



Diagnostics of Rare Diseases: Beyond the Exome

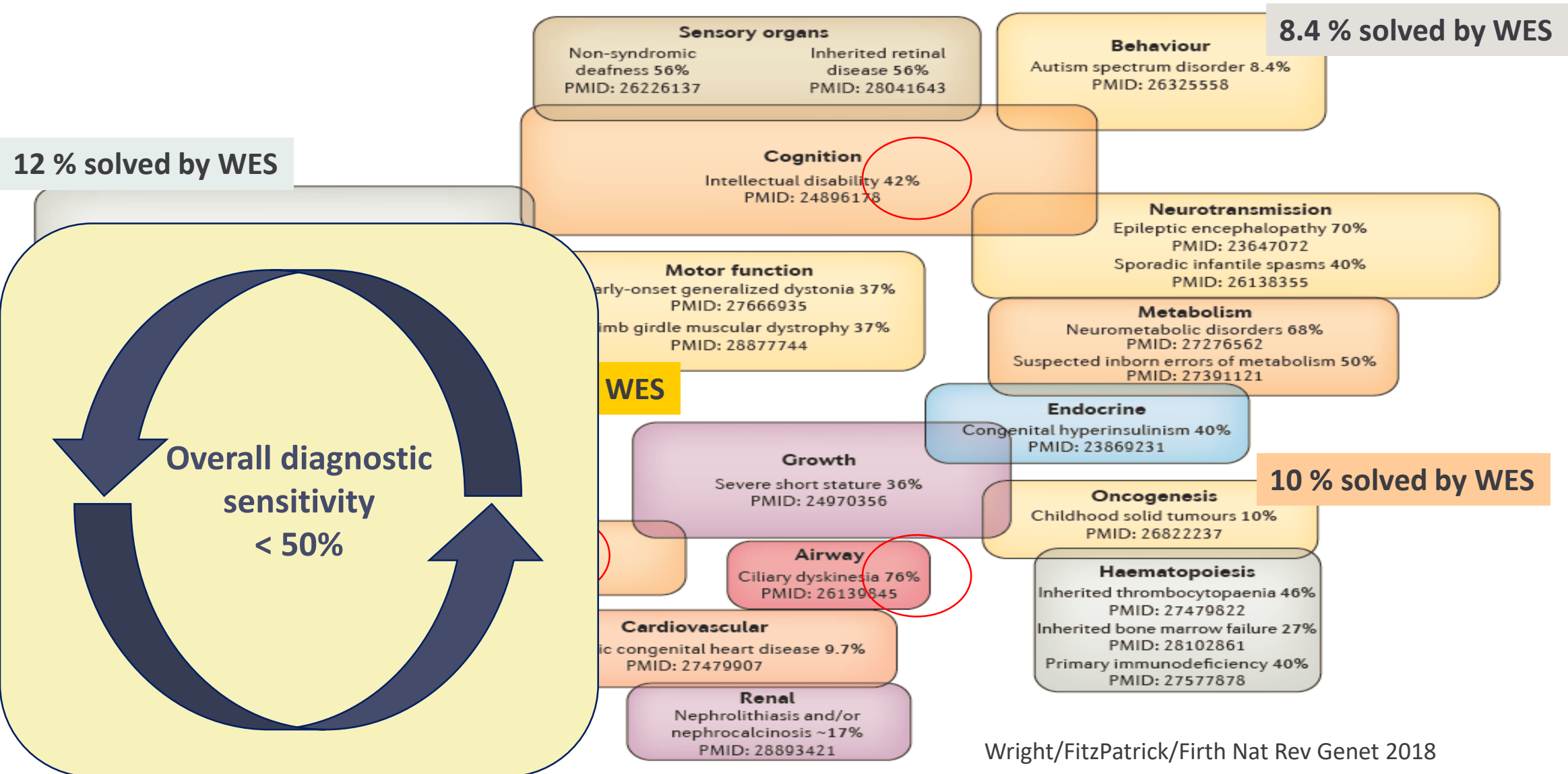
Olaf Riess

11.04.2021

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Diagnostic sensitivity of today's **Whole Exome Sequencing** in pediatric diseases



UNSOLVED after WES:

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



**300 Mio RD patients worldwide
150 Mio patients unsolved**



**30 Mio patients in Europe
15 Mio unsolved**



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

Use case: Rare diseases

Diagnosis: A disease has to have a name to

- Communicate with doctors, relatives, authorities, health insurance
- Better define disease impact and progression and disease management
- Potentially apply disease-specific treatment
- Select disease information via internet



Solving the Unsolved: Rare diseases

Major **limitations** to increase diagnostic sensitivity:

- Bureaucracy, ethical hurdles, data protection regulations
- Financing
- Limited knowledge
- Clinical processes in patient tissue/sample collection
- But presumably **NOT** technical limitations



From Exome to Genome

Limitations of Whole Exome Sequencing (WES)

Important: Type of enrichment system: SureSelectXT Human All Exon v6

Statistics of coverage: complete coding sequence +/-5bp intronic region
depth of sequencing (at least 20 fold)
coverage: 98.99%

Bacon: 6222806
Ausgewählte Gene (1884): A2ML1, AACB, AACS, ABAT, ABCA13, ABCA2, ABCC1, ABCD1, ABCD4, ABDH4, ABIB2, ABIZ, ABL1, ACACA, ACACB, ACAD9, ACADS, ACSBD5, ACSBD6, ACER3, ACO2, ACOX1, ACOX2, ACP5, ACSF3, ACSL4, ACTB, ACTG1, ACTL6A, ACTR1B, ACTR11, ADAM22, ADAM33, ADAR, ADAT3, ADCY5, ADDRG2, ADCIPOR1, ADK, ADNP, ADRA2B, ADSL, AFF2, AFF3, AFF4, AFG3L2, AGA, AGK, AGMO, AGO1, AGPAT2, AGPS, AGTR2, AHGY, AHUCL1, AHI1, AHSQ, AIEM1, AIMP1, AIR AK1, AKT3, ALDH1B1, ALDH1A3, ALDH3A2, ALDH4A1, ALDH5A1, ALDH7A1, ALDOA, ALG1, ALG11, ALG12, ALG13, ALG2, ALG3, ALG8, ALG9, ALMS1, ALS2, AMER2, AMMECR1, AMPD2, AMT, AMZ2, ANAPC2, ANK2, ANK3, ANKH, ANKLE2, ANKRDI1, ANO10, ANOS, AP1S1, AP1S2, AP3B1, AP3B2, AP4B1, AP4E1, AP4M1, AP4M2, AP4L2, ARPP11, JPH, RPL2L, RFX, RPLC1, RPTOR, RPL3, RPL37, RPL38, RPL39, RPL40, RPL41, RPL42, RPL43, RPL44, RPL45, RPL46, RPL47, RPL48, RPL49, RPL50, RPL51, RPL52, RPL53, RPL54, RPL55, RPL56, RPL57, RPL58, RPL59, RPL60, RPL61, RPL62, RPL63, RPL64, RPL65, RPL66, RPL67, RPL68, RPL69, 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Limitations of WES:

Coverage

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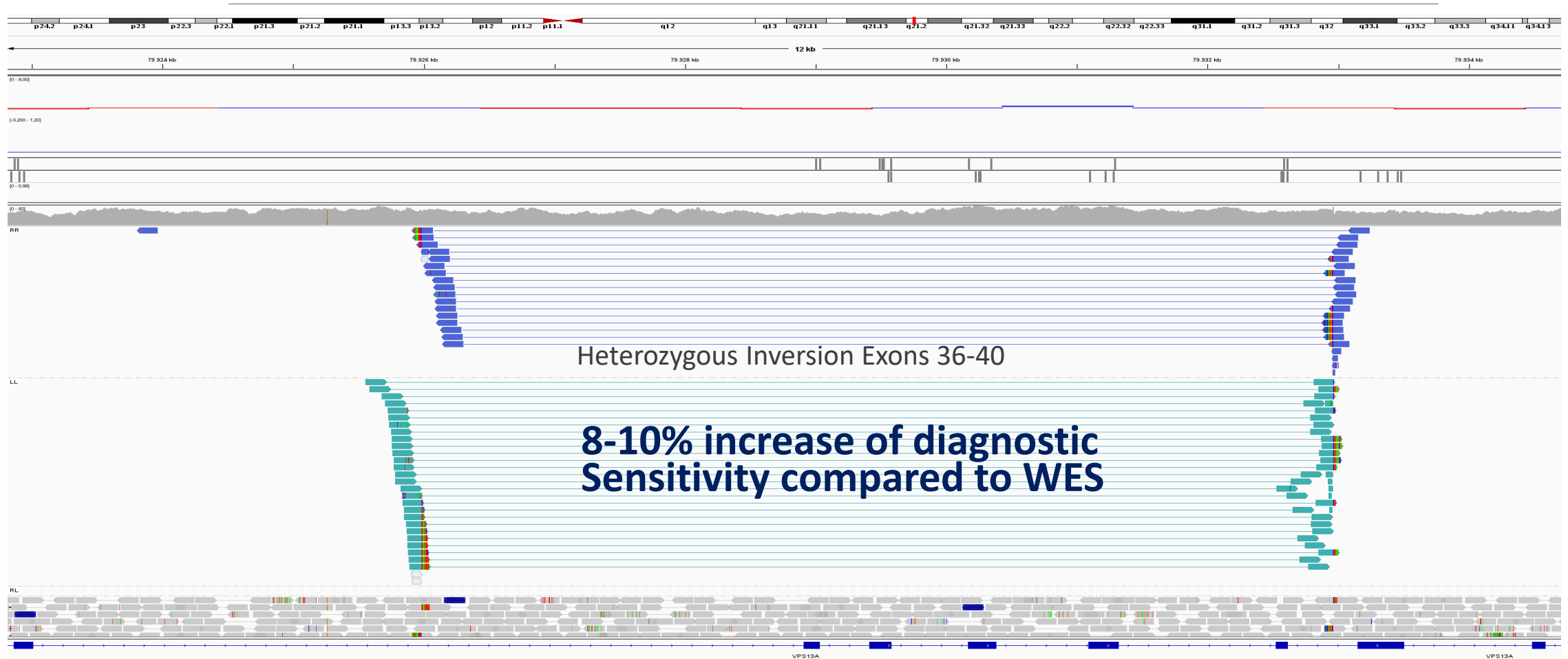
Aberrant splicing

Structural aberrations

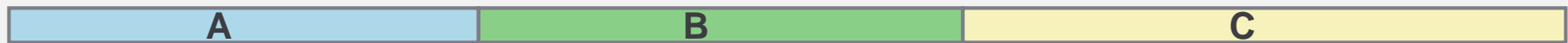
Regulatory regions

COXRED1, FRAS1, FREM2, FRKX3, FRMD4A, FRMPD4, FRRS1L, FRY, FSCN1L, FTL, FTO, FTSJ1, FUCAL1, G6PD, GABBR2, GABRA1, GABRA3, GABRB1, GABRB2, GABRB3, GABRG2, GAD1, GALT, GALT1B, GALT2, GALT, GAMT, GAN, GATAD2B, GATM, GBA2, GCC2, GCDH, GCH1, GCN1, GCSH, GDI1, GEMIN4, GFAP, GFM1, GPFPT2, GGN, GIGYF1, GIGYF2, GIMAPB, GJA1, GJC2, GK, GLB1, GLDC, GLI2, GLI3, GLRA2, GLS, GLUL, GLYCTC, GM2A, GNMN, GMPFA, GMPBP, GNAI1, GNAI2, GNAO1, GNAQ, GNAS, GNB1, GNB5, GNE, GNPAT, GNPTAB, GNPTG, GNS, GONAL, GPR41, GPC2, GPHN, GPI, GPM6A, GPR138, GPR37, GPR52, GPS1, GPSM2, GP2, GRAMD1B, GRA1, GRA2, GRA3, GRG2, GRK2, GRK2, GRIN1, GRIN2A, GRIN2B, GRIN2D, GRM1, GRM1, GSS, GSTT1, GTF2E2, GTF2H5, GTF3C3, GTPBP3, GUCY2D, GURF1, GUSB, HAC1, HAC1L, HAX1, HCC5, HCCF1, HCN1, HDAC4, HDAC8, HDAC9, HECW2, HEMK1, HERPACAM, HERC1, HERC2, HESX1, HEXA, HEXB, HGSNAT, HHAT, HEBH, HIKESH, H1ST1HE, H1ST1HB, H1ST1HB, H1ST1H3, HIVEP2, HIVEP3, HYL1, ILCS, IMB5, HMCN2, HMG20A, HMG2B, HMGCL, HMGCLL1, HNF1B, HNF4A, HNF4, HNFMT, HNRNP1H1, 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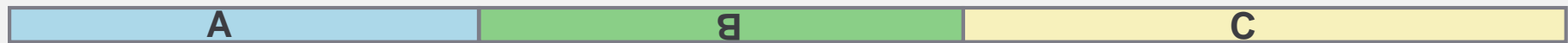
WGS in unsolved chorea acanthocytosis: Structural variants in VPS13A



