



Solving the unsolved Rare Diseases

How Genomics solves undiagnosed patients

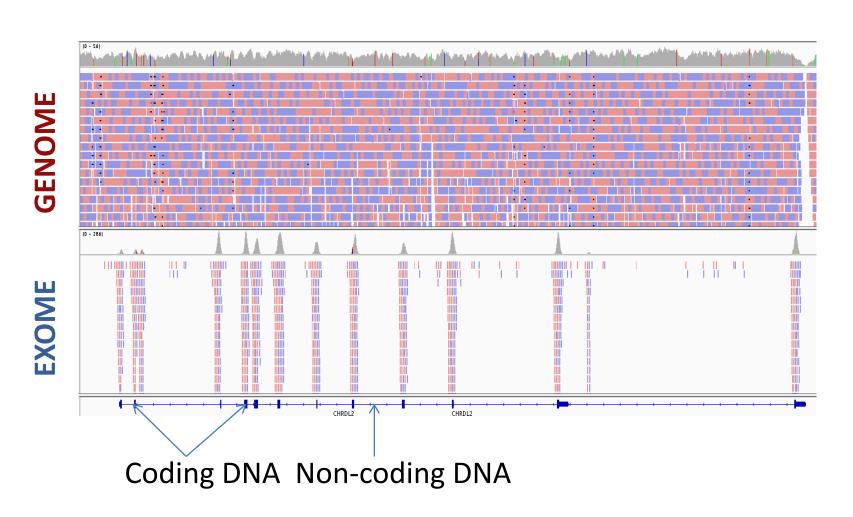
This project receives funding from the European Union's Horizon 2020 research and innovation programme

Start date: January 2018, Duration: 5 years

Conflict of interest:

I declare no potential conflict of interest in relation to this presentation.

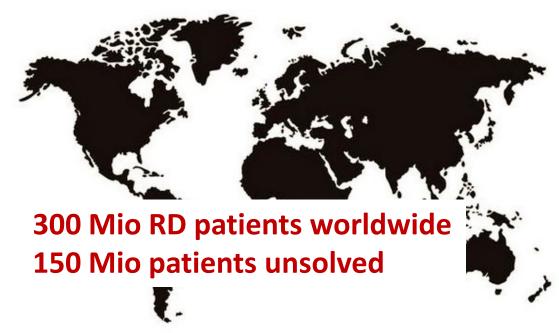
SOLVE-RD: Beyond the exome

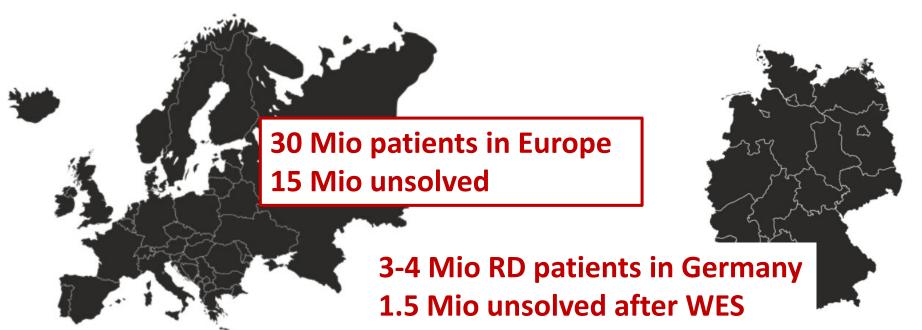


The exome is only 1-2% of our entire genome!

UNSOLVED after WES:

