Workshop genomDE

Herausforderung der Genomdiagnostik:

Seltene Erkrankungen

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genomDE

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Medical Need

UNSOLVED after WES:

>50% of all patients with a rare disease will not have access to health care without having a clear diagnosis

Not all monogenic disease genes known yet
Not all disease mechanisms known
Technical limitations
Limited target region sequenced
Epigenome?

300 Mio RD patients worldwide
150 Mio patients unsolved

30 Mio patients in Europe
15 Mio unsolved

Medical Need

Whole Genome Sequencing is the next logical consequence

3-4 Mio RD patients in Germany
1.5 Mio unsolved after WES

Potential der Genomsequenzierung

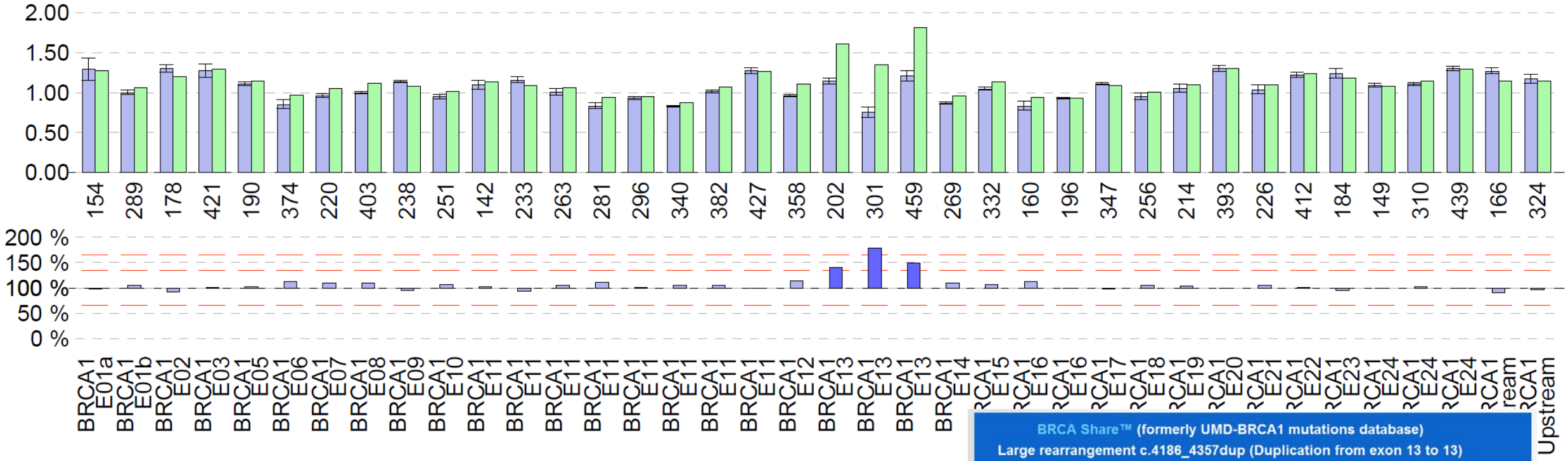
genom

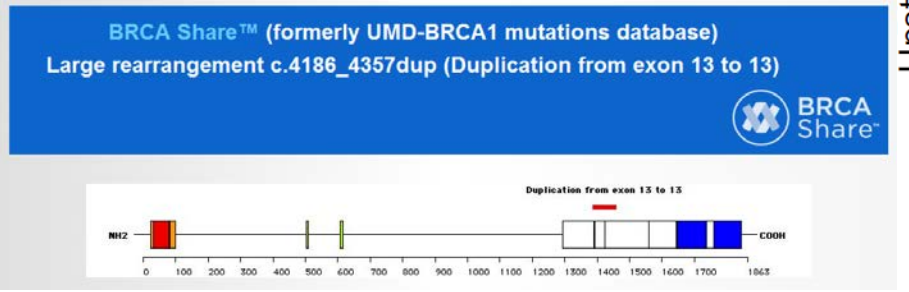
		Exome analysis	Short read Genome analysis	Long read Genome
Genomics	Point mutations	Coding only	„Complete“ genome	
	Small InDels	Coding only	„Complete“ genome	
	Copy number variations		„Complete“ genome	„Haplo-typing“
	Structural variations		„Complete“ genome	Complete genome
	Repeat expansions		Short repeats only	All repeat expansions
Transcriptomics	Aberrant expression		Short read	Long read
	Aberrant splicing		Short read	Long read
	Gene fusion		Cancer	
Epigenomics	Methylation	Pilot → Extend of contribution for SE unclear	Methylseq	All in one?