Referenz



Patient



Genome: Regulatory regions



Regulatory regions: Enhancers

B

Neural Tube

Forebrain

Forebrain includes all parts of the



hs1043



hs238



hs169



hs230



hs311



hs769



hs200



hs595



hs853



hs692



hs840

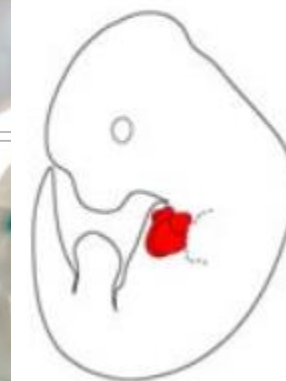


hs742



hs798

Heart



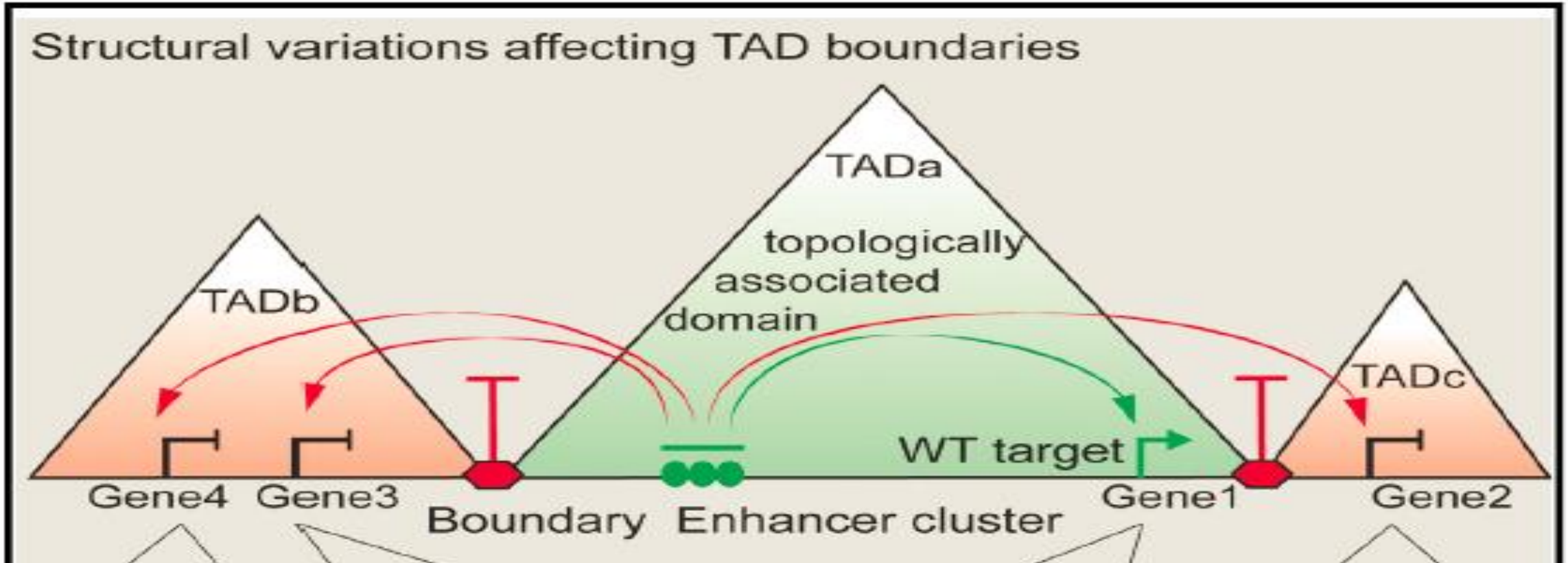
hs222



hs229

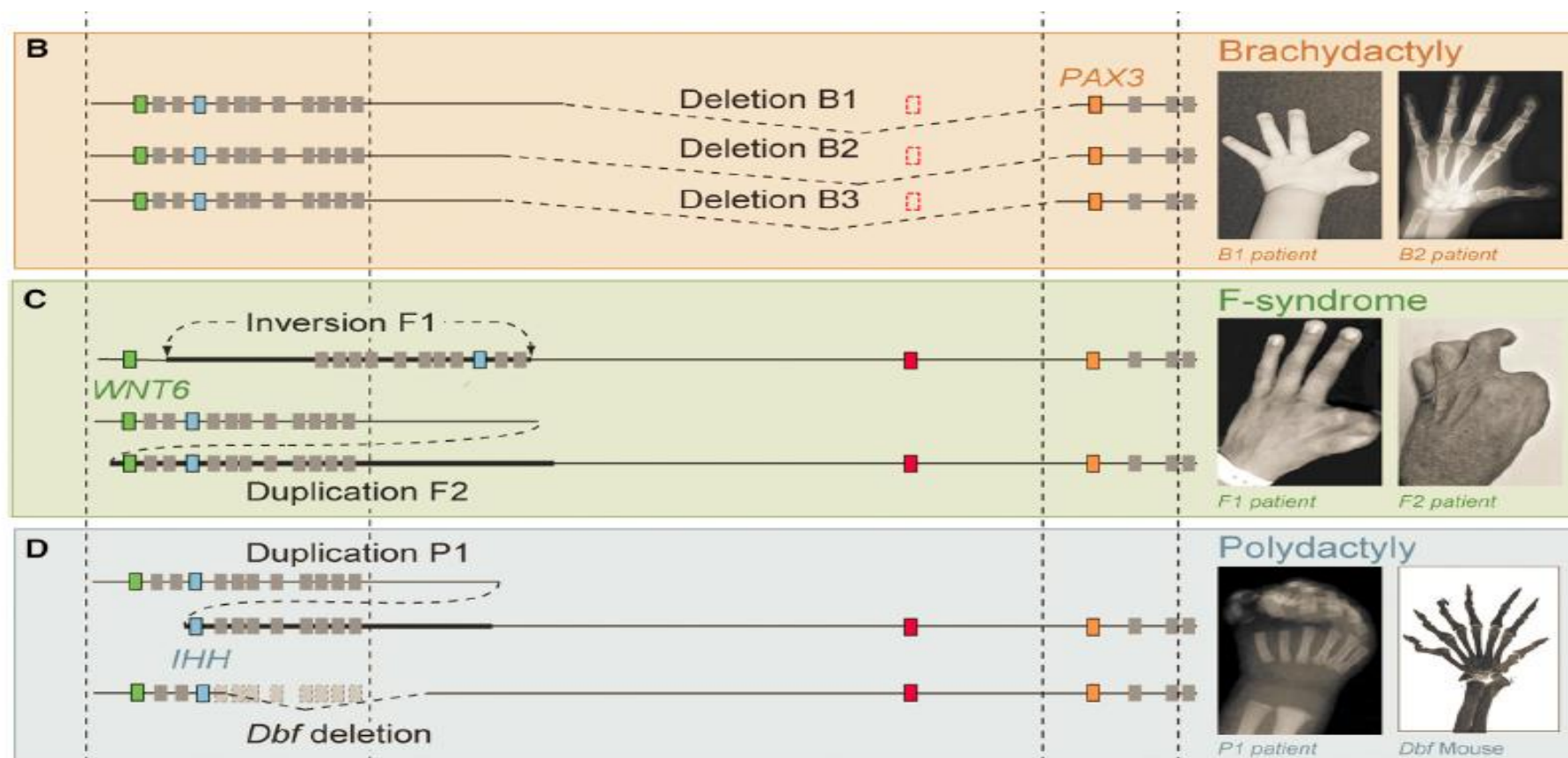
Disruptions of Topological Chromatin Domains Cause Pathogenic Rewiring of Gene-Enhancer Interactions

Darío G. Lupiáñez,^{1,2} Katerina Kraft,^{1,2} Verena Heinrich,² Peter Krawitz,^{1,2} Francesco Brancati,³ Eva Klopocki,⁴ Denise Horn,² Hülya Kayserili,⁵ John M. Opitz,⁶ Renata Laxova,⁶ Fernando Santos-Simarro,^{7,8} Brigitte Gilbert-Dussardier,⁹ Lars Wittler,¹⁰ Marina Borschiwer,¹ Stefan A. Haas,¹¹ Marco Osterwalder,¹² Martin Franke,^{1,2} Bernd Timmermann,¹³ Jochen Hecht,^{1,14} Malte Spielmann,^{1,2,14} Axel Visel,^{12,15,16} and Stefan Mundlos^{1,2,14,*}



Disruptions of Topological Chromatin Domains Cause Pathogenic Rewiring of Gene-Enhancer Interactions

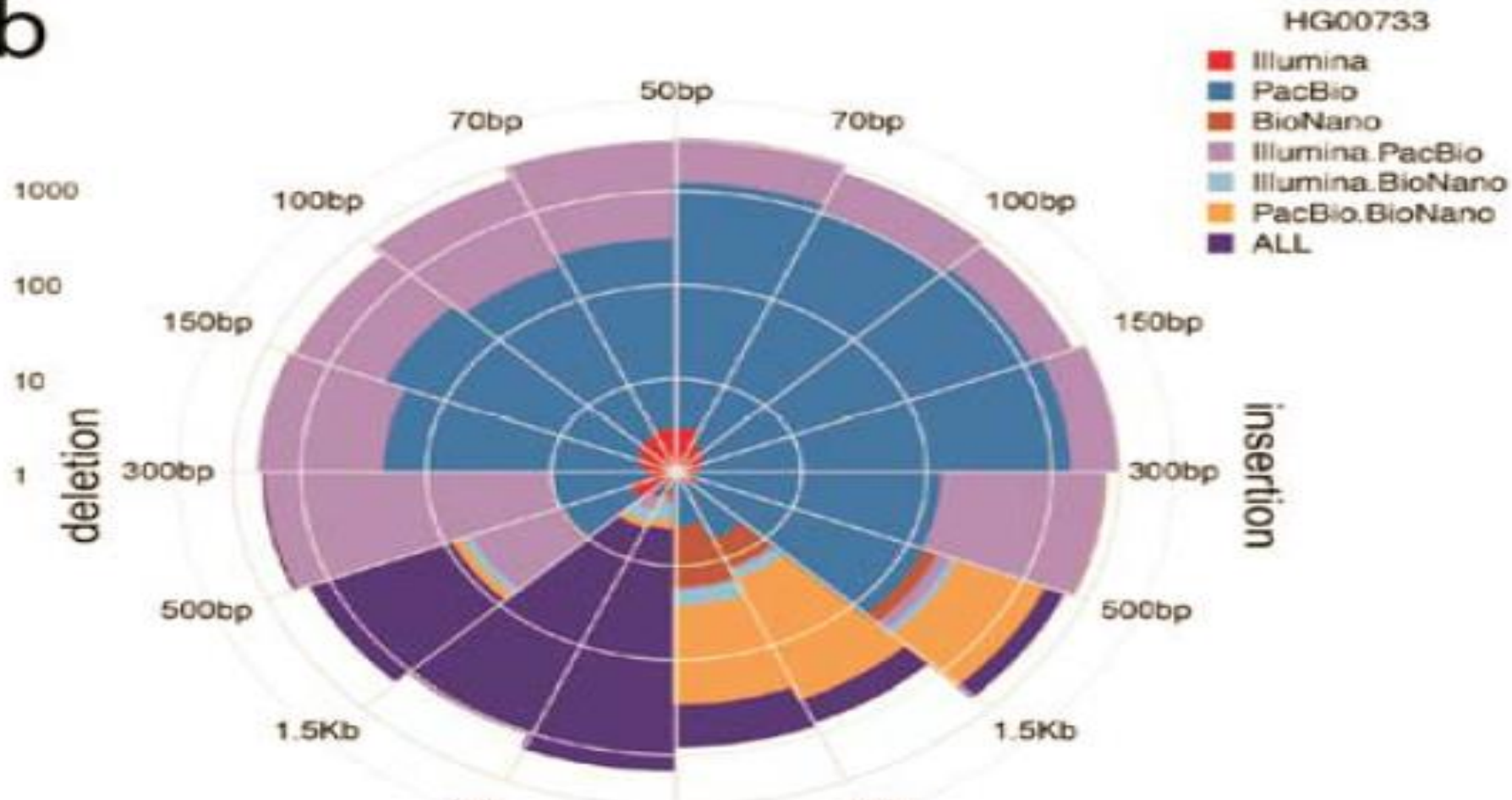
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Genome: Towards long read sequencing