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





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%

Ausgewertste Gene (1884): A2ML1, AACS, AARS, AASS, ABAT, ABCA13, ABCA2, ABCC8, ABCC9, ABCD1, ABCD4, ABHD5, ABIZ, ABIL1, ACACA, ACACB, ACACB, ACADB, ACADB, ACBD6, ACBD6, ACER3, ACOZ, ACOX1, ACOX2, ACP5, ACSF3, ACSI.4 ACTR. ACTG1, ACTURA, ACTURA, ACTURA, ACTURA, ACTURA, ACCURA, ADAMEZ, ADAMES, ADARR, ADATS, ADCYS, ADGRIGI, ADGR ADK, ADNP, ADRA2B, ADSL, AFF2, AFF3, AFF4, AFG3L2, AGA, AGK, AGMO, AGO1, AGPAT2, AGP8, AGTR2, AHLV1, AHLV1, AH1, AHSG, AIFM1, AIMP1, AIP, AK1, AKT3, ALDH18A1, ALDH1A3, ALDH3A2, ALDH4A1, ALDH5A1, ALDH7A1, ALDDA ALG1, ALG11, ALG12, ALG13, ALG2, ALG3, ALG6, ALG8, ALGR, ALMS1, ALS2, AMER2, AMMECR1, AMPD2, AMT, AM22 ANAPC2, ANK2, ANK3, ANKH, ANKLE2, ANKRD11, ANO10, ANO5, AP1\$1, AP1\$2, AP3B1, AP3B2, AP4B1, AP4E1, AP4M1 AP481, APUZ, APUPTI, APP, APPLA, APTX, ARCLI, ARTGERS, ARRS, ARIGAPSS, ARROADSS, ARROADSS, ARROADSS ARHGEFS, ARHGEFS, ARID1A, ARID1B, ARID2, ARIH1, ARL13B, ARL14EP, ARLB, ARMCS, ARSA, ARSE, ARV1, ARX ASAH1, ASCC1, ASCC3, ASCL1, ASH1L, ASL, ASNS, ASPA, ASPM, ASS1, ASTNO, ADXL1, ADXL2, A ATFIR ATIC, ATM, ATM1, ATCH1, ATP100, ATP1A2, ATP1A3, ATP1B1, ATP2A1, ATP2A2, ATP2B3, ATP2C2, ATPSJ, ATP6AP2,

ATPEVOA2, ATPEV182, ATP7A, ATP8A2, ATPAI B4GALT1, B4GALT7, B9D2, BAZ1A, BBIP1, BB BCKDHA, RCKDHB, BCL11A, BCOR, BCORL1 BRAT1, BRD3, BRF1, BRPF1, BRSK2, BRWD1 C120RF67, C120RF65, C150RF38-AP382, C CA2, CA5A, CA8, CACNA1A, CACNA1C, CAC GAGNOS, CAD, CADDOS, CAMESA, CAMESA CBS, CBX4, CC2D1A, CC2D2A, CCAR1, CCB CCNA2, CCND2, CCNL2, CCNT2, CDC42BPB CDK5RAP2, CDK6, CDK9, CDKL5, CDKN2AIP CEP152, CEP19, CEP290, CEP41, CEP55, CE CHD2, CHD3, CHD4, CHD5, CHD7, CHD8, CH CKAP2L, CLCN4, CLHC1, CLIC2, CLIP1, CLM CNNM2, CNOT3, CNOT4, CNPY3, CNTN4, CN COG6, COG7, COG8, COL18A1, COL3A1, CO COX14, COX15, COX20, COX5A, DUXBB1, UX CRYL1, CSDE1, CSNK2A1, CSPP1, CSTB, CS CTSA, CTSD, CTTNBP2, CUL3, CUL4B, CUX DARS, DARSZ, DBT, DCAF17, DCC, DCHS1 DEAF1, DENNOSA, DEPOCS, DHCR24, DHCR

DHC1, DLAT, DLC1, DLD, DLG2, DLG3, DLG4 DNAJC12, DNAJC19, DNAJC6, DNHD1, DNM1, DNM1L, DNM3 DOLK, DONSON, DOPEY1, DPAGT1, DPH1, DPM1, DPM2, DP DYNC1H1, DYRK1A, EARS2, EBF3, EBP, ECHS1, ECM2, EDC EIF2AK3, EIF2S3, EIF4A2, EIF4G1, ELAC2, ELMO2, ELOVL4, EOGT, EP300, EP841L1, EP841L4A, EPG5, EPH82, EPH86, E ERCC8, ERF, ERLIN2, ERMARD, ESCO2, ETHE1, ETV1, EXC EZR. FA2H, FAAH2, FAM120AOS, FAM126A, FAM177A1, FAM2 FANCD2, FANCE, FANCF, FANCG, FANCL, FANCM, FAR1, FA FBXO31, FBXO47, FBXW4, FCRL6, FDPS, FDXR, FERMT1, F FKRP, FKTN, FLG, FLNA, FLVCR1, FLVCR2, FMN2, FMOD, FM