Short read

Genome and Transcriptome

Missed by WES: Examples





Klinik:
Mammakarzinom (ED 30)

Kausale Varianten:

Gen	Typ	Genotyp	Variante	Erbgang	c.	p.	gnomAD	NGSD hom/het	Kommentar 1. Auswerter	Kommentar 2. Auswerter	Klasse	In Report

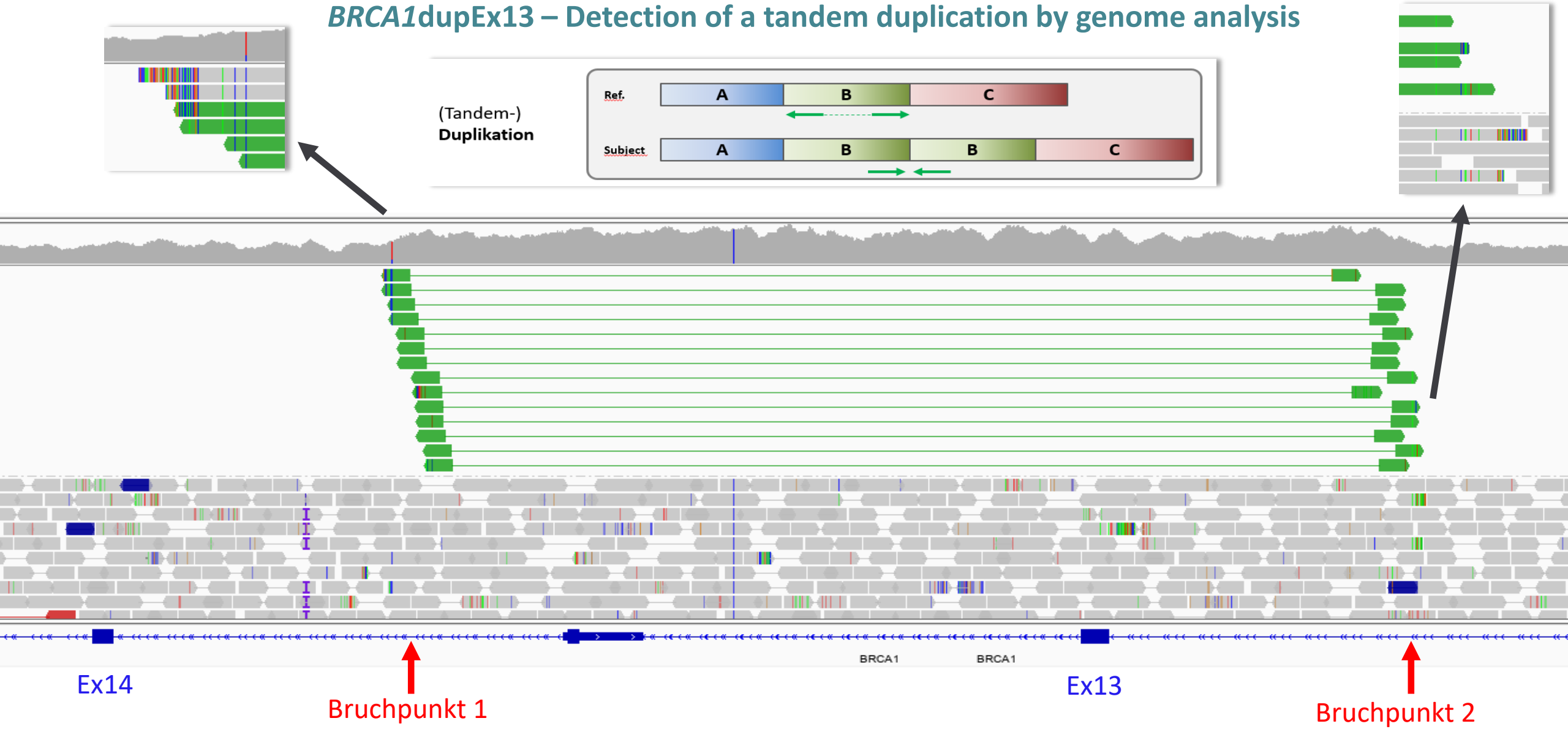
Sonstige Varianten:

Gen	Typ	Genotyp	Variante	Erbgang	Ausschlussgrund	gnomAD	NGSD hom/het	Kommentar 1. Auswerter	Kommentar 2. Auswerter	Klasse	In Report
HFE	missense_variant	het	chr6:26093141-26093141 G>A	n/a	Anderer (siehe Kommentare)	0.0383	27 / 1262	AR		5	nein (incidental finding)
RYR1	synonymous_variant	het	chr19:39071036-39071036 G>C	n/a	Anderer (siehe Kommentare)	0.0001	0 / 10	ACMG-VUS		3	nein (incidental finding)

Kausale CNVs:

CNV	copy-number	Gene	Erbgang	Infos	Kommentar 1. Auswerter	Kommentar 2. Auswerter	Klasse	In Report
chr17:41230209-41236209	3	BRCA1, RPL21P4	AD	regions:6 size:6.001kb	BIC, UMD, LOVD, Literatur pathogen		5	ja (diagnostic variant)

*BRCA1*dupEx13 – Detection of a tandem duplication by genome analysis



BRCA1 ENST357654

WGS in unsolved chorea acanthocytosis: Structural variants in VPS13A

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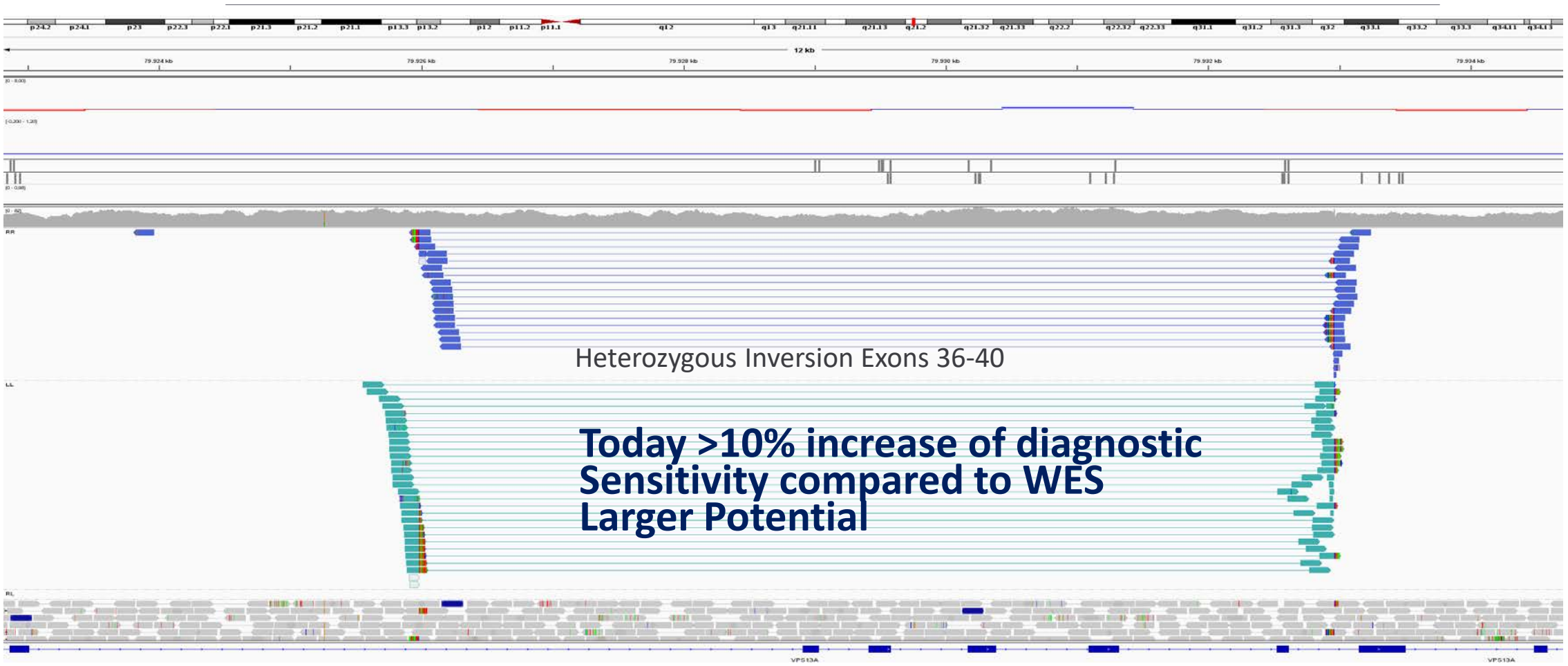
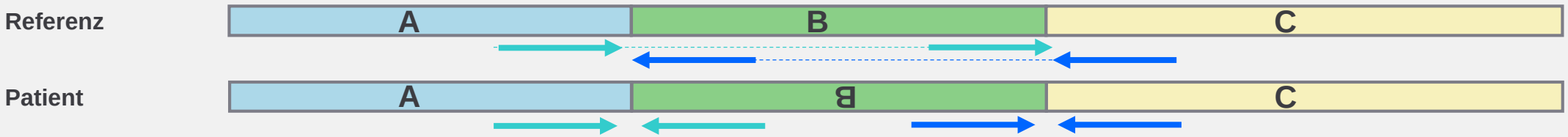
UNIVERSITÄT
TÜBINGEN