Combination of short and long read NGS Required for sensitive detection of deletions and insertions

b



Multi-platform discovery of haplotype-resolved structural variation in human genomes

Mark J.P. Chaisson^{1,2*}, Ashley D. Sanders^{3*}, Xuefang Zhao^{4,5*}, Ankit Malhotra^{6†}, David

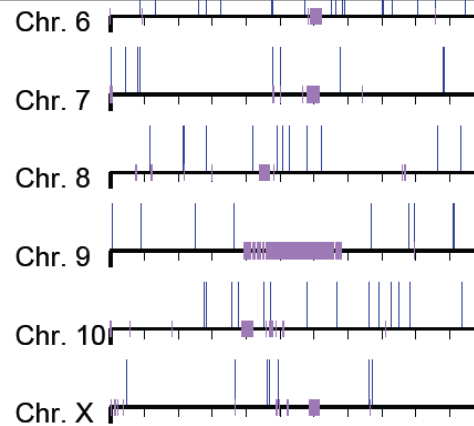
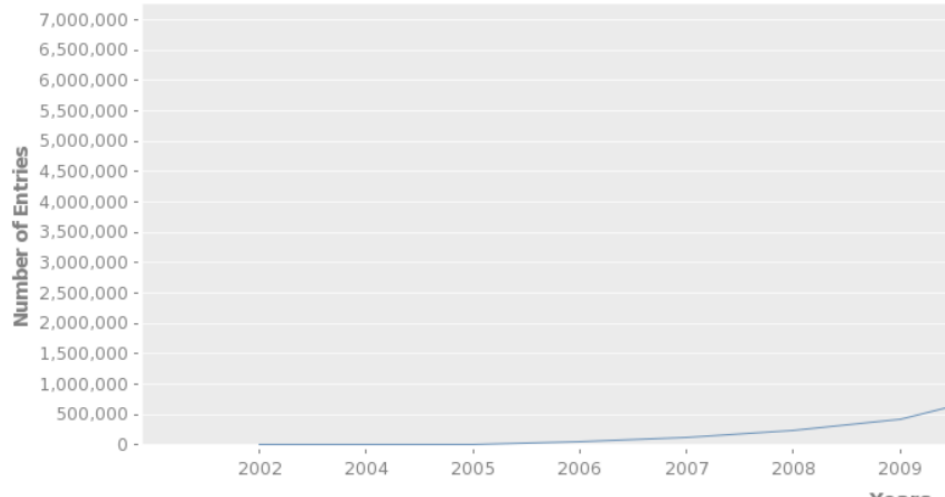
Characterization of missing human genome sequences and copy-number polymorphic insertions

Jeffrey M Kidd¹, Nick Sampas², Francesca Antonacci¹, Tina Graves³, Robert Fulton³, Hillary S Hayden¹, Can Alkan¹, Maika Malig¹, Mario Ventura⁴, Giuliana Giannuzzi⁴, Joelle Kallicki³, Paige Anderson², Anya Tsalenko², N Alice Yamada², Peter Tsang², Rajinder Kaul¹, Richard K Wilson³, Laurakay Bruhn² & Evan E Eichler^{1,5}

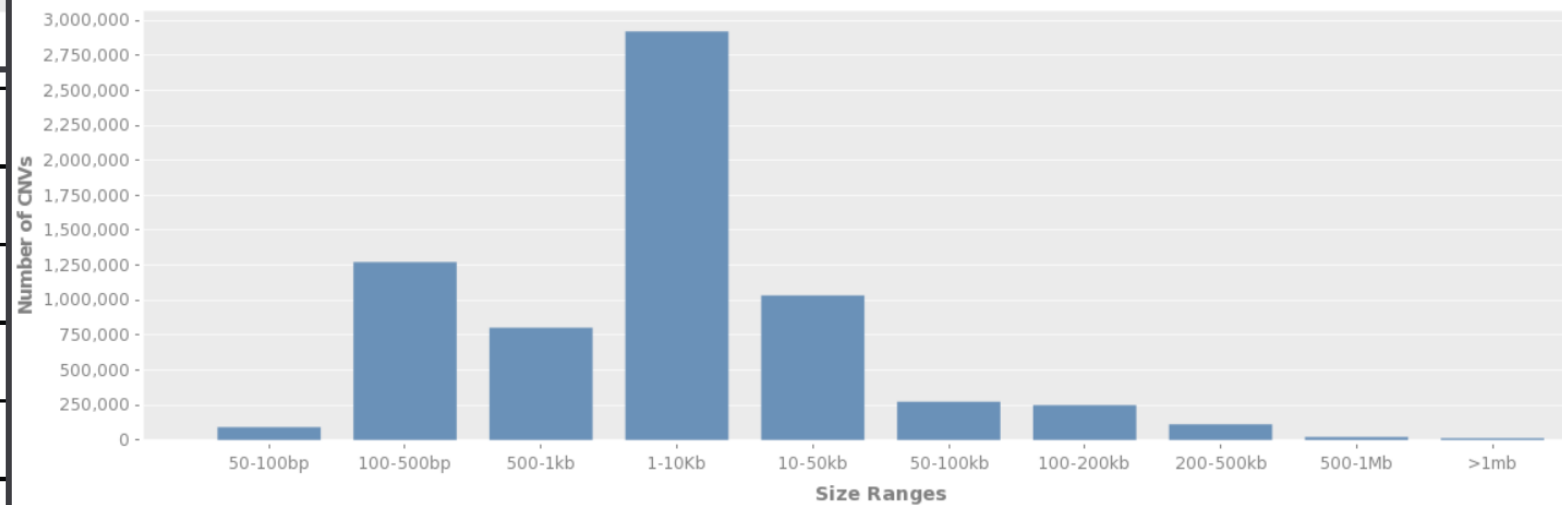
NATURE METHODS | VOL.7 NO.5 | MAY 2010

2,363 new insertion sequences
corresponding to **720** genomic loci.

Increase in Variation Data



Size Distribution of CNVs in DGV2



Highly Homologous Genes neglected in Short-Read WGS

HiFi reads to distinguish functional genes (GBA) from its pseudogenes (ψ GBA) and allocate variants
--> Targeted Application with Amplicons: HiFi reads to screen a large cohort

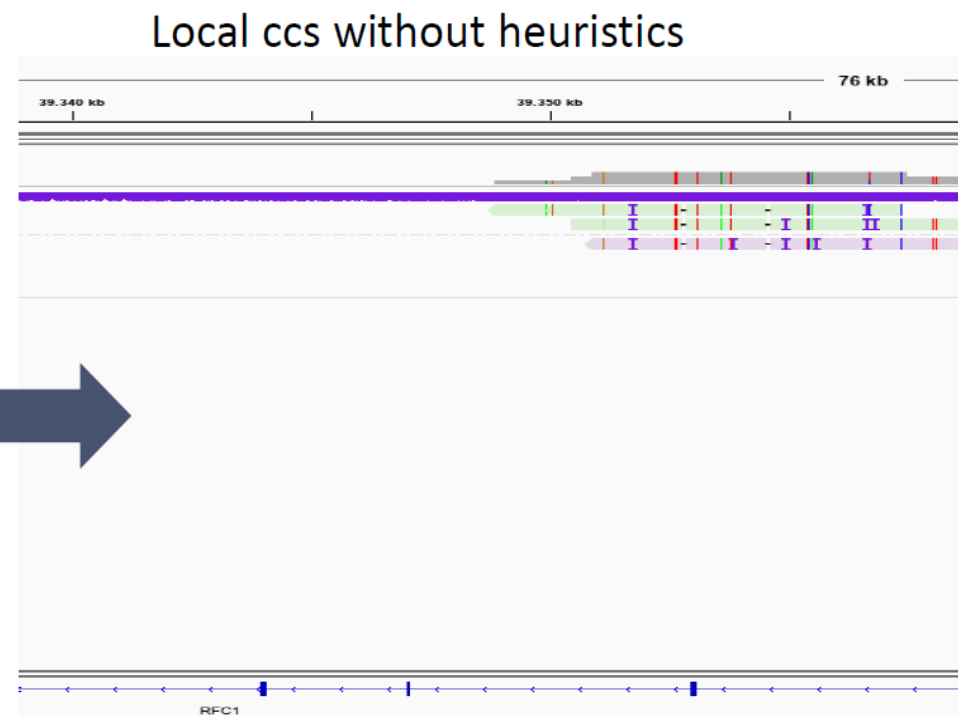
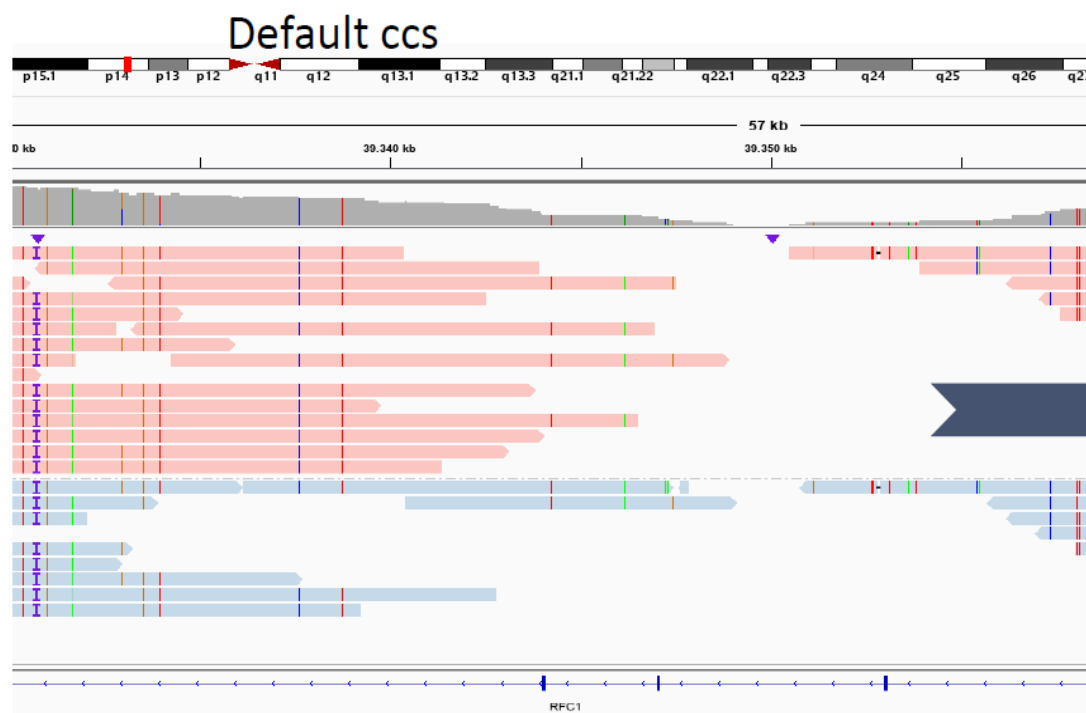


Bad mapping quality

WGS – Variant Calling: HiFi data from NCCT establishment

RFC1 Repeat Expansion

I19D045a01



No reads mapping to RFC1

Problem: Not enough reads in close distance to RFC1 region.
Currently all steps are repeated with **larger window** around RE site.