Limitations of WES:

Coverage Copy number **Aberrant splicing**

TMEMS, TMEMS, TMEMSO, TMEMSSB, TMEMS2, TMEMS4, TMTC3, TNIK, TNKS, TNPO2, TNPO3, TNR, TNROSB, TNS3, TOE1, TOP28, TOP38, TOR1A, TP53TG5, TP11, TP0, TPP1, THAIP; TRAP1, TRAPPC11, TRAPPC12, TRAPPC68, TRAPPC9. IBEX1, TRIM17, TRIM32, TRIM36, TRIM37, TRIM47, TRIM8, TRIO, TRIP12, TRIP4, TRIT1, TRMT1, TRMT10A, TRMT10C, TROVEZ TRIPMS, INPST, IRRUP, ISCI, TOCK, TOCKIO, TREND, TESMA, TOCKIA, TOCKIA, TOCKIA, TOCKIA, TOCKIA, TOCKIA, TSPAN7, TTC19, TTC5, TTC6, TTI2, TTN, TUB, TUBA1A, TUBA3E, TUBA8, TUBAL3, TUBB, TUBB2A, TUBB2B, TUBB3 TUBB4A, TUBG1, TUBGCP4, TUBGCP6, TUSC3, TWIST1, TWNK, U2AF2, UBA5, UBA7, UBE2A, UBE2A, UBE3A, UBE3B, IBE3C, UBE4A, UBN2, UBR1, UBR4, UBR7, UBTD2, UBTF, UFM1, UMPS, UNC80, UPB1, UPF1, UPF38, UQCRQ, UROC1 OSP16, USP278, USP44, USP1, USP4K, WOLLA, WHILL, YER, YERLE, VIRSH, VIRS WWA3B, WAC, WAR82, WASHC4, WASHC5, WDFY3, WDPCP, WDR13, WDR26, WDR45, WDR45B, WDR62, WDR73, WDR81, WDR83, WFDC1, WFS1, WNT1, WWOX, WWP2, XIRP1, XPA, XPNPEP2, XPCT, XRCC4, XYLT2, YARS, YME1L1, YWRAG, YWRAZ, YY1, YY1AP1, ZBTB11, ZBTB18, ZBTR18, ZBTB20, ZBTB40, ZC3H14, ZC3H4, ZC4H2, ZCCHC8, ZDHHC15, ZDHHC9, ZEB2, ZFAND2B, ZFHX4, ZFYVE26, ZIC2, ZMYM3, ZMYM5, ZMYM6, ZMYND11, ZNF148, ZNF292, ZNE335, ZNF41, ZNF420, ZNF526, ZNF528, ZNF589, ZNF582, ZNF599, ZNF574, ZNF711, ZNF713, ZNF81, ZSCAN25, ZSWIM6, ZSWIM8