Bedeutung des EU Netzwerkes

SolveRD

für genomeDE

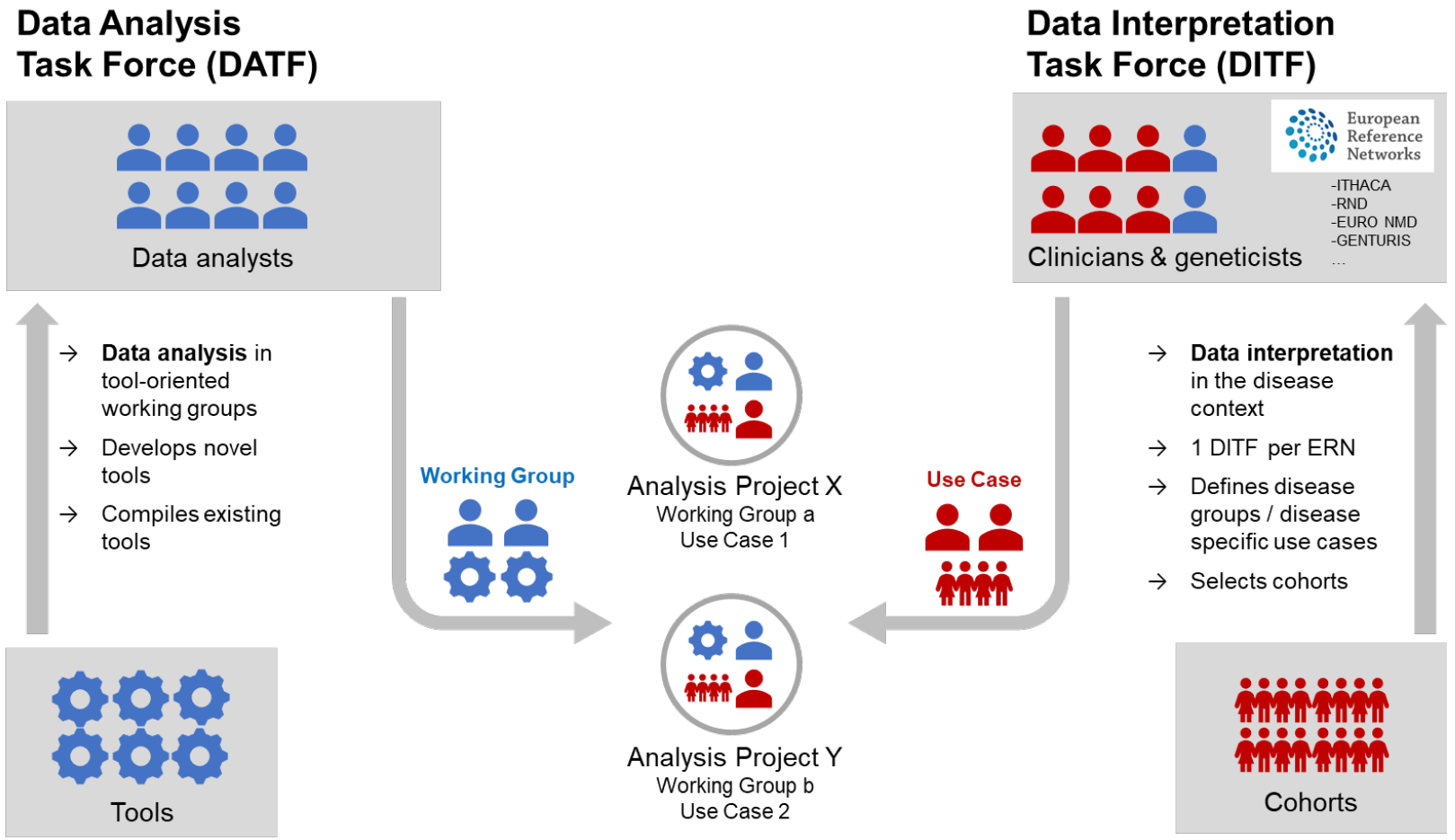
genomDE

21.07.2022

Implementation of a data re-analysis infrastructure

DATA ANALYSIS TASK FORCE WORKING GROUPS (WG)

WG1	SNVs / indel
WG2	CNVs
WG3	RoH/relatedness
WG4	*de novo* mutations
WG5	Meta-analysis
WG6	epigenomics
WG7	RNAseq
WG8	Somatic mutations
WG9	Structural variants



Unsolved cases

21,346 datasets collected from 20,807 individuals

Data Freeze 1: 7,447 datasets from 7,382 individuals

Data Freeze 2: 3,125 datasets from 3,070 individuals

Data Freeze 3: 10,774 datasets from 10,604 individuals

Data freeze 1+2 re-analysis (6,232 families)

745 newly solved families

11,95 % **SOLVED!**

Potential der Genomsequenzierung

Strategische Diskussionspunkte

21.07.2022

Critical Ill Infants

202 neonates: 45% neuromuscular, 22% respiratory, 18% immunologic/infect.

Trio-rGS: ~37% diagnostic yield

TAT: 7d

Metagenome: pathogenic microbes in 6 infants with symptoms of sepsis

(2 x Pseudomonas, Mycobact. Tuberculosis / h MastadenovirusB, h betaherpesvirus6A, h gammaherpesvirus4)

Wu et al. Application of full-spectrum rapid clinical genome sequencing improves diagnostic rate and clinical outcomes in critically ill infants in the China Neonatal Genomes project.
Critical Care Medicine 2021

21.07.2022

China Neonatal Genome Project

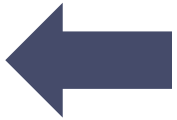
Repeat expansion Detection in srWGS diagnostics