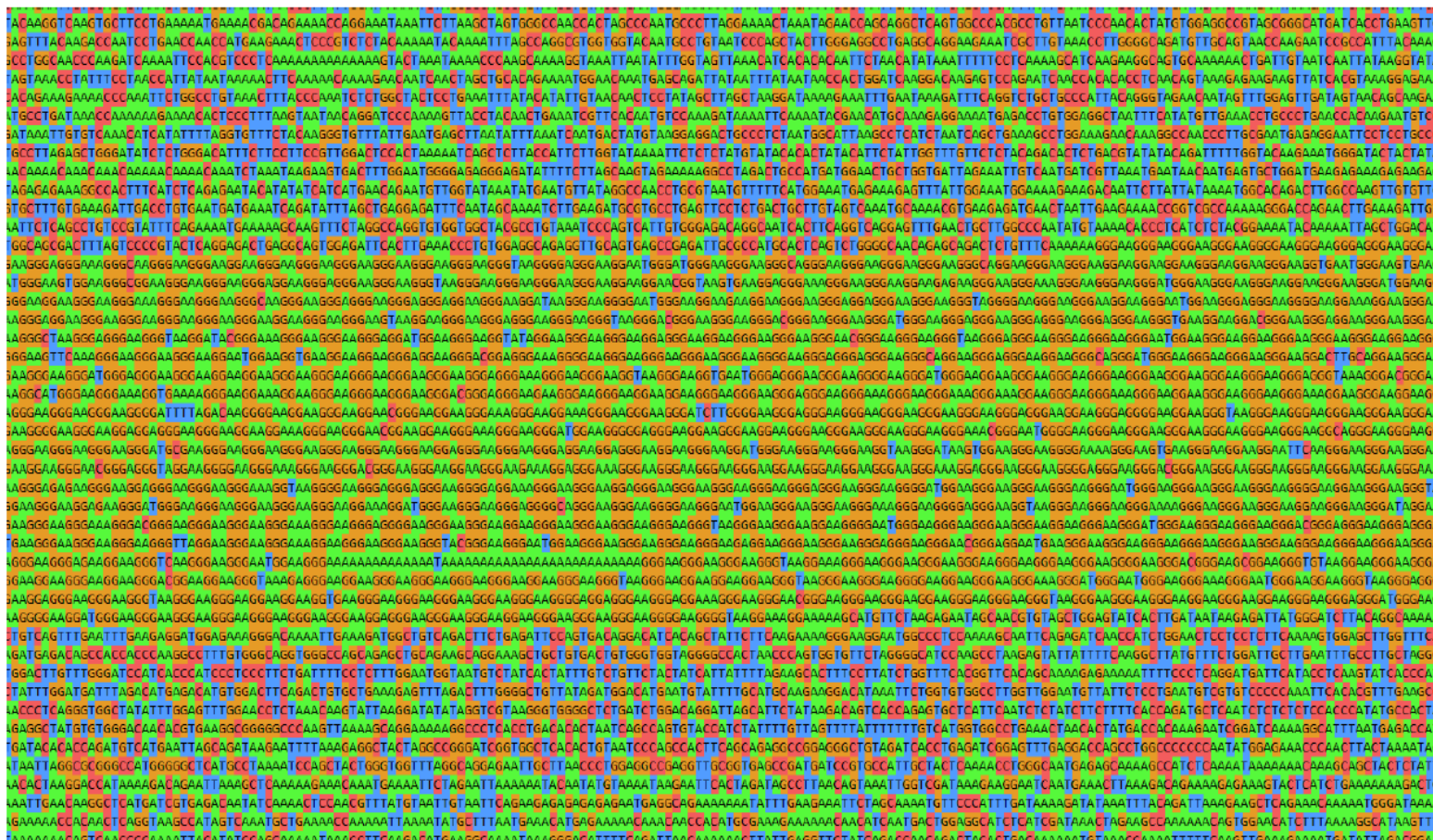
WGS – Variant Calling: HiFi data from NCCT establishment

RFC1 Repeat Expansion

I19D045a01

Local assembly confirms repeat expansion undetected by mapping

Length:
3753 bases

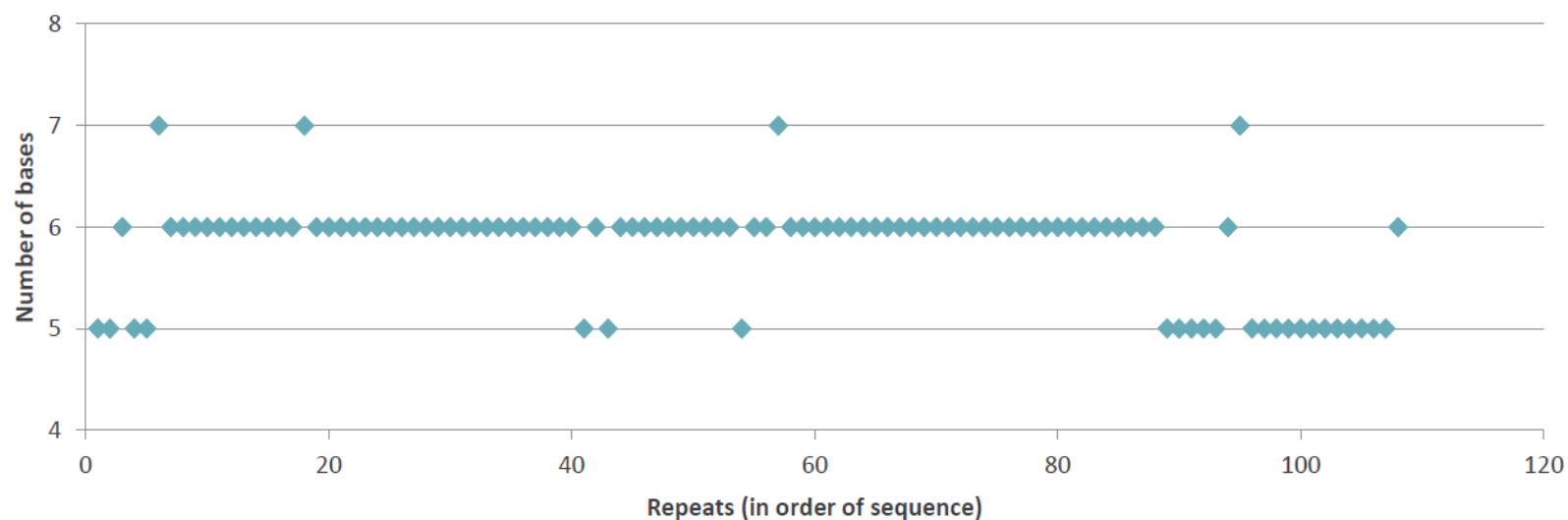


WGS – Variant Calling: HiFi data from NCCT establishment

RFC1 Repeat Expansion

I19D045a02

HiFi Read (mapped) Sequence of the 571-bp Insertion and flanking bases

[illegible]

Complexity of analysing genomes in the clinical context

TOPMed study (Trans-Omics for Precision Medicine)

53,831 Genomes analysed

>400 Mio variants

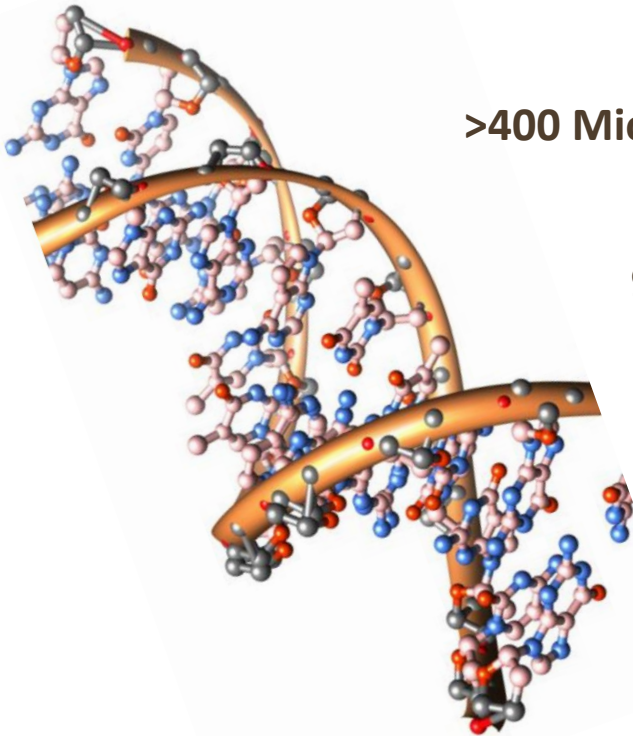
**97% of the variants
less than 1%**

**53% present
in only 1 individual**

- > 4,6 Mio protein-coding variants
- Total 230,000 putative loss of function variants in 18.493 genes
- > 104,000 frameshift variants
- > 97,000 putative splice and truncation variants

2.5 unique pLOF per individual

Additional read out needed !



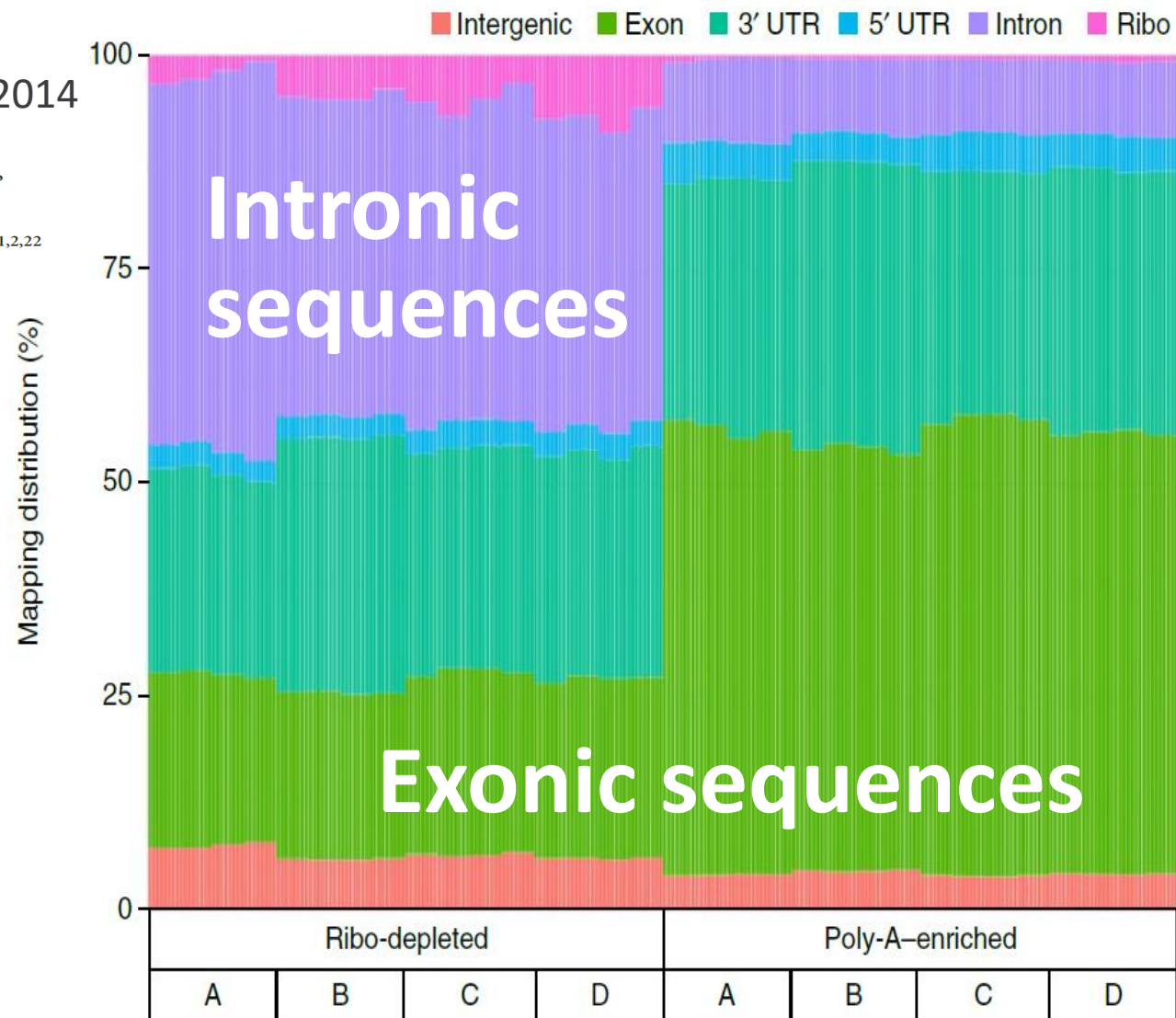
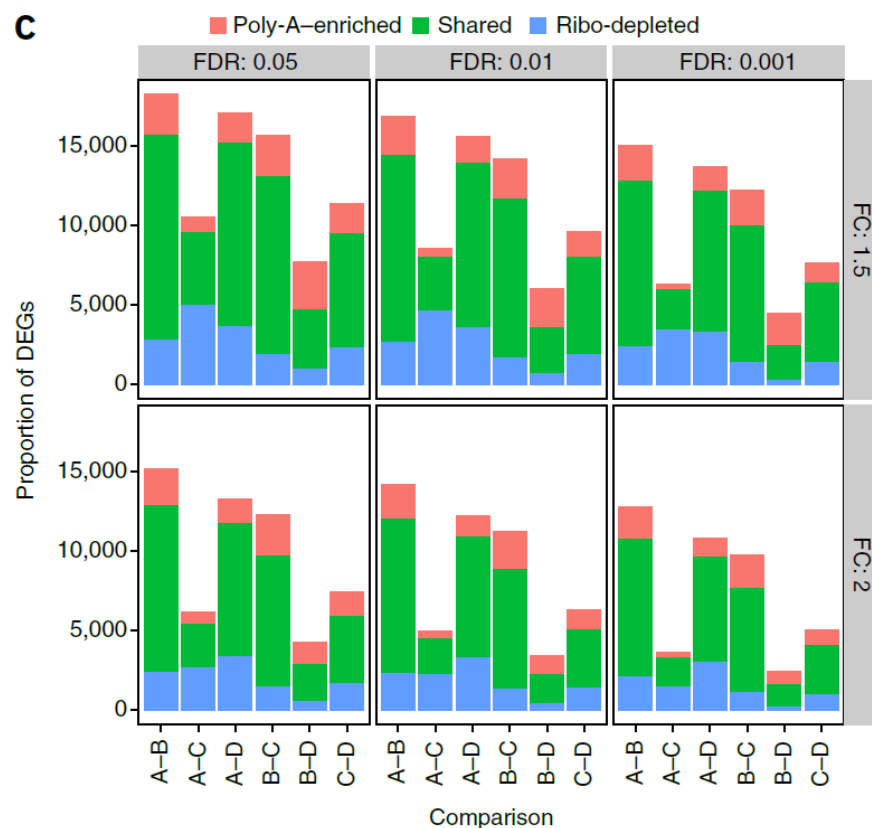
Genome: Complemented with RNAseq