FOXRED1, FRAS1, FREM2, FREM3, FRMD4A, FRMPD4, FRRS1L, FRY, FSCN1, FTL, FTO, FTSJ1, FUCA1, G6PD, GABBR2 GABRA1, GABRA3, GABRB1, GABRB2, GABRB3, GABRG2, GAD1, GALC, GALE, GALNT18, GALNT2, GALT, GANT, GAN, GATAD2B, GATM, GBA2, GCC2, GCDH, GCH1, GCN1, GCSH, GDI1, GEMINA, GFAP, GFM1, GFPT2, GGN, GIGYF1, GIGYF2, GIMAPS, GJA1, GJC2, GK, GLB1, GLDC, GLI2, GLI3, GLRA2, GLS, GLUL, GLYCTK, GM2A, GMNN, GMPPA, GMPPB, GNAI1 GNAQ, GNAO1, GNAQ, GNAS, GNB1, GNB5, GNE, GNPAT, GNPTAB, GNPTG, GNS, GON4L, GPAA1, GPC3, GPHN, GPI, GPMSA, GPR138, GPR37, GPR52, GPS1, GPSM2, GPT2, GRAMD1B, GRIA1, GRIA2, GRIA3, GRID2, GRIK2, GRIN1, GRIN2A GRIN2B, GRIN2D, GRM1, GRM7, GSS, GSTT1, GTF2E2, GTF2H5, GTF3C3, GTPBP3, GUCY2D, GUF1, GUSB, HACE1, HACL1, HAX1, HCCS, HCFC1, HCN1, HDAC4, HDAC6, HDAC6, HECW2, HEMK1, HEPACAM, HERC1, HERC2, HESX1 HEXA, HEXB, HGSNAT, HHAT, HIBCH, HIKESHI, HIST1H1E, HIST1H4B, HIST1H4C, HIST3H3, HIVEP2, HIVEP3, HK1, HLCS HMBS, HMCN2, HMG20A, HMGBS, HMGCL, HMGCLL1, HNF1B, HNF4A, HNMT, HNRNPH1, HNRNPH2, HNRNPK, HNRNPI HNRNPU, HCXA1, HPD, HPRT1, HRAS, HSD17B10, HSD17B4, HSPD1, HSPG2, HTR7, HTRA2, HTT, HUWE1, HYLS1, IARS, BA57, ICE2, IDH2, IDH3A, IDS, IDUA, IER3IP1, IFIH1, IFT172, IFT27, IFT57, IGBP1, IGF1, IKBKG, IL1R2, IL1RAPL1, ILF2, ILF3, IMPA1, INIP, INO80, INPP4A, INPP5E, INPP5K, INTS1, INTS13, INTS8, INTU, IPP, IQSEC2, IRX5, ISCA2, ISPD, ITCH ITGA7, ITGAV, ITIH6, ITPA, ITPR1, ITSN1, IVD, JAG1, JAK2, JAM3, KALRN, KANK1, KANSL1, KAT6A, KAT6B, KATNAL2, KATNB1, KCNA2, KCNA4, KCNB1, KCNC1, KCNC3, KCND3, KCNH1, KCNJ10, KCNJ11, KCNJ8, KCNK8, KCNK9, KCNMA1 KCNQ2, KCNQ3, KCNQ5, KCNT1, KCNT2, KCNV1, KCTD18, KCTD3, KCTD7, KDM1A, KDM5A, KDM5B, KDM5C, KDM6A KOM6B, KIAA0232, KIAA0556, KIAA0586, KIAA0753, KIAA1109, KIAA1217, KIDINS220, KIF11, KIF14, KIF16, KIF1A, KIF18F KIF26A, KIF2A, KIF4A, KIF6C, KIF7, KIRREL3, KLHL15, KLHL33, KLHL40, KMT2A, KMT2B, KMT2C, KMT2D, KMT2E, KMT5B KNL1, KPNA7, KPTN, KRAS, KRBOX4, KY, L1CAM, L2HGDH, LAGE3, LAMA1, LAMA2, LAMA5, LAMB1, LAMC1, LAMC3,

N2L LMNB2, LMTK3, LONP1, LRCH3, LRP1 LZTR1, MARSILI, MARSILS, MADO, MAR VBA, MAOA, MAP2, MAP2K1, MAP2K2, IN4. MBD5. MBNL3. MBOAT7, MBTPS2 MDM2_MECP2_MECR_MED12_MED13L ETTL4. METTL5. MFF, MFRP, MFSD2A LC1, MILIT3, MLYCD, MMAA, MMAB, ICI, MCMSC MCY17, MOSHI, MRPI MT-CO2, MT-CO3, MT-ND5, MT-TE, MT-TK MIRR, MISSIL, MUCSA, MUT, MVK, MYCH, NACC1, NAGA, NAGILU, NAGS, NALCN, NOARH NOKAPI NOKAPI NOORI NDEI. DUFAF1, NDUFAF2, NDUFAF3, NDUFAF5. S4. NDUFS8. NDUFS7, NDUFS8, NDUFV1. EXMIE NET. NETA. NEIX. NEUT. NEXLT. LGN3, NLGN4X, NLRP3, NLRP4, NME1, NME7 UZ. NRAS, NRG3, NRXXN1, NPXXN2, NBD1 N1, OR2A12, ORC1, OTC, OTUDS, OTUDSE SQ. PAX1, PAX5, PAX6, PAX7, PAX8, PC, PCOLCE, PDCD1, PDE10A, PDE4D, PDE4DIP ET100, PET117, PEX1, PEX10, PEX11B. X7, PGA5, PGAM5, PGAP1, PGAP2, PGAP3, FILEFF, FLICKED, FIRMS, FIRBS, FIEBER, K3R1, PIK3R2, PIP6K1A, PLA2G6, PLAA,