Repeat Expansions of single-sample analysis DX21100052_02

	chr	start	end	repeat_id	repeat_unit	repeats	wt_repeat	repeat_ci	filter	locus_coverage	reads_flanking	reads_in_repeat	reads_spanning
1	chr1	149390802	149390841	NOTCH2NL	GGC	18/21	13	18-18/21-21	PASS	53.01	35/41	0/0	29/18
2	chr2	190880872	190880920	GLS	GCA	8/15	16	8-8/15-15	PASS	46.66	12/22	0/0	30/12
3	chr3	63912684	63912714	ATXN7	GCA	10/10	10	10-10/10-10	PASS	54.12	24/24	0/0	46/46
4	chr3	63912714	63912726	ATXN7_GCC	GCC	4/4	4	4-4/4-4	PASS	54.12	12/12	0/0	51/51
5	chr3	129172576	129172656	CNBP	CAGG	15/15	20	15-15/15-15	PASS	41.77	46/46	0/0	19/19
6	chr3	129172656	129172696	CNBP_CAGA	CAGA	10/9	10	10-10/9-9	PASS	41.77	27/25	0/0	13/14
7	chr3	129172696	129172732	CNBP_CA	CA	20/20	18	20-20/20-20	PASS	41.77	21/21	0/0	34/34
8	chr4	3074876	3074933	HTT	CAG	17/20	19	17-17/20-20	PASS	53.52	43/49	0/0	22/16
9	chr4	3074939	3074966	HTT_CCG	CCG	9/12	9	9-9/12-12	PASS	53.52	25/29	0/0	21/14
10	chr4	39348424	39348479	RFC1	AARRG	11/34	11	11-11/34-43	PASS	41.77	35/52	0/2	10/0
11	chr4	41745972	41746032	PHOX2B	GCN	20/20	20	20-20/20-20	PASS	51.04	52/52	1/1	42/42
12	chr5	146878727	146878757	PPP2R2B	GCT	10/10	10	10-10/10-10	PASS	50.35	32/32	0/0	42/42
13	chr6	16327633	16327723	ATXN1	TGC	31/32	30	31-31/32-32	PASS	53.52	87/87	0/0	13/9
14	chr6	170561906	170562017	TBP	GCA	37/36	37	37-37/36-36	PASS	40.92	54/53	0/0	4/6
15	chr9	27573528	27573546	C9ORF72	GGCCCC	5/460	3	5-5/425-676	PASS	48.03	18/39	0/350	23/0
16	chr9	69037261	69037286	FXN_A	A	26/27	25	26-26/27-27	PASS	45.12	17/17	0/0	15/20
17	chr9	69037286	69037304	FXN	GAA	9/25	6	9-9/25-25	PASS	45.12	20/25	0/1	22/9
18	chr11	119206289	119206322	CBL	CGG	11/11	11	11-11/11-11	PASS	51.38	45/45	0/0	66/66
19	chr12	6936716	6936773	ATN1	CAG	12/16	19	12-12/16-16	PASS	46.49	18/21	0/0	12/19
20	chr12	50505001	50505022	DIP2B	GGC	7/7	7	7-7/7-7	PASS	51.98	16/16	0/0	50/50
21	chr12	111598949	111599018	ATXN2	GCT	22/27	23	22-22/27-27	PASS	62.27	59/67	0/0	19/16
22	chr13	70139353	70139383	ATXN8OS_CTA	CTA	9/9	10	9-9/9-9	PASS	43.40	15/15	0/0	40/40
23	chr13	70139383	70139428	ATXN8OS	CTG	15/14	15	15-15/14-14	PASS	43.40	30/29	0/0	22/15
24	chr14	23321472	23321490	PABPN1	GCG	6/6	6	6-6/6-6	PASS	52.24	18/18	0/0	49/49
25	chr14	92071009	92071042	ATXN3	GCT	18/25	11	18-18/25-25	PASS	43.23	37/38	0/0	14/13
26	chr15	22786677	22786701	NIPA1	GCG	8/8	8	8-8/8-8	PASS	60.56	21/21	0/0	61/61
27	chr16	87604287	87604329	JPH3	CTG	14/14	14	14-14/14-14	PASS	56.36	27/27	0/0	48/48
28	chr18	55586155	55586227	TCF4	CAG	11/12	24	11-11/12-12	PASS	35.17	10/11	0/0	16/12
29	chr19	13207858	13207897	CACNA1A	CTG	13/11	13	13-13/11-11	PASS	52.32	37/33	0/0	28/18
30	chr19	45770204	45770264	DMPK	CAG	11/12	20	11-11/12-12	PASS	65.70	37/37	0/0	25/32
31	chr20	2652733	2652757	NOP56	GGCCTG	7/9	4	7-7/9-9	PASS	65.62	36/39	0/0	37/27
32	chr20	2652757	2652775	NOP56_CGCCTG	CGCCTG	2/2	3	2-2/2-2	PASS	65.62	6/6	0/0	80/80
33	chr21	43776443	43776479	CSTB	CGCGGGGCGGGG	3/3	3	3-3/3-3	PASS	56.70	30/30	0/0	54/54
34	chr22	45795354	45795424	ATXN10	ATTCT	14/11	14	14-14/11-11	PASS	41.26	26/25	0/0	10/15
35	chrX	67545316	67545385	AR	GCA	26	23	26-26	PASS	23.93	27	0	13
36	chrX	147912050	147912110	FMR1	CGG	30	20	30-30	PASS	26.68	34	0	13
37	chrX	148500631	148500691	AFF2	GCC	20	20	20-20	PASS	31.35	24	0	18

