Genetische Diagnostik in der Neuropädiatrie

Univ. Doz. DDr. Denisa Weis

Institut für Medizinische Genetik
Kepler Universitätsklinikum, Linz

JOHANNES KEPLER UNIVERSITÄT LINZ
Altenberger Straße 69
4040 Linz, Österreich
jku.at

Themen

Syndromologische Diagnostik

Konventionelle Zytogenetik

Molekulare Zytogenetik (FISH)

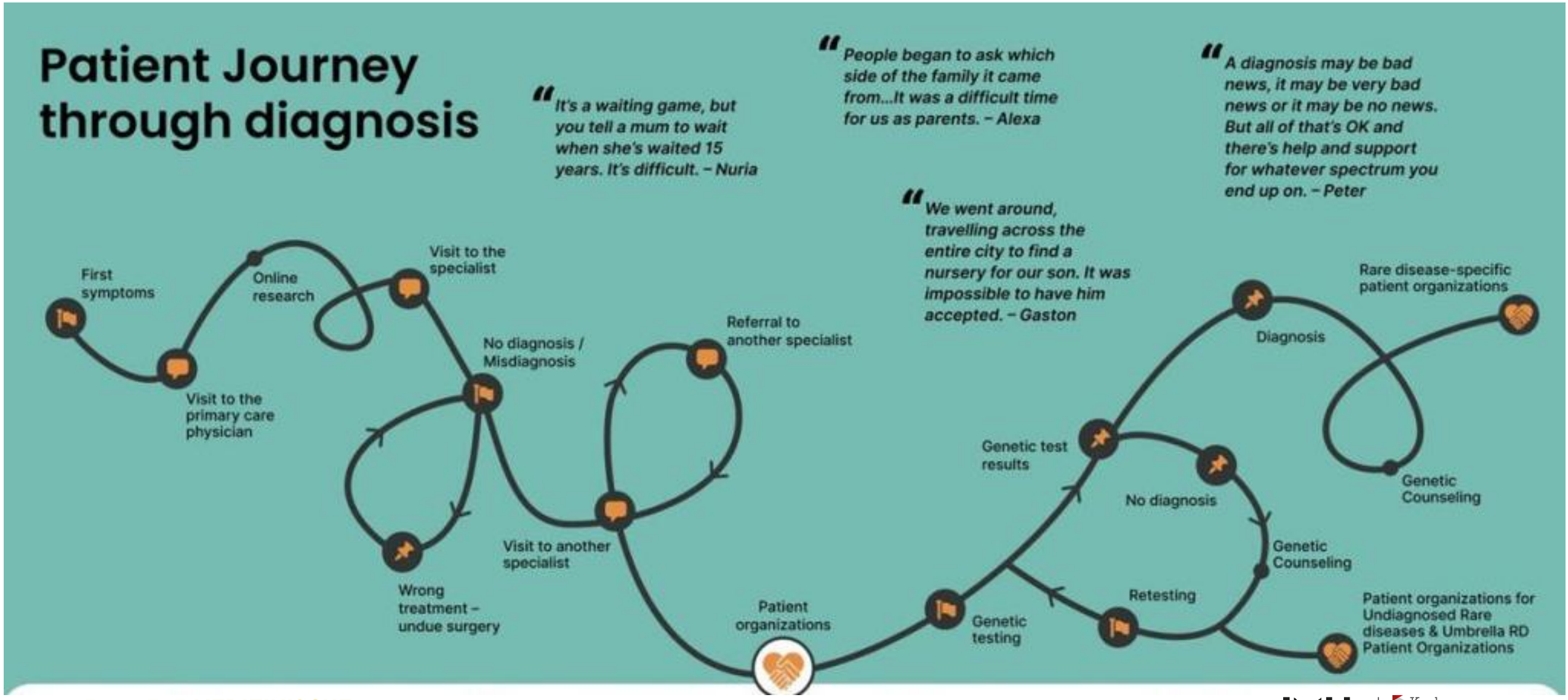
SNP Array

MLPA

Sequenzierung (Sanger, WES, WGS)