OX, PPP1CB, PPP1R15B, PPP1R35, PPP2R1A, PPP2R5B, PPP2R5C, PPP2R5D, PPP3CA PRIMAT, PRIMATUS, PRICOS, PRICOT, FRUGA, FRUGA, FRUTTY, FRUTTY, FRUSANT, FR T2. PRRX1, PRSS12, PRUNE1, PSAP, PSMA7, PSMD12, PSPH, PTCH1, PTCHD1, PTDSS1 PTPN11, PTPN23, PTPRK, PTPRT, PTRH2, PTRHD1, PTS, PUF60, PUM2, PURA, PUS1, PUS3 QDPR, QRFPR, QRICH1, RAB11A, RAB11B, RAB18, RAB23, RAB27A, RAB2A, RAB39B, AUAL, FURBUS DI, FARBLO, RADI, RADEI, RADEILI, RABGO, RACI, RAVI, RALGOS, RAMOS RBBP8, RBFOX1, RBM28, RBMS3, RBPJ, RCBTB1, RDH11, REEP1, RELN, RERE, RFT1 HOSETEZ, RIMST, RINZ, RIPPLYT, RITT, RUM, RWINDT, RWINF, RIMOCTION, RIMOGERA 13A, RNF125, RNF135, RNF158, RNF38, RNF5, RNFT2, RNU12, RNU4ATAC, ROCK2, ROGD PS6KA3, RRM2B, RSPRY1, RTEL1, RTN4IP1, RTTN, RUSC2, RXRB, RYR3, SALL1, SAMD9, ISS, SATRZ SBF1, SCSD, SCAPER, SCHIP1, SCN1DA, SCN1A, SCN1B, SCNZA, SCNBA, SCO1 1, SOCCAGS, SDHA, SDHAP1, SDHB, SURZ, SEUZJIP, SEUZJU, SEUBJU1, BENADE, OCHAGO SET, SETBP1, SETD1A, SETD1B, SETD2, SETD5, SF1, SFXNA, SGMS1, SGPL1, SGSH, SHANKS SHH SHOCZ SHROOMA SKI, SILI, SINJA, SIX3, SKAI, SKI, SKIDAI, SLAINI, 8, SLC13A5, SLC16A2, SLC17A5, SLC18A3, SLC18A1, SLUTAZ, SLUTAA, SLUZBATZ, 1410, ELCOMADO SI CORADO SLC25A23, SLC25A24, SLC25A26, SLC25A36, SLC25A44, 5, SLC30A9, SLC31A1, SLC33A1, SLC35A1, SLC35A2, SLC35A3, SLC35C1, SLC33A1, 1, SLC45A1, SLC46A1, SLC4A4, SLC5A7, SLC5A1, SLC5A17, SLC5A3, SLC6A8, SLC6A9 SMAD4, SMAD6, SMARCA1, SMARCA2, SMARCA4, SMARCB1, SMARCC2, SMARCE1, SMC1A