Transcriptome analysis in research diagnostics

Need for transcriptome analysis to complement WGS data



Bericht zur Transkriptomsequenzierung aus PAXgene Blut bei V. a. hereditären Brust- und Eierstockkrebs (HBOC)

Patientin: [redacted] Befunddatum: [FREIGABEDATUM] Befund-ID: 47329

Labor-Nr. (A
Material:
Klinische An
Familienana

Sehr geehr

in einer Ex
ID 43252).
durchgefüh
Auswertun
Exomseque

ZUSAMME

- Vorb
  den a
- Vorb
- Die g
  Gens
- Aufgr
  ATM

Bericht zur Transkriptomsequenzierung aus PAXgene Blut bei Vorliegen einer Entwicklungsverzögerung

Patientin: [redacted] Befunddatum: [FREIGABEDATUM] Befund-ID: 46576

Labor-Nr. (Ablage-Nr.): **RNA-2200246 A1** (20208093) Probeneingang: 12.01.2022
Material: EDTA-Blut 2 ml + 2 ml, Paxgene 8 ml Auftragsfreigabe: 18.02.2022
Klinische Angaben: Leichte geistige Behinderung, Sprachentwicklungsstörung, Verhaltensauffälligkeiten
Fam

Sehr geehrte Kollegin Roggia,

in einer Exomsequenzierung wurde eine Near-Spleiß-Variante unklarer Signifikanz im *FRAS1*-Gen identifiziert (unser Befund vom 13.01.2022, Befund-ID 40632). Es wurde auf die Möglichkeit einer Transkriptomsequenzierung zur weiteren Einschätzung des Effekts der Variante auf die *FRAS1*-mRNA hingewiesen.

Auswertung: Im Rahmen der Fragestellung wurden die Sequenzdaten der Transkriptomsequenzierung zusammen mit den Daten der Exomsequenzierung im wissenschaftlichen Kontext ausgewertet und beurteilt.

Un
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BCL

Bericht zur Transkriptomsequenzierung aus archiviertem Schilddrüsen und Lungengewebe bei Vorliegen einer bilateralen Nierenagenesie

Patientin: [redacted] Befunddatum: [FREIGABEDATUM] Befund-ID: 46339

Labor-Nr. (Ablage-Nr.): **RNA-2201860 A1** (20204784) Probeneingang: 03.03.2022
Externe Nummer: 4007
Material: RNA 50 µl + 50 µl Auftragsfreigabe: 10.03.2022
Klinische Angaben: Z.n. med. Interruptio bei Geminigravidität und fetaler Nierenagenesie, Potter-Sequenz
Familienanamnese: Zwillingsbruder ebenfalls betroffen

Aberrantes Spleißen => Pathogenität bewiesen

ZUSAMMENFASSUNG:

- **Vorbefundlich im Kontext der Fragestellung kein Nachweis pathogener oder wahrscheinlich pathogener DNA-Varianten, die den angegebenen Phänotyp hinreichend erklären**
- **Vorbefundlich wurde eine Near-Spleiß-Variante unklarer Signifikanz im *FRAS1*-Gen identifiziert (unser Befund vom 13.01.2022)**
- **Aufgrund der vorbefundlich identifizierten Near-Spleiß-Variante im *FRAS1*-Gen kommt es zu einer Retention von Intron 43 der *FRAS1*-mRNA**
- Varianten im FRAS1-Gen sind mit einem autosomal-rezessiv vererbbaren Fraser-Syndrom (OMIM #219000) assoziiert worden
- In Zusammenschau mit den vorliegenden phänotypischen Informationen ist diese Veränderung zum derzeitigen Kenntnisstand als wahrscheinlich ursächlich für die Erkrankung anzusehen

The GeMed diagnostic approach

Combining clinical genetics with genomic health

GeHealth Prevention

Why:
- Diagnostic sensitivity
- Automatization
- Data

Genome First
(Cancer syndromes, Rare Diseases)

+ **Transcriptome („always“) Pax tubes / blood**

Disease causing mutation

+ **Verified in Transcriptome?**

unsolved

+ **ACMG73**
5% aller
WES/WGS

+ **PRS**
Breast Cancer
Diabetes Type 2
Ca. 2% each

Exceptions:
Children
Patients with psychiatric or neurodegenerative diseases

Exceptions: Clear
Exce
eml
- Familial
index p

Exceptions: *de novo*
- ID/DD in children: Trio-Exome

negative

Genome+

Universitätsklinikum Tübingen

„Organisational Complexity“ of clinical care and diagnostic pathways in human genetics

Common diseases
Genomic Health
Newborn screening

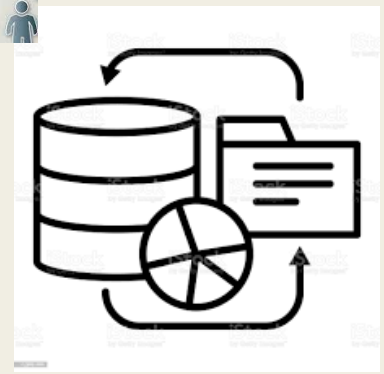
Emerging new fields

Communication flow

Patient's autonomy
Global informed consent

Data flow

Documentalist



Data base entries
Stewardship

GHGA
1+MEGA
MII
ML/AI

Clinical Geneticist

Sample flow

Genetic Nurse

Case Manager

Study Manager

long read
DNA
RNAseq
Biomarker

Treatment

decision

Information flow

Actionable genes
Polygenic Risk Scores

Structures to immediately enhance diagnostic sensitivity in RD

Strategic Discussion Points

21.07.2022

Not data alone, but data interpretation and clinical integration are important!

- SOLVE-RD GERMANY / SOLVE-GD GERMANY
  - German DATF excellence network, coordinated interaction with German ERN-organized DITFs
  - Technically sophisticated but diagnostically experienced NGS+ („multiOmics“)
- GD Diagnostic Competence Centers
  - Latest technologies, semiautomated, high throughput, interconnected, „accessible“

Integration in and interaction with: national ERNs,
RD research networks, 1+MG, AI Genomics & MultiOmics

genomDE

Need in diagnostic care of RD/GD:
Data of unclear clinical implication: How to proceed?

- Variant Interpretation Data Base
  - Focus: Variants of unknown clinical significance; VUS class 3, expert networks
- Variant Validation Groups
  - Gene pathway focused experts experimentally validating VUS class 3
- German RDMM
  - Disease modeling network for ultra-rare diseases, establishing novel disease genes or novel disease mechanisms

21/07/22

SolveRD

ZSE TÜBINGEN
Zentrum für Seltene Erkrankungen

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I declare to receive an explorative grant from Illumina for implementation of WGS into clinical care.

I receive further funding for genome analysis from the EU and the German Research Foundation (NGS Competence Center).

genomDE

Institut für Medizinische Genetik und Angewandte Genomik

NCCT
NGS Competence Center Tübingen

Deutschland Land der Ideen
Ausgewählter Ort 2011