1, TTC37, TTC7A, TYK2, UBE2T, UNC119, UNC13D, UNC93B1, UNG, USB1, USP18, VAV1, VPS13B, VPS45, WAS, WDR1, WIPF1, WRAP53, XIAP, XRCC2, ZAP70, ZBTB24, 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