RNAseq in research and diagnostics – Technical „details“

Multi-platform assessment of transcriptome profiling using RNA-seq in the ABRF next-generation sequencing study

Nature Biotechnol 2014

Sheng Li^{1,2,24}, Scott W Tighe^{3,24}, Charles M Nicolet⁴, Deborah Grove⁵, Shawn Levy⁶, William Farmerie⁷, Agnes Viale⁸, Chris Wright⁹, Peter A Schweitzer¹⁰, Yuan Gao¹¹, Dewey Kim¹¹, Joe Boland¹², Belynda Hicks¹², Ryan Kim^{13,23}, Sagar Chhangawala^{1,2}, Nadereh Jafari¹⁴, Nalini Raghavachari¹⁵, Jorge Gandara^{1,2}, Natàlia Garcia-Reyero¹⁶, Cynthia Hendrickson⁶, David Roberson¹², Jeffrey A Rosenfeld¹⁷, Todd Smith¹⁸, Jason G Underwood¹⁹, May Wang²⁰, Paul Zumbo^{1,2}, Don A Baldwin²¹, George S Grills¹⁰ & Christopher E Mason^{1,2,22}



RNAseq in diagnostics

94 patients / 1600 controls

343 outliers per sample

7.5% diagnostic rate

Additional 16.7% improved
candidate gene resolution



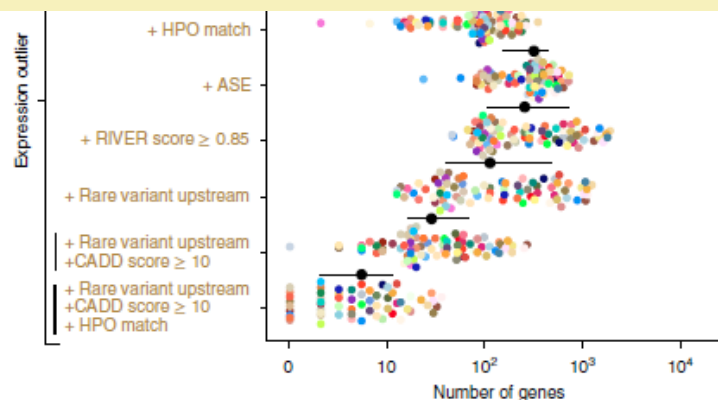
RNAseq control data:

909: Depression Genes and Network (DGN)

65: Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS)

620: Genotype-Tissue Expression Consortium (GTEx vs 7)

Expression
outliers



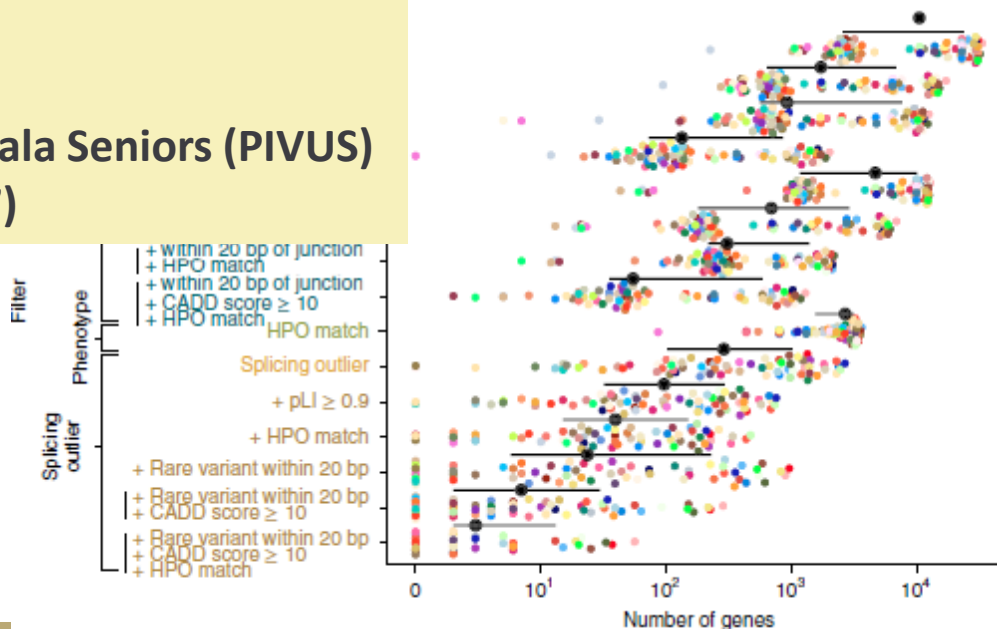
nature
medicine

LETTERS

<https://doi.org/10.1038/s41591-019-0457-8>

Identification of rare-disease genes using blood transcriptome sequencing and large control cohorts

Laure Frésard^{1*}, Craig Smail², Nicole M. Ferraro², Nicole A. Teran³, Xin Li¹, Kevin S. Smith¹, Devon Bonner⁴, Kristin D. Kernohan⁵, Shruti Marwaha^{4,6}, Zachary Zappala³, Brunilda Balliu¹, Joe R. Davis³, Boxiang Liu⁷, Cameron J. Prybol³, Jennefer N. Kohler⁴, Diane B. Zastrow⁴, Chloe M. Reuter⁴, Dianna G. Fisk⁸, Megan E. Grove⁸, Jean M. Davidson⁴, Taila Hartley⁹, Ruchi Joshi⁸, Benjamin J. Strober¹⁰, Sowmithri Utiramerur⁸, Undiagnosed Diseases Network¹¹, Care4Rare Canada Consortium¹¹, Lars Lind¹², Erik Ingelsson^{6,13}, Alexis Battle^{10,14}, Gill Bejerano^{15,16,17,18}, Jonathan A. Bernstein¹⁶, Euan A. Ashley^{3,4,13}, Kym M. Boycott⁹, Jason D. Merker^{1,8,19}, Matthew T. Wheeler^{4,6} and Stephen B. Montgomery^{1,3*}



Splicing
outliers

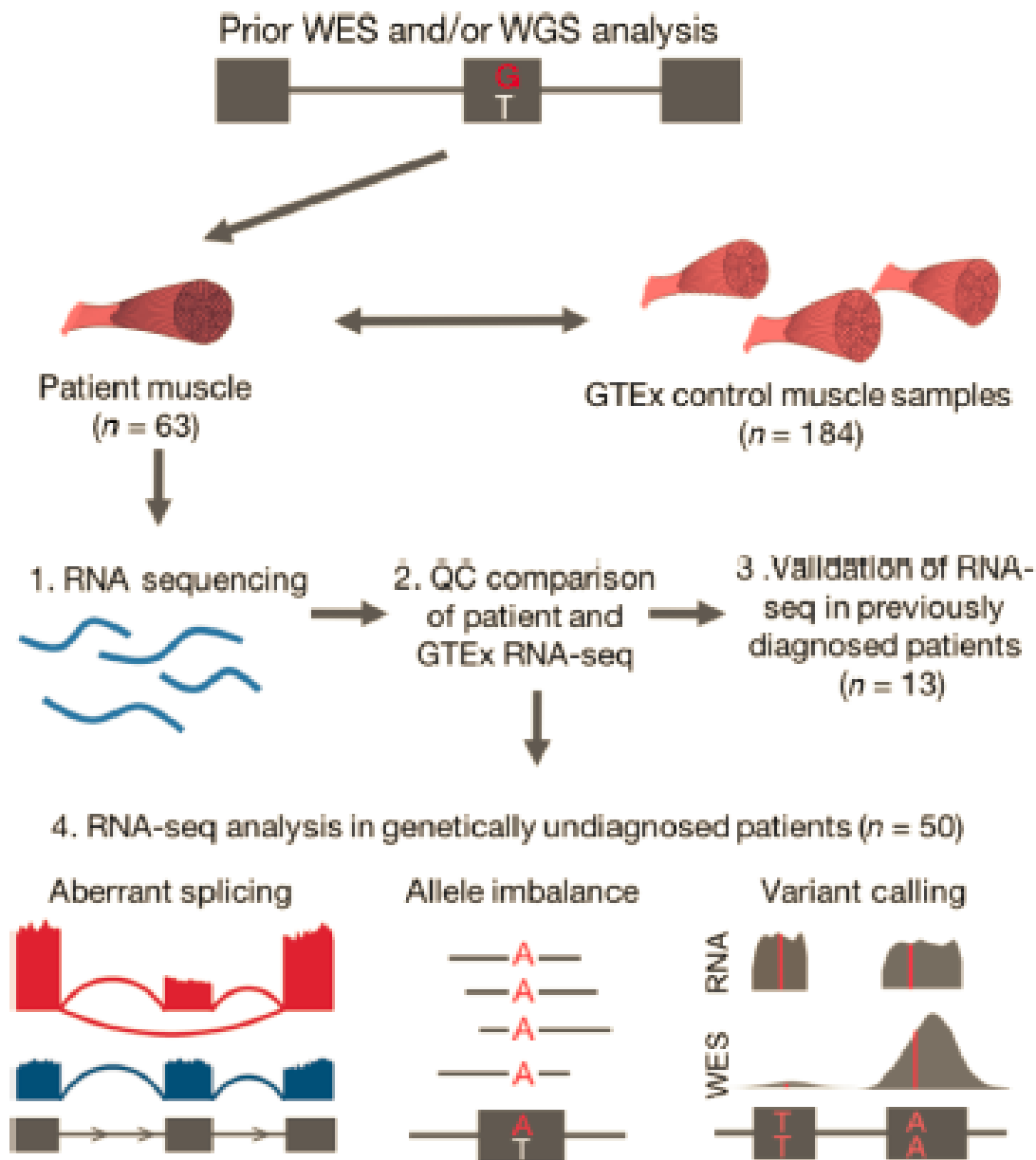
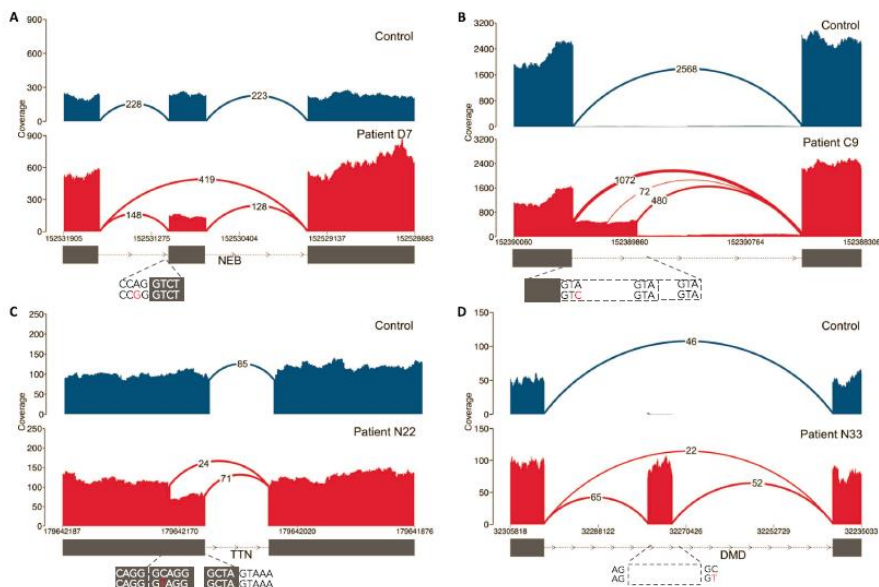
RNAseq in diagnostics