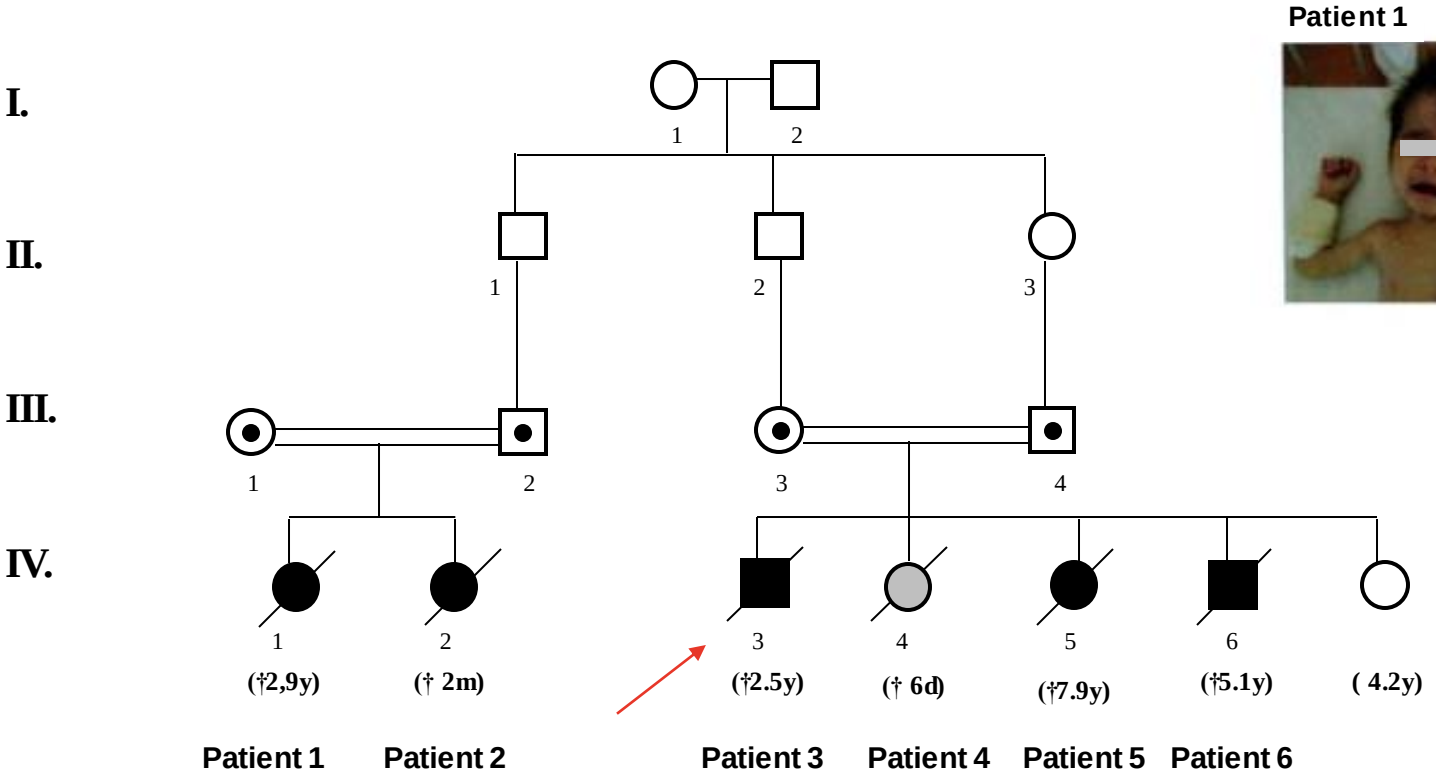
Forschungsinitiativen

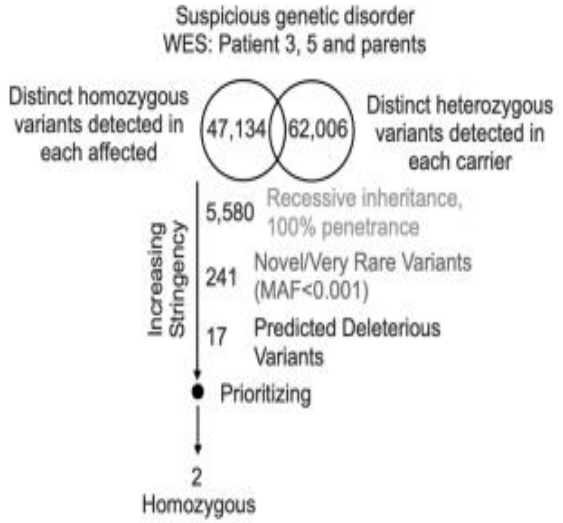
Genetische Diagnostik – oft eine diagnostische Odyssee