SMUS, SMOS, SMUST, SMS, SMURF2, SMYD5, SNAP25, SNAP29, SNIP1, SNORD118, SNRPB, SNTG1, SNX14,

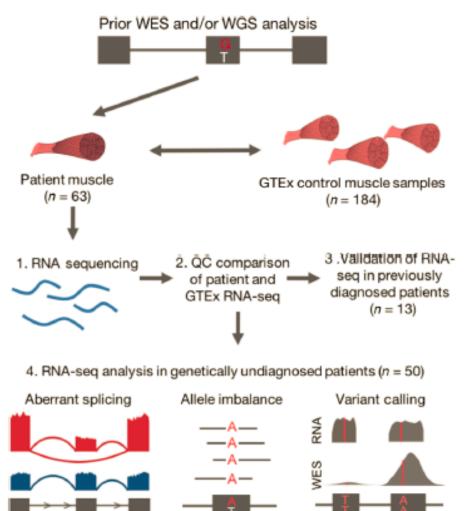
RNAseq in diagnostics

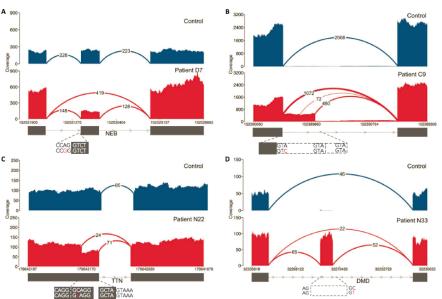
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

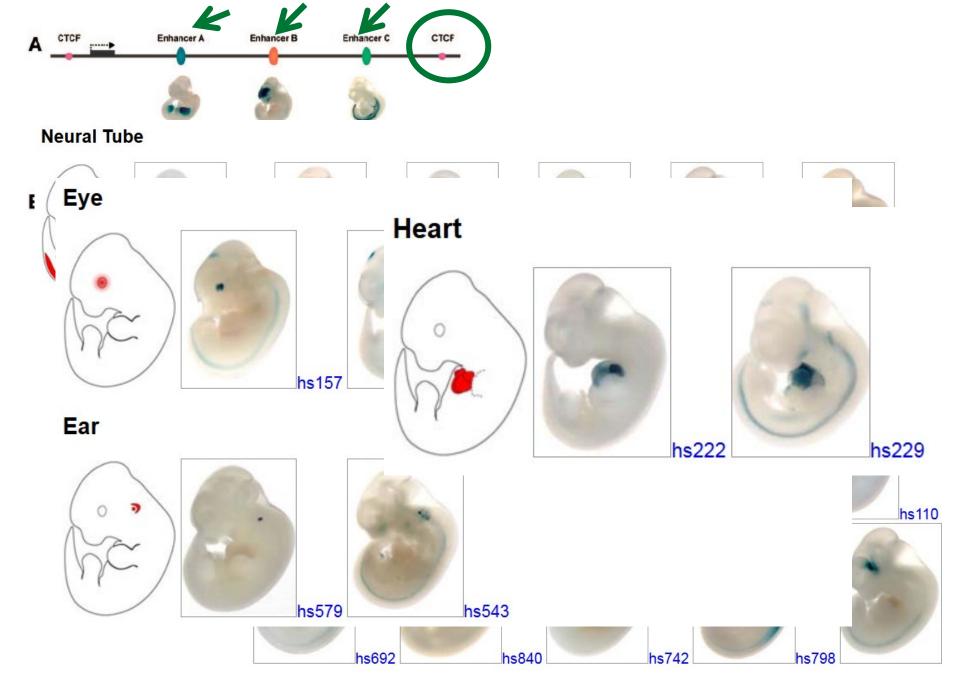
GENETIC DIAGNOSIS

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 Hernan 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†}



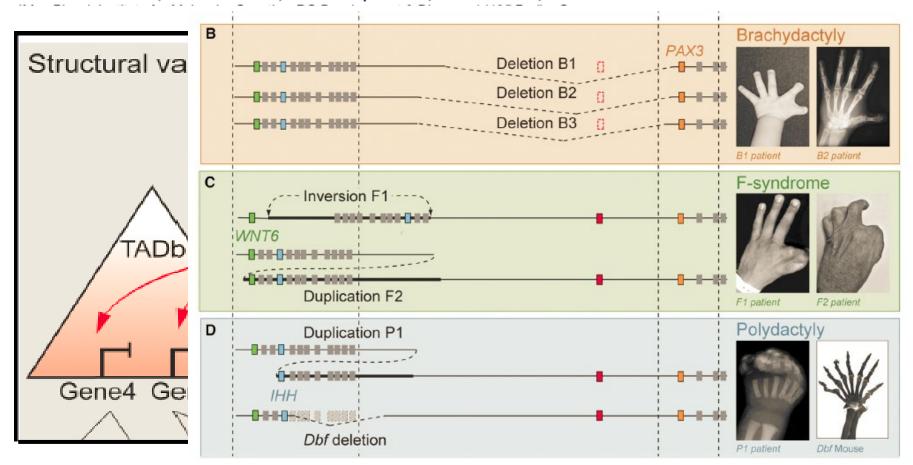




https://enhancer.lbl.gov/gallery_n.html

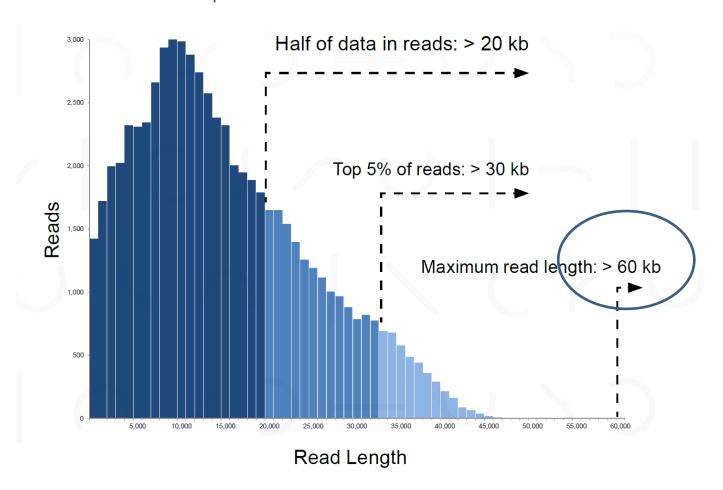
Disruptions of Topological Chromatin Domains Cause Pathogenic Rewiring of Gene-Enhancer Interactions

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,*}



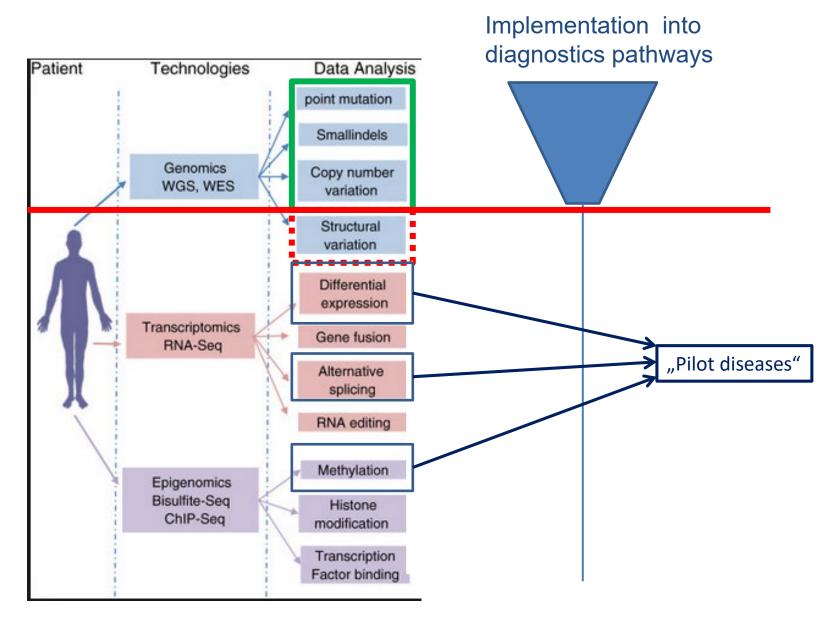
PacBio sequencing reads

Read lengths > 20 kb Data per SMRT Cell: 750 Mb - 1.25 Gb

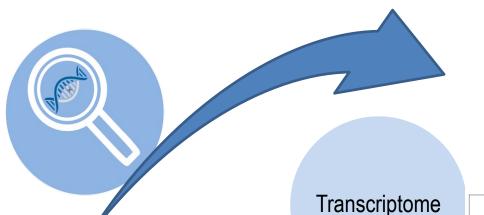


From: http://www.pacificbiosciences.com

Technical hurdles in diagnostics



Technological hurdles in diagnostics



Solve-RD - Solving the unsolved Rare Diseases



European Union funding



Solving the unsolved Rare Diseases

Challenge in Diagnostic Transition: From genome analysis towards "System Diagnostics"

Proteome

Metabolome





"in numbers"

- 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
- Long-read genomes for 500 cases with smartly chosen phenotypes such as anticipated repeat expansion disorders (SBMA; DM1 and DM2)
- Novel omics approaches (transcriptome, epigenome, proteome, metabolome, deep WES, deep molecular phenotyping) for more than 2.000 cases
- Multi-Omics approaches for 120 "unsolvable syndromes"

Main implementation steps

Challenge 2: New and improved approaches for the discovery of novel molecular causes

	inolecular causes					
3	Reanalyse exomes / genomes	 Data mining on the variants and regions detected with Solve-RD standard analysis pipelines Approaches: (i) a data driven approach, (ii) an expert driven approach. 				
4	molecular	 → Solve unsolved diseases from unique RD cohorts provided by 4 ERNs with unique phenotypes applying novel (multi-) omics tools → Solve ultra-rare diseases presenting with novel phenotypes by holding phenotype-jamborees' → ,Solve the unsolvable syndromes' with joined power of clinical ERN and genomics experts applying all available latest romics tools 				
5	Functional analysis	 → Validate up to 50 novel candidate genes identified by a resequencing those in even larger cohorts of relevant clinical samples (n=5,000) → Implement an innovative brokerage system which allows gene/model/pathway experts to verify pathogenicity of new genes or new disease mechanisms quickly 				

Resources and infrastructures

Core group of 4 European Reference Networks: ERN-RND,

ERN-EURO-NMD, ERN-ITHACA, ERN-GENTURIS

Associated networks: 6 additional ERNs and 2 Undiagnosed Patient Programmes (Italy, Spain)

Existing RD infrastructures: RD-Connect/ELIXIR, Orphanet, HPO, EuroGentest, Canadian Models and Mechanisms Network

Patient organisations: EURORDIS, Genetic Alliance UK



Solving the unsolved Rare Diseases

Coordinators: Olaf Riess, Holm Graessner (Tübingen)

Co-coordinators: Han Brunner (Nijmegen), Anthony Brookes (Leicester)

Partic-	Participant Organisation Name	Short	Country
ipant N°		Name	
1	Eberhard Karls Universitaet Tuebingen	EKUT	Germany
2	Stichting Katholieke Universiteit Nijmegen	RUMC	Netherland
3	University of Leicester	ULEIC	U.K.
4	University of Newcastle upon Tyne	UNEW	U.K.
5	Central Manchester University Hospitals NHS Foundation Trust	MUH	U.K.
6	Centre Hospitalier Reg Universitaire Dijon	DIJON	France
7	Fundacio Centre de Regulacio Genomica	CRG-CNAG	Spain
8	EURORDIS – European Organisation for Rare Diseases Association	EURORDIS	France
9	Institut National de la Sante et de la Recherche Medicale	INSERM	France
10	Univerzita Karlova	CUP	Czech Republic
11	European Molecular Biology Laboratory	EMBL-EBI	U.K.
12	The Jackson Laboratory Non Profit Corporation	JAX	USA
13	King's College London	KCL	U.K.
14	University College London	UCL	U.K.
15	Universiteit Antwerpen	UA	Belgium
16	Universita degli Studi della Campania Luigi Vanvitelli	Uni Naples	Italy
17	Universita degli Studi di Ferrara	UNIFE	Italy
18	Universitaetsklinikum Bonn	UHB	Germany
19	IPATIMUP – Instituto de Patologia Eimunologia Molecular da	UoP	Portugal
	Universidade do Porto PCUP		· ·
20	Academisch Ziekenhuis Groningen	UMCG	Netherlands
21	Charite – Universitaetsmedizin Berlin	Charité	Germany