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

GENETIC DIAGNOSIS

Improving genetic diagnosis in Mendelian disease with transcriptome sequencing

Beryl B. Cummings,^{1,2,3} Jamie L. Marshall,^{1,2} Taru Tukiainen,^{1,2} Monkol Lek,^{1,2,4,5} Sandra Donkervoort,⁶ A. Reghan Foley,⁶ Veronique Bolduc,⁶ Leigh B. Waddell,^{4,5} Sarah A. Sandaradura,^{4,5} Gina L. O'Grady,^{4,5} Elicia Estrella,⁷ Hemakumar M. Reddy,⁸ Fengmei Zhao,^{1,2} Ben Weisburd,^{1,2} Konrad J. Karczewski,^{1,2} Anne H. O'Donnell-Luria,^{1,2} Daniel Birnbaum,^{1,2} Anna Sarkozy,⁹ Ying Hu,⁶ Herman Gonorazky,¹⁰ Kristl Claeys,¹¹ Himanshu Joshi,⁵ Adam Bournazos,^{4,5} Emily C. Oates,^{4,5} Roula Ghaoui,^{4,5} Mark R. Davis,¹² Nigel G. Laing,^{12,13} Ana Topf,¹⁴ Genotype-Tissue Expression Consortium, Peter B. Kang,^{7,8} Alan H. Beggs,⁷ Kathryn N. North,¹⁵ Volker Straub,¹⁴ James J. Dowling,¹⁰ Francesco Muntoni,⁹ Nigel F. Clarke,^{4,5*} Sandra T. Cooper,^{4,5} Carsten G. Bönnemann,⁶ Daniel G. MacArthur^{1,2†}



RNAseq in diagnostics

RNASeq revealed clear indication of
loss of the second allele



Ataxia patient

WES defined heterozygous frameshift

Mutation of one allele in SIL1

If both alleles affected, it would cause

Marinesco-Sjörgen-Syndrome

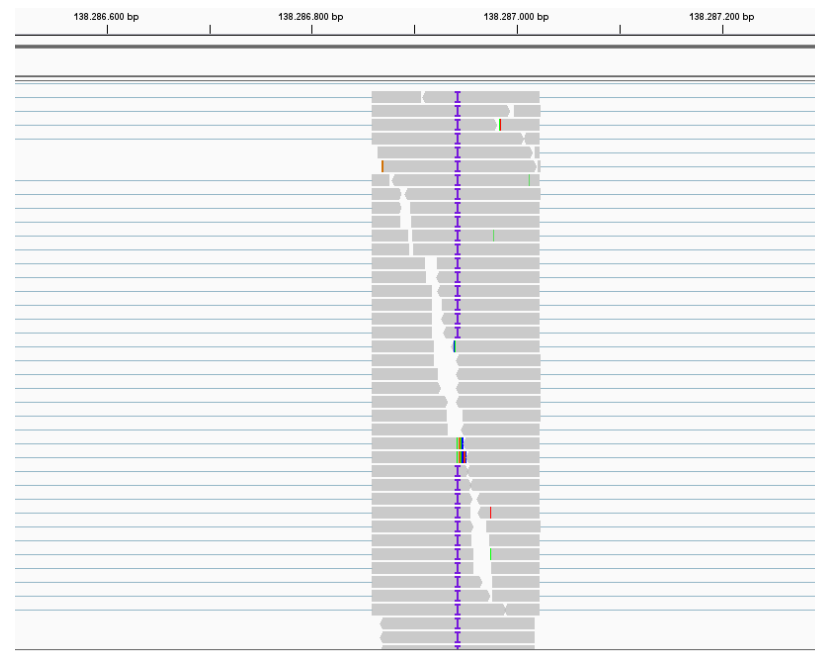
No indication of deletion of the second allele

Phenotype of the patient would fit MSS

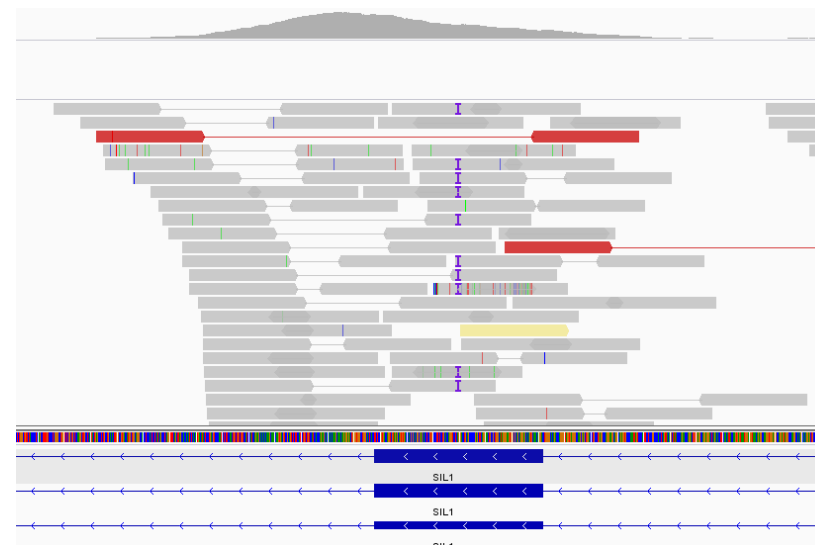
Variant 1 (frameshift)

DX174053_01

RNAseq

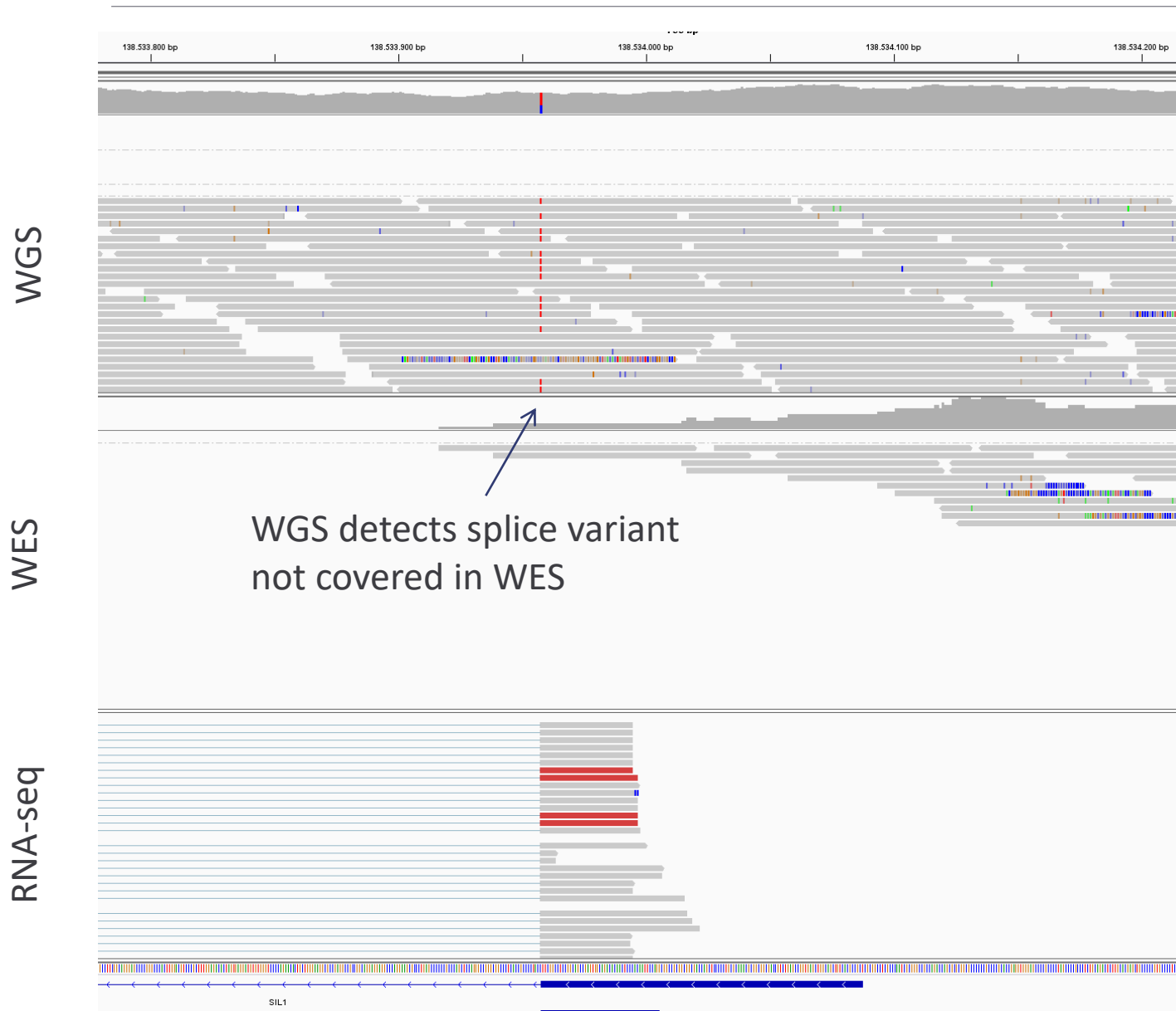


WES



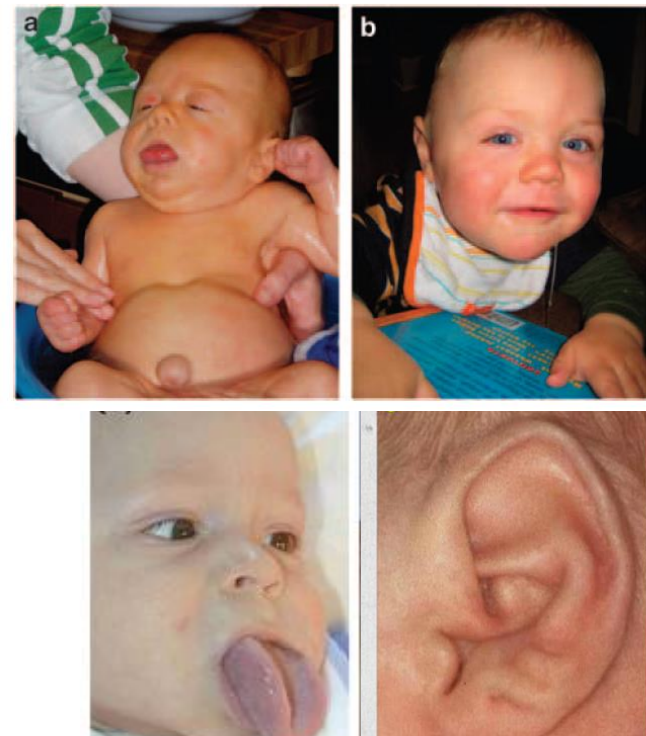
RNAseq in diagnostics

WGS finds Second Hit



The Genome is not „naked“: The Epigenome

Don't forget the epigenome in diagnostics !



Beckwith-Wiedemann-Syndrome (BWS)
Transient Neonatal Diabetes mellitus
Birk-Barel intellectual disability syndrome
Silver-Russel syndrome
Temple syndrome
Kagami-Ogata syndrome
Central Precocious Puberty
Prader-Willi syndrome
Angelman syndrome
Schaaf-Yang syndrome
Mulchandani-Bhoi-Conlin syndrome
Pseudo-hypoparathyroidism

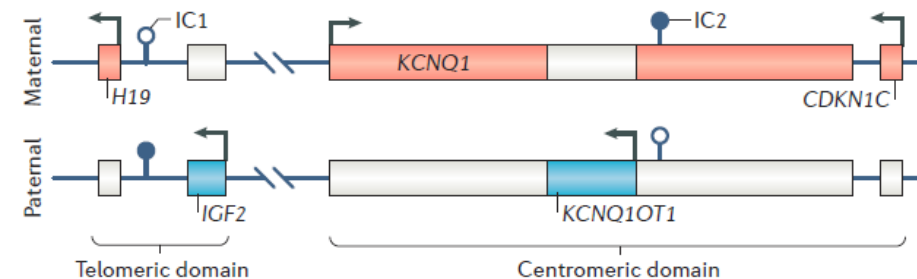
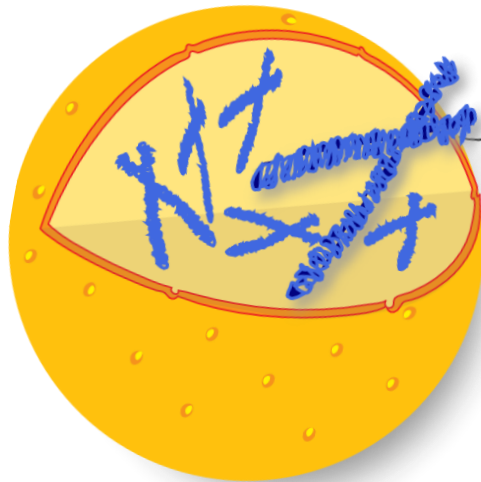


Figure 3 | The Beckwith-Wiedemann syndrome locus at chromosome 11p15.5.