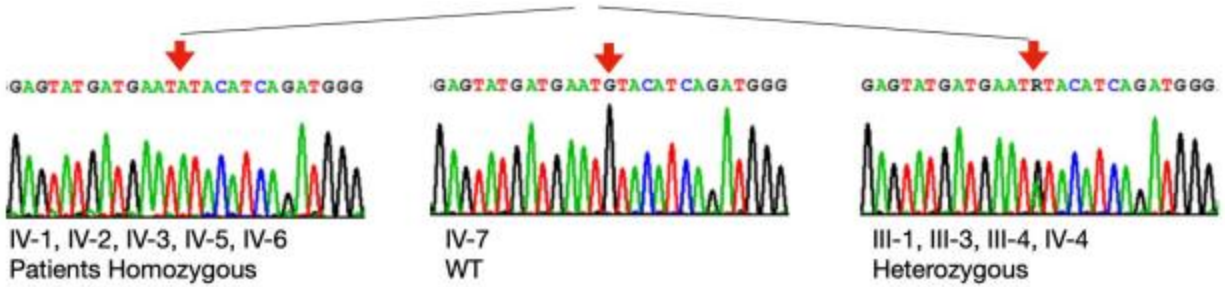


ILLUSTRATIONS: JAVIER MARIA TRIGO GONZALEZ

Langer Weg zur Diagnose







Chr14:
g.81.972.504C>T,
ENST00000336735.4:**c.422G>A**,
ENSP00000337053.4:**p.Cys141Tyr** (p.C141Y, codons tGt>tAt) in exon 4/21

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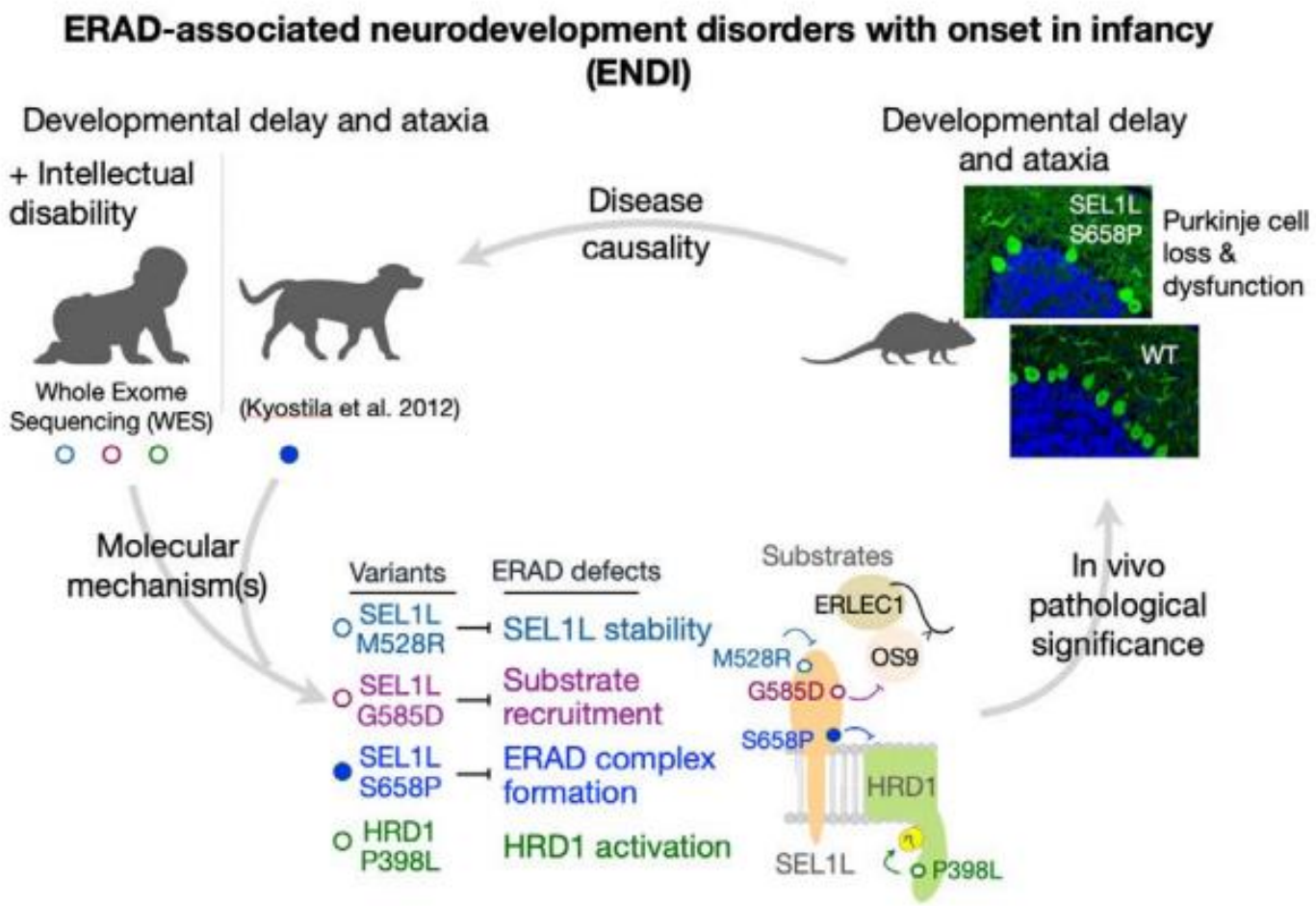
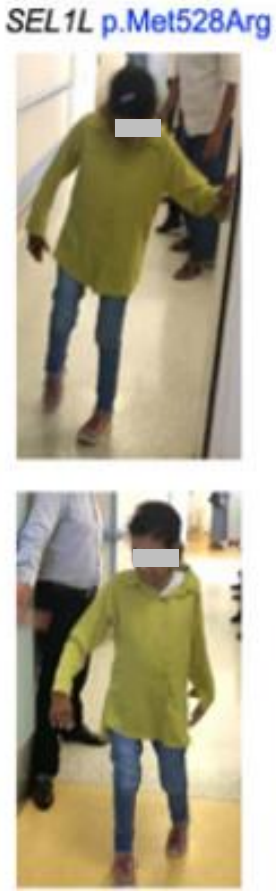
GeneMatcher - ENDI





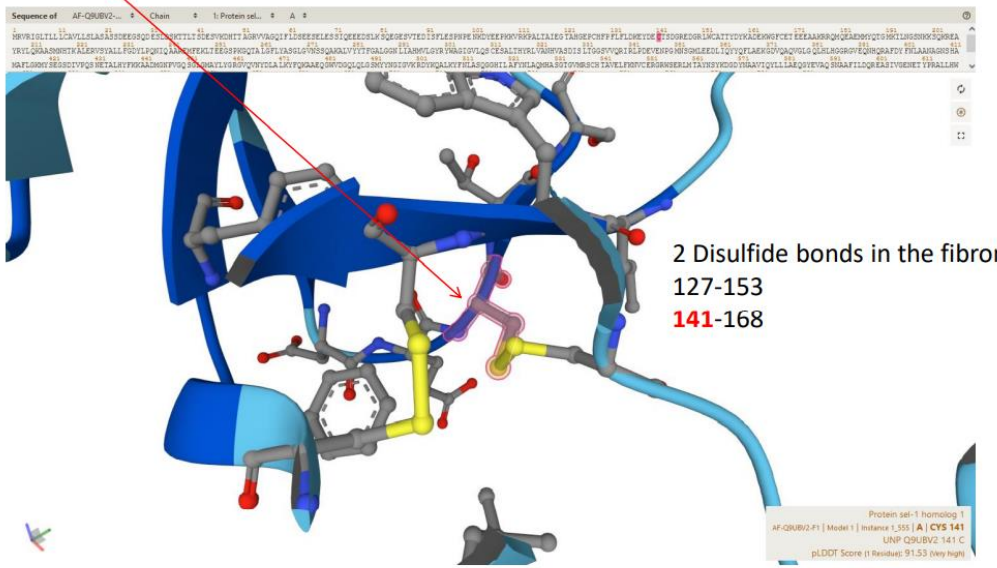
Hypomorphic variants of SEL1L-HRD1 ER-associated degradation are associated with neurodevelopmental disorders

Huilun H. Wang, ... , Fowzan S. Alkuraya, Ling Qi

ER-associated degradation-associated neurodevelopmental disorder with onset in infancy is associated with hypomorphic variants of SEL1L and HRD1.

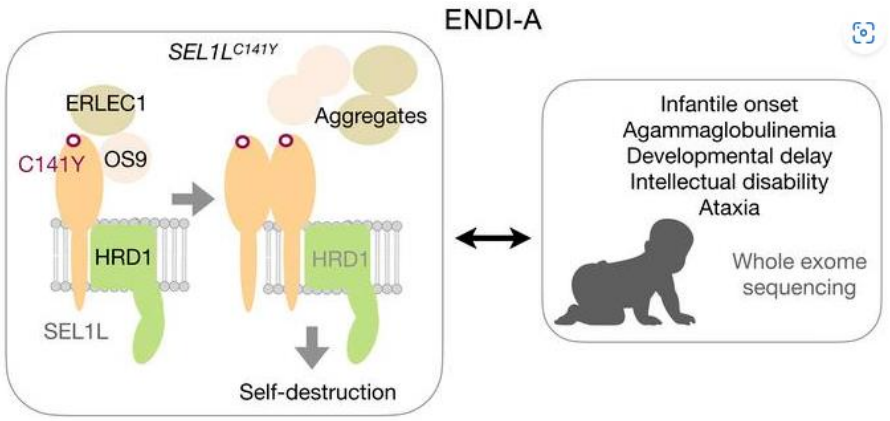
ENDI-A

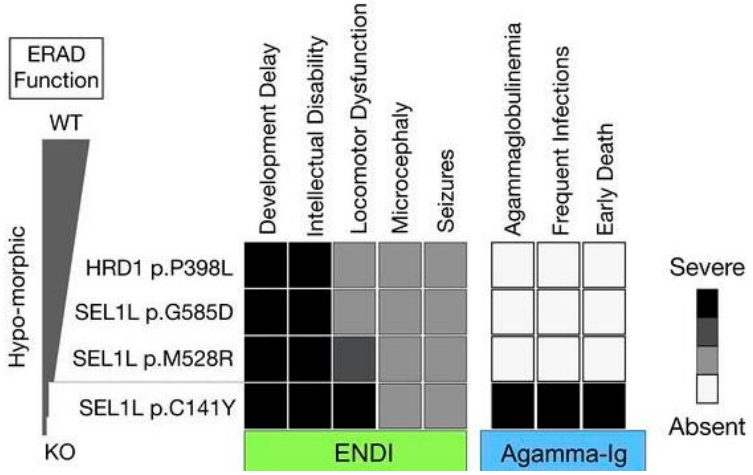
SEL1L protein
Cysteine at position 141 is required for the formation of a conserved **disulfide bond** in the **fibronectin type II** domain



2 Disulfide bonds in the fibronectin type II domain
127-153
141-168

https://alphafold.ebi.ac.uk/entry/Q9UBV2





Biallelic Cys141Tyr variant of *SEL1L* is associated with neurodevelopmental disorders, agammaglobulinemia, and premature death

Denisa Weis, … , Johannes A. Mayr, Ling Qi

Published November 9, 2023

Citation Information: *J Clin Invest*. 2024;134(2):e170882. https://doi.org/10.1172/JCI170882.

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Syndromsuche

Symptome

Muskuläre Hypotonie

Muscular dystrophy

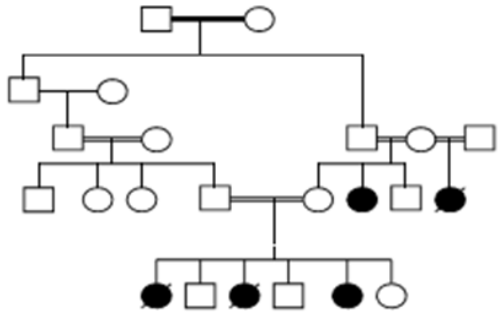
Scapula allata

Developmental delay

Synophyris

CK-ämie (elevation of creatine kinase)

Stammbaum



Syndromologische Databanken

Symptome:www.symptomsuche.at

www.simulconsult.com

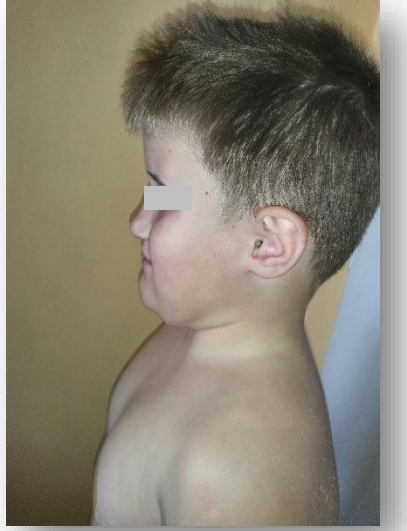
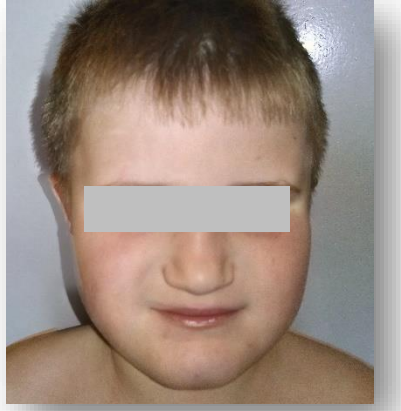
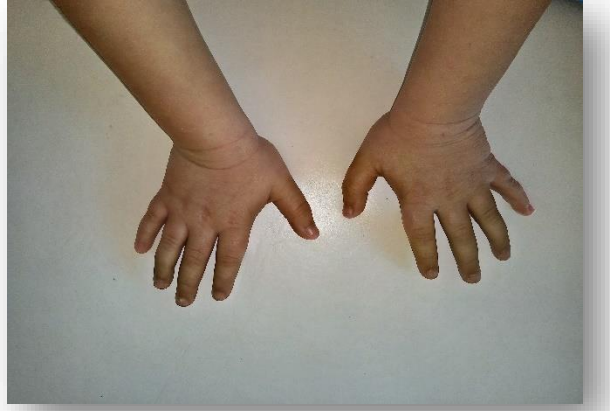
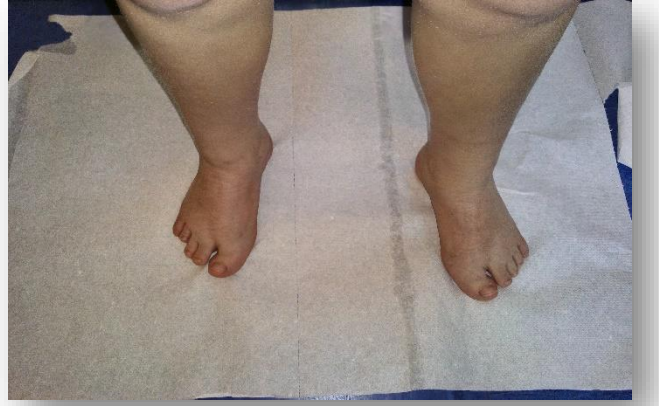
https://www.omim.org/

www.possumcore.com

faziale Dysmorphie:www.face2gene.com

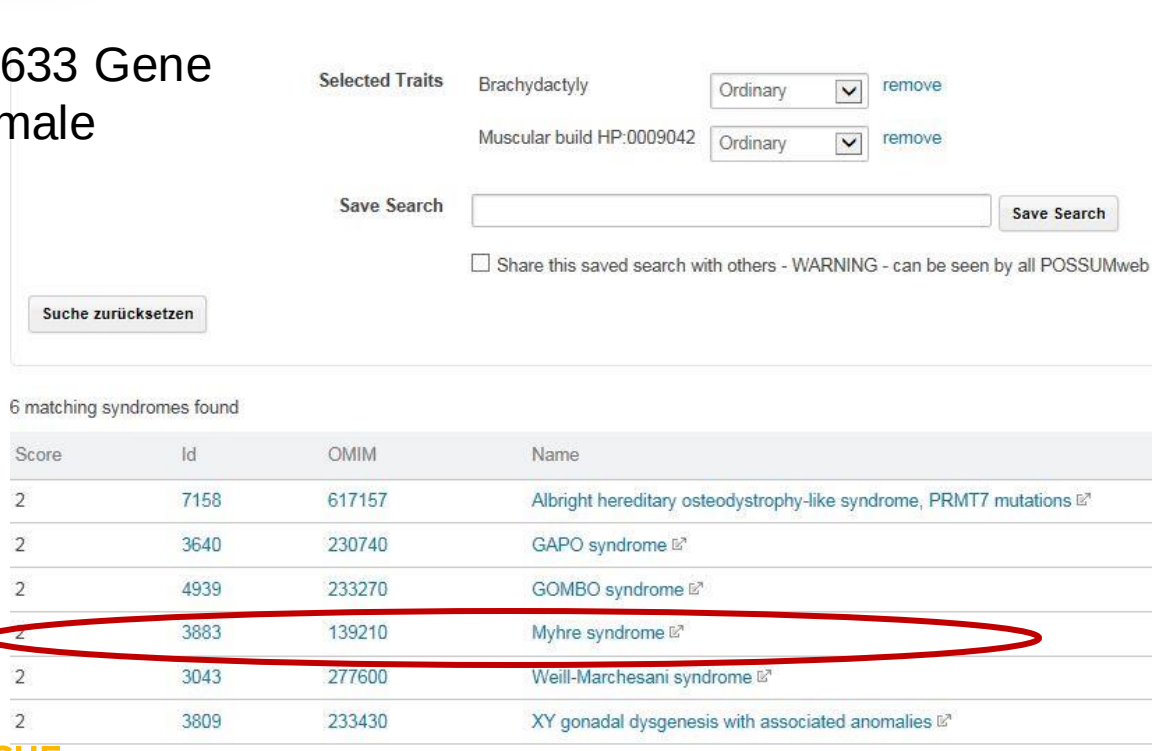
www.gestaltmacher.com

http://www.orpha.net/consor/cgi-bin/index.php

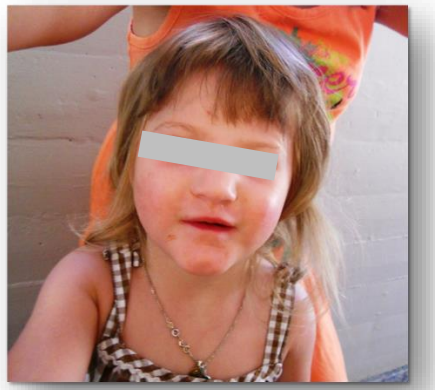
Johannes, 8 J., Kleinwuchs (10 P), faziale Dysmorphie, Spitzengang, harte Muskel, Lordose, Brachydaktylie, normaler Intelekt

OMIM: Brachydactyly: 633 Gene
Possumweb: 2-3 Merkmale



Mutation in *SMAD4* Gen

c.1498A>G
p.Ile500Val 59%

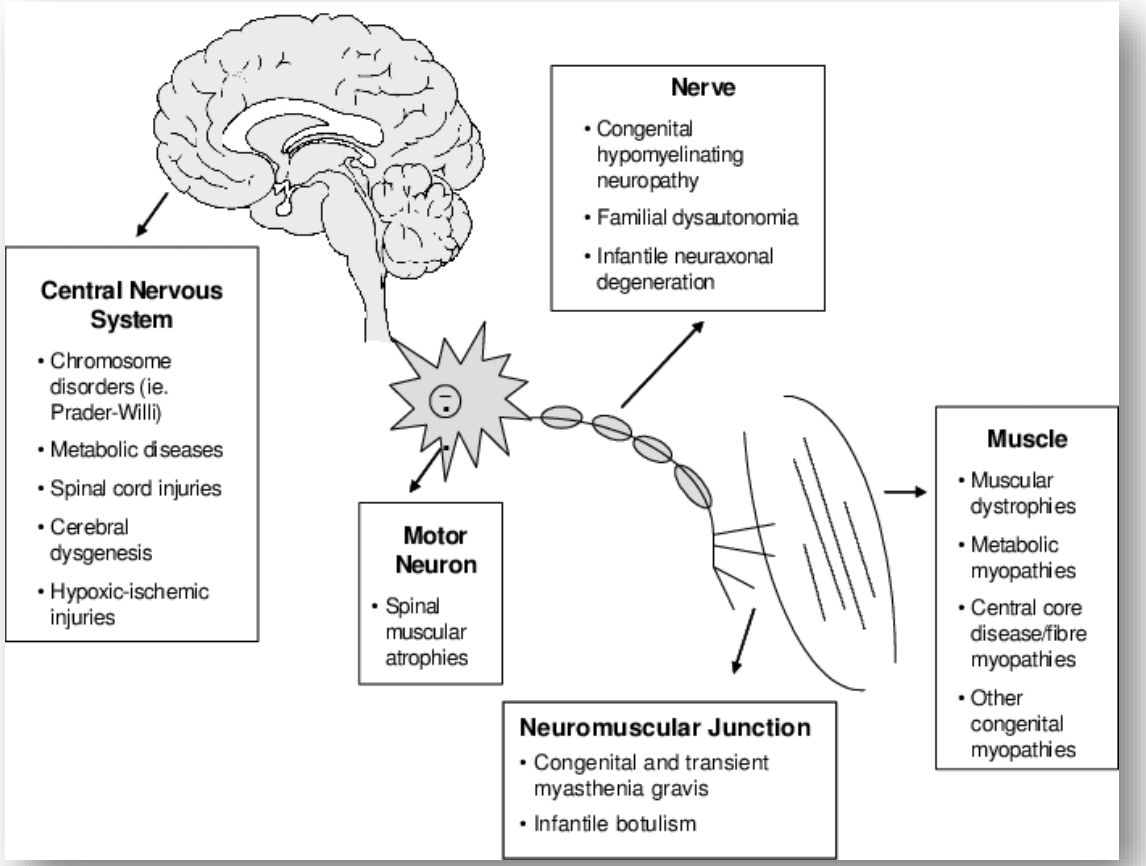


Entwicklungsneurologie und Neuropädiatrie

- breites Spektrum der Erkrankungen (Developmental delay 1950 Gene, Hypotonia 1843 Gene)

Neuromuskuläre Erkrankungen 955 Gene

- 535 verschiedene Gene,
  davon 56 sind mitochondrial
- 69 mappierte Loci warten
  auf die Identifikation



*Leynaar et al, Paediatr Child Health, 2005*http://www.musclegenetable.fr/.

Gemeinsamer diagnostische Zugang

Radiologie

Biochemie

Elektro-physiologie

Pathologie

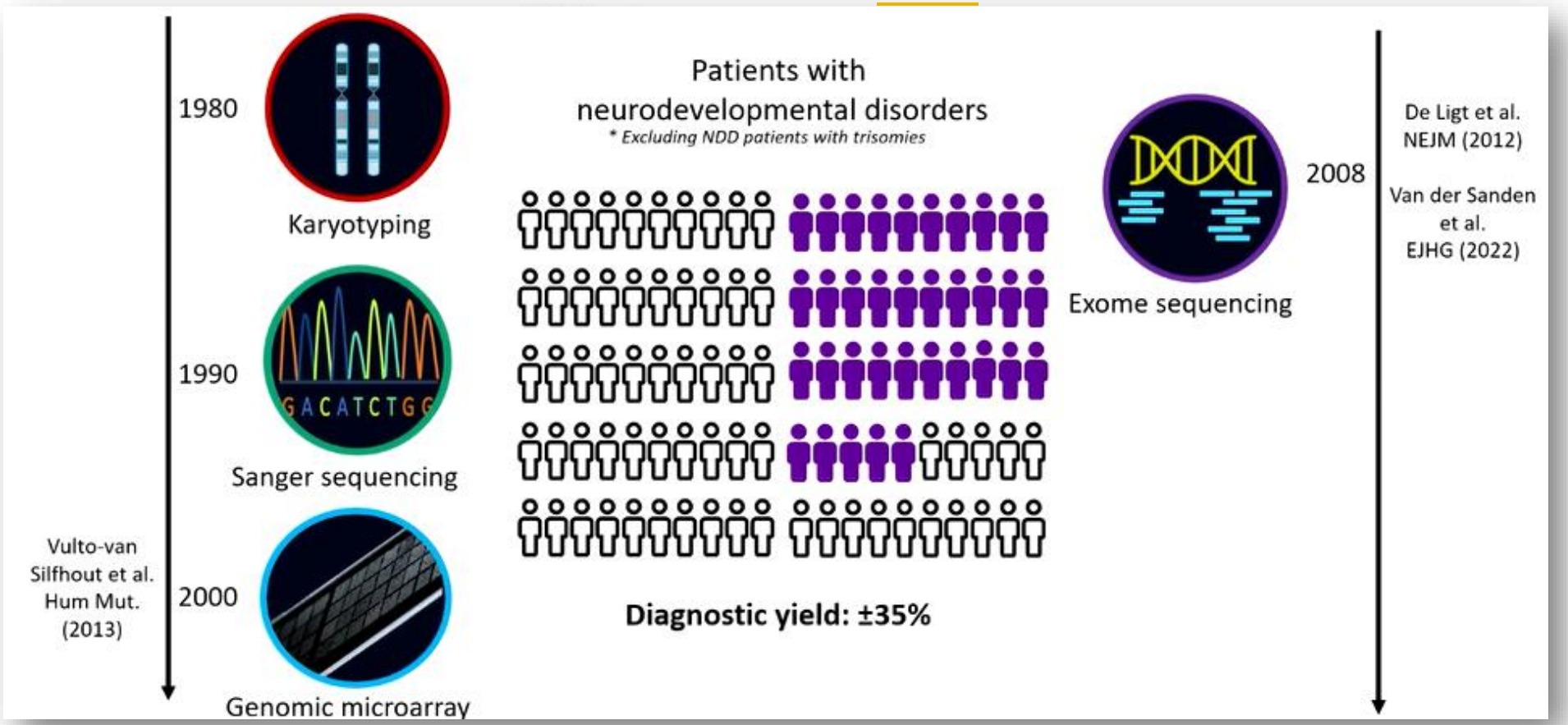
Klinik

Genetik

Genom:

>3 Milliarden Nukleotide (>3000 Mb)

Exom:



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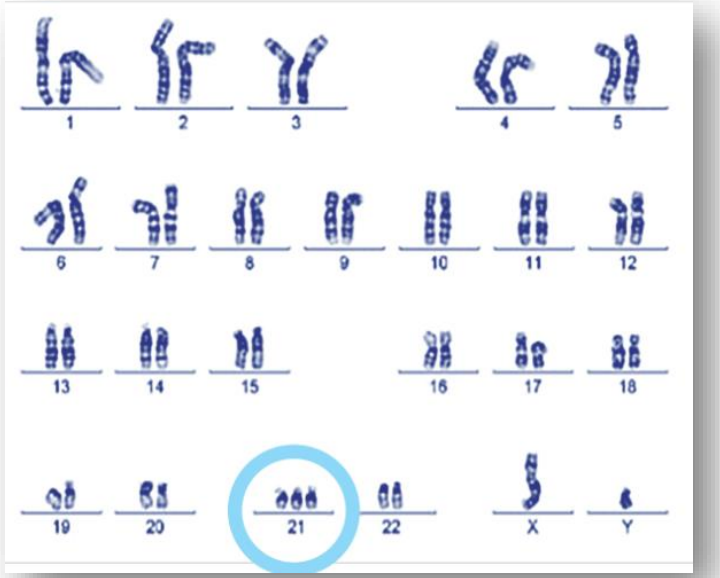
Konventionelle Zytogenetik - Karyogramm