Epigenome Profiling using NGS

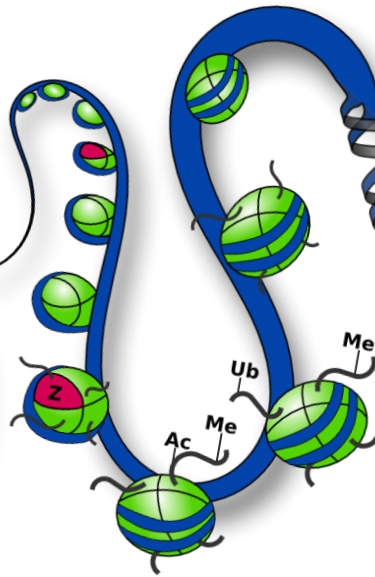
Chromatin

- structure
- domains
- accessibility



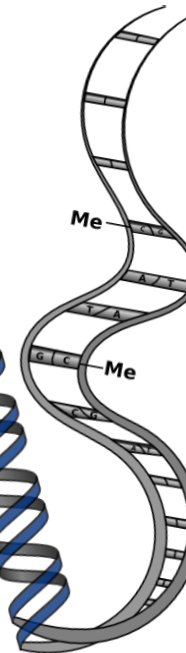
Histone

- modifications
- variants



DNA

- modifications



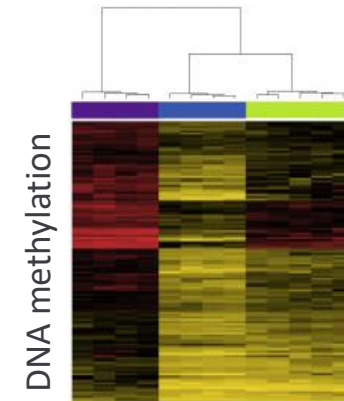
3C, 4C, 5C, Hi-C,
ChIA-PET, DNase-seq,
FAIRE-seq, ATAC-seq

ChIP-seq,
Mnase-seq

WGBS, RRBS,
MeDIP-seq,
Methyl-seq

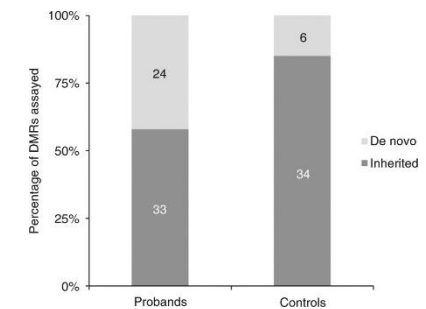
Rare Diseases

Episignatures



Erfan Aref-Eshghi *et al* 2020

Epivariants



Mafalda Barbosa *et al* 2018

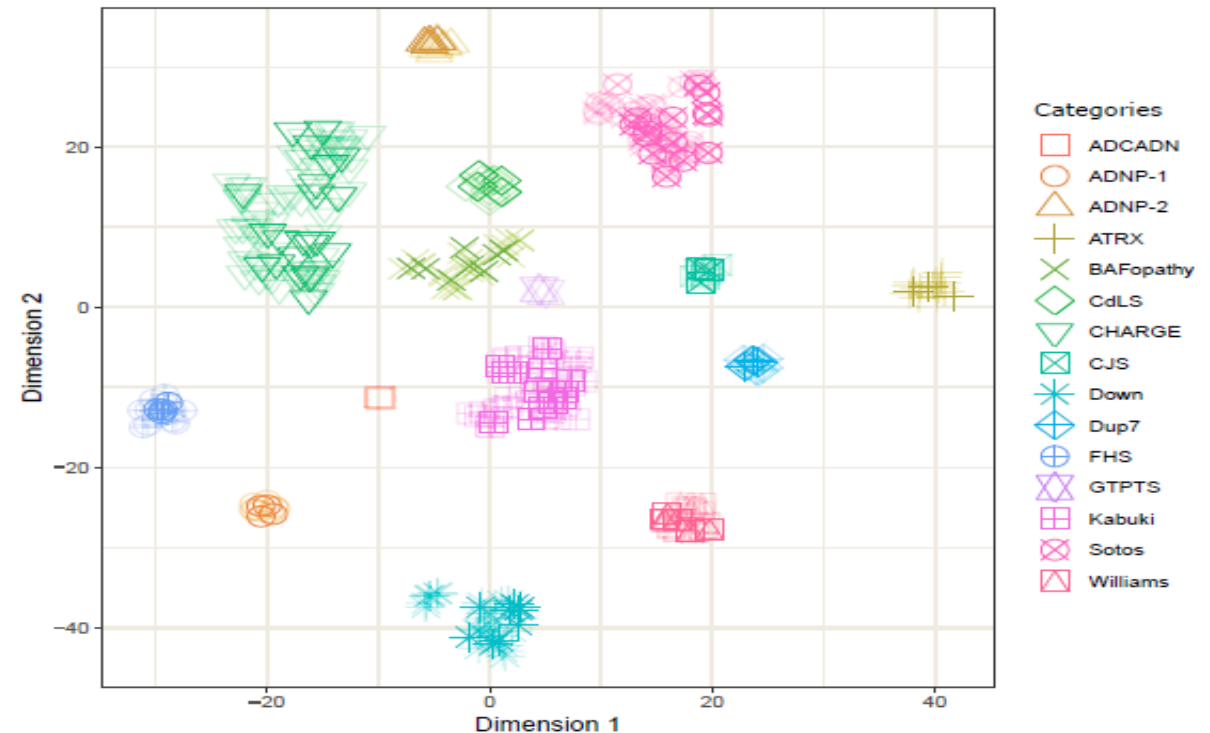
Methylation-sensitive arrays for detection of epigenomic disease specific profiles

Diagnostic Utility of Genome-wide DNA Methylation Testing in Genetically Unsolved Individuals with Suspected Hereditary Conditions

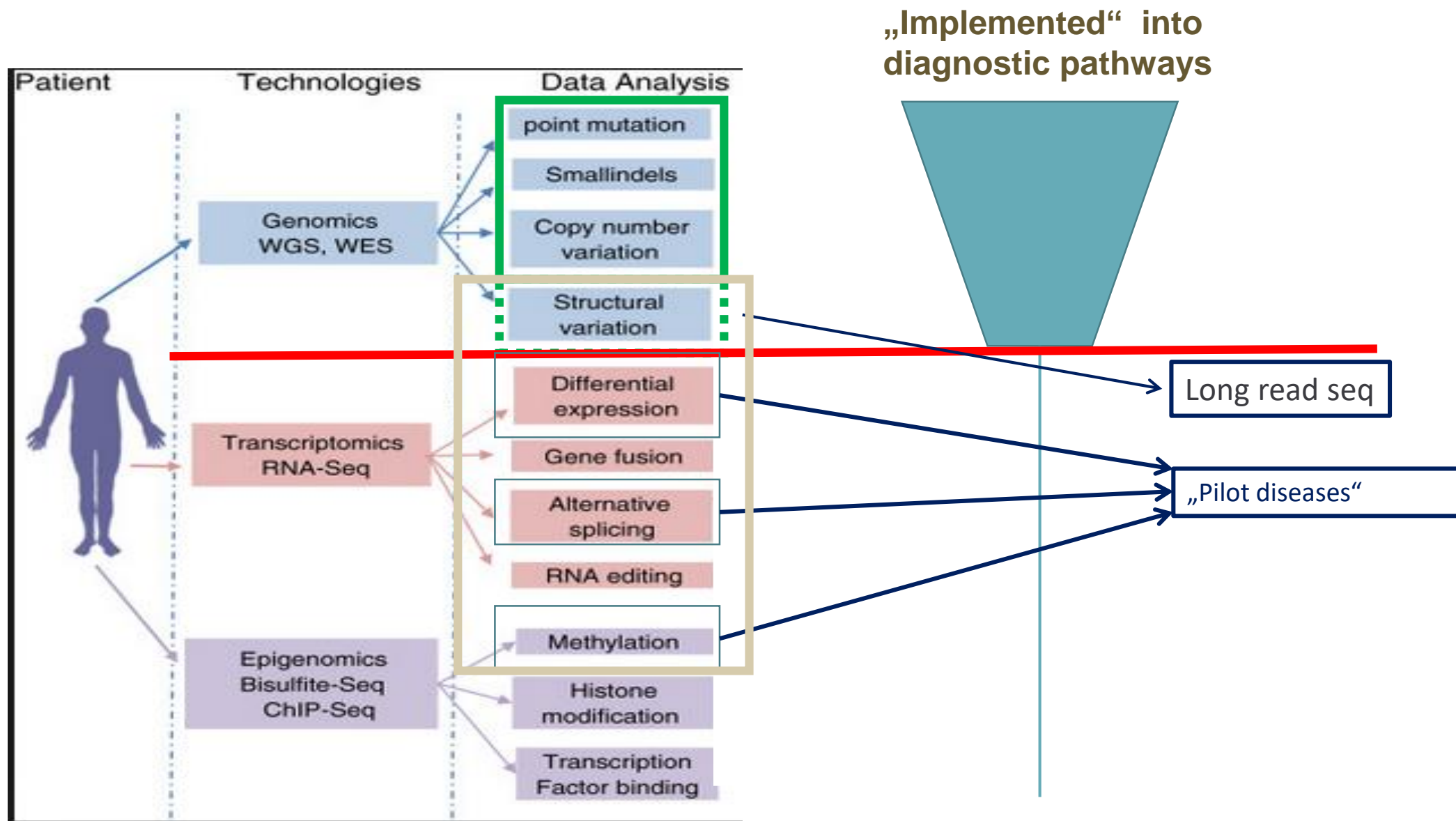
Erfan Aref-Eshghi,^{1,2} Eric G. Bend,³ Samantha Colaiacovo,⁴ Michelle Caudle,⁴ Rana Chakrabarti,⁴ Melanie Napier,⁴ Lauren Brick,⁵ Lauren Brady,⁵ Deanna Alexis Carere,² Michael A. Levy,^{1,2} Jennifer Kerkhof,² Alan Stuart,² Maha Saleh,⁴ Arthur L. Beaudet,⁶ Chumei Li,⁵ Maryia Kozenko,⁵ Natalya Karp,⁴ Chitra Prasad,⁴ Victoria Mok Siu,⁴ Mark A. Tarnopolsky,⁵ Peter J. Ainsworth,^{1,2} Hanxin Lin,^{1,2} David I. Rodenhiser,⁷ Ian D. Krantz,⁸ Matthew A. Deardorff,⁸ Charles E. Schwartz,³ and Bekim Sadikovic^{1,2,*}

MethBank 450K Array > 5000 controls

MethSeq control data:
Epigenie data base:



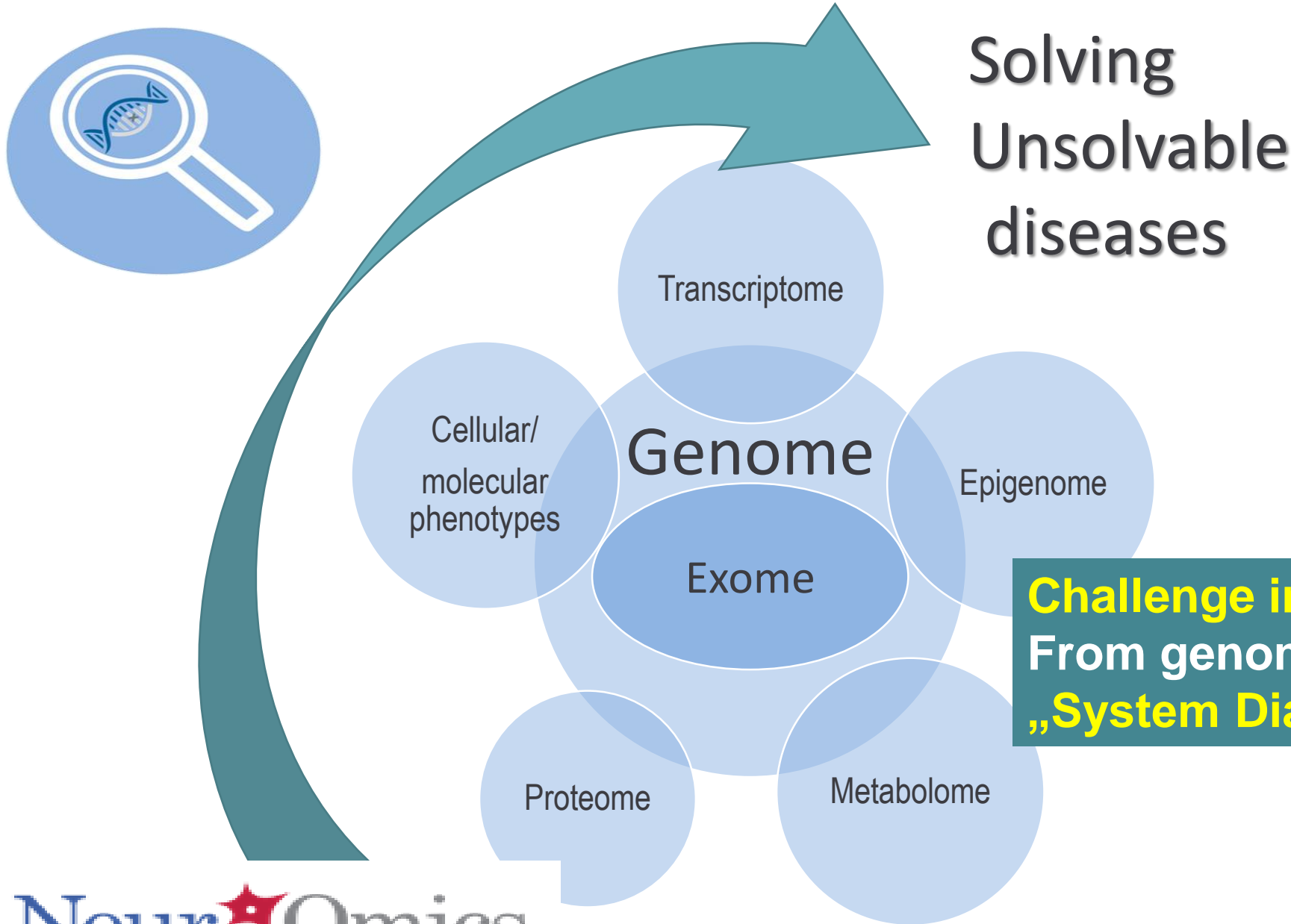
Technical hurdles in diagnostics



SolveORD



Solving Unsolvable diseases

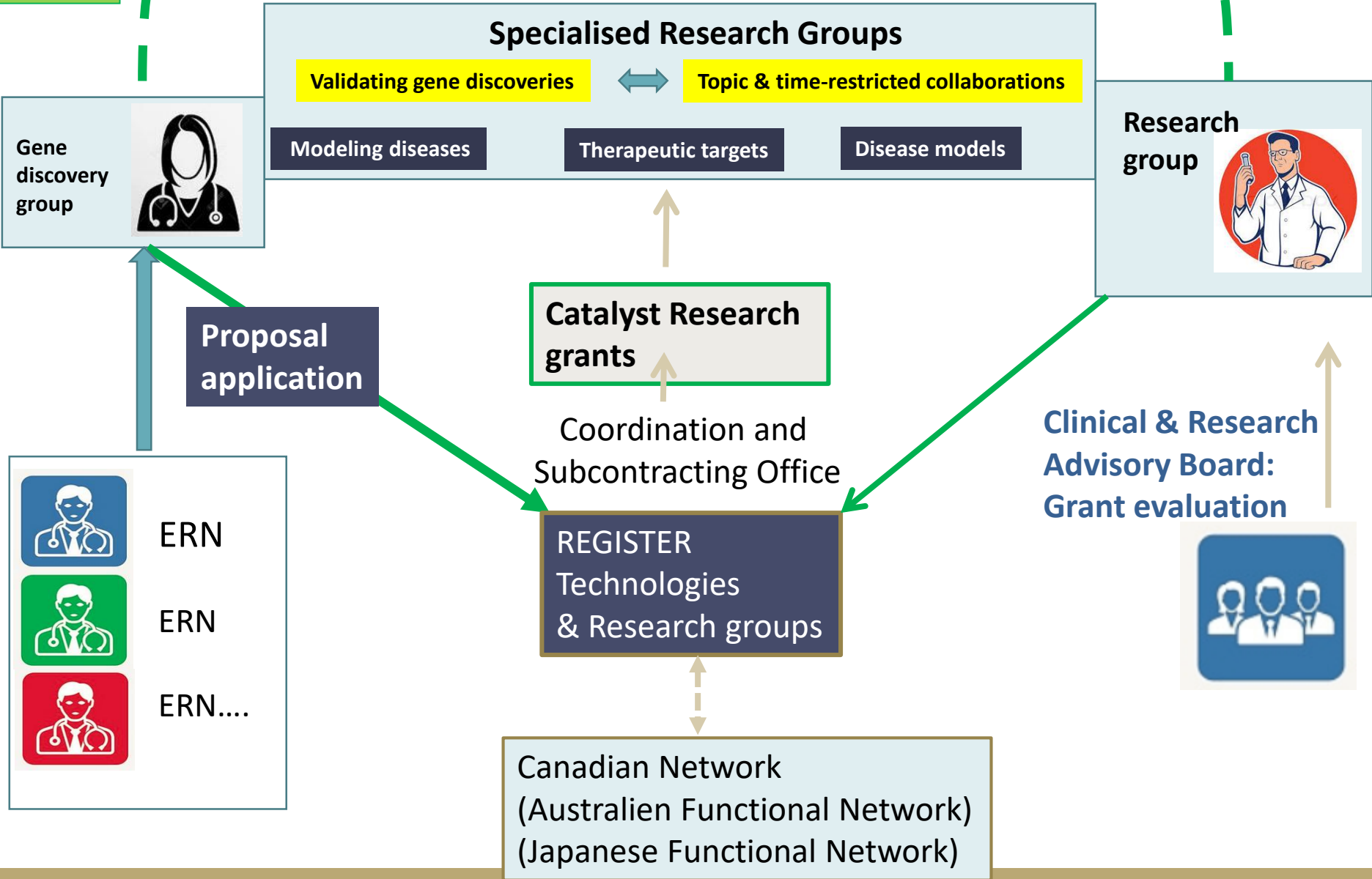


Challenge in Diagnostic Transition:
From genome analysis towards
„System Diagnostics“

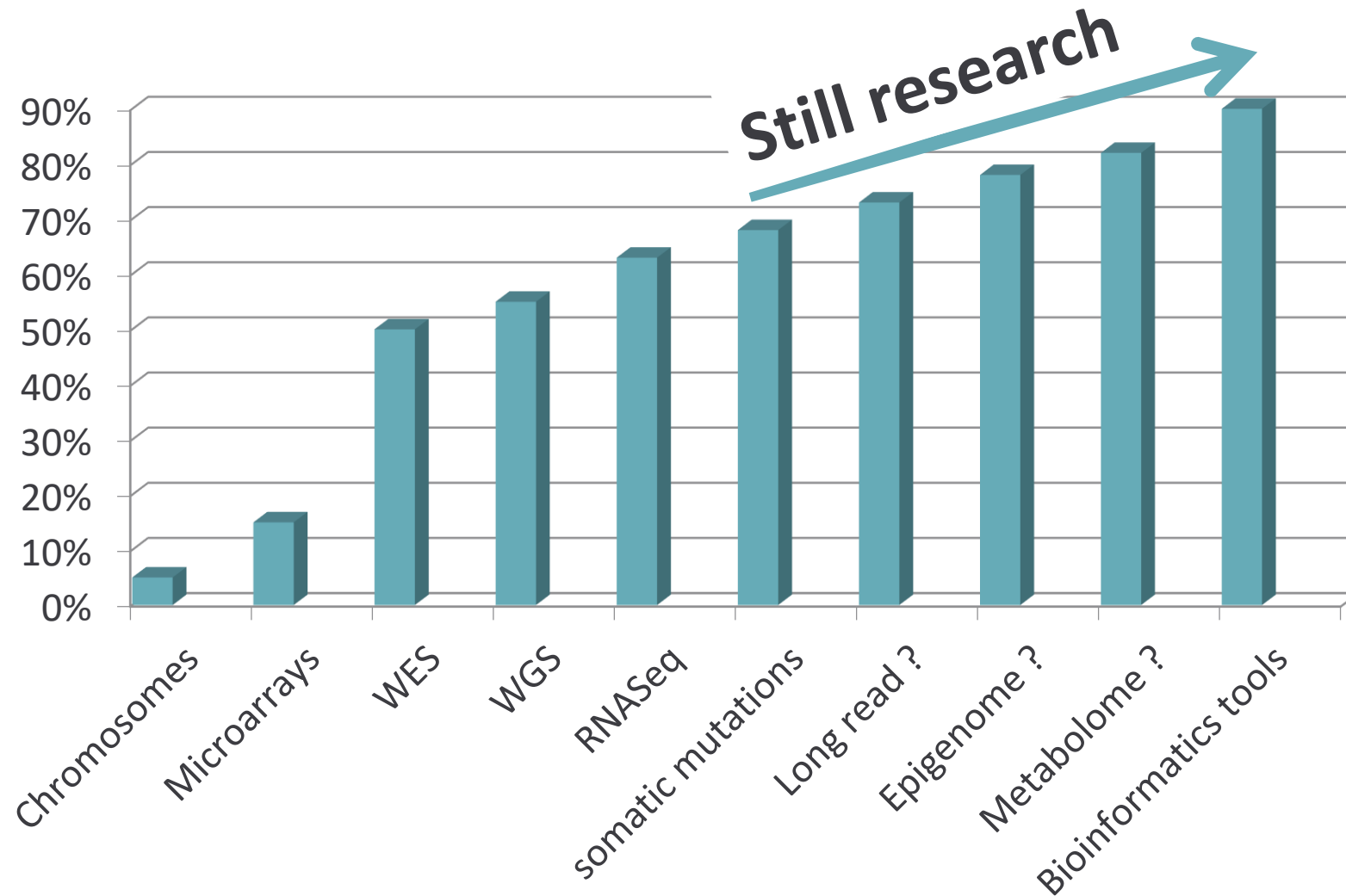
- Re-analysis of **19.000** exomes of unsolved cases
- **800 ultra-rare** RD patients presenting new phenotypes that will undergo WES/WGS
- **WGS for 2.000 cases** to achieve a more complete coding sequence
- **Novel omics approaches** (transcriptome, epigenome, proteome, metabolome, deep WES, deep molecular phenotyping) for more than **2.000 cases**
- **Long-read genomes for 500 cases** with smartly chosen phenotypes such as anticipated repeat expansion disorders (SBMA; DM1 and DM2, disorders with phenotype anticipation)
- **Multi-Omics approaches for 120 „unsolvable syndromes“**

**50 projects –
20.000€ each**

Innovative and internationally interoperable small funding service
RDMM Europe



Diagnostic yield of genetic technologies in rare diseases



Cave: 80% of all RD have a clear genetic cause

Conclusions

1. Patient cohort of „Undiagnosed diseases“ should be **subgrouped** to define different diagnostic follow-up strategies
2. Development and availability of **novel Omics technologies will contribute** to deciphering unsolved and unsolvable diseases
3. Next steps in Omics diagnostics (**system diagnostics**) will be a challenge and require further strong support of research consortia
4. Implementing Machine learning and AI algorithms will greatly improve diagnostic work up
5. Whereas WES/WGS are more „global“ approaches of entire patient cohorts, next steps in terms of technology, methods, and analytics will be more patient **individualized and require time**
6. Special functional attention should be on **solving VUS**





Olaf Riess

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

www.ncct.life



 **Institut für
Medizinische Genetik und
Angewandte Genomik**



NGS Competence Center Tübingen

**Deutschland
Land der Ideen**

Ausgewählter Ort 2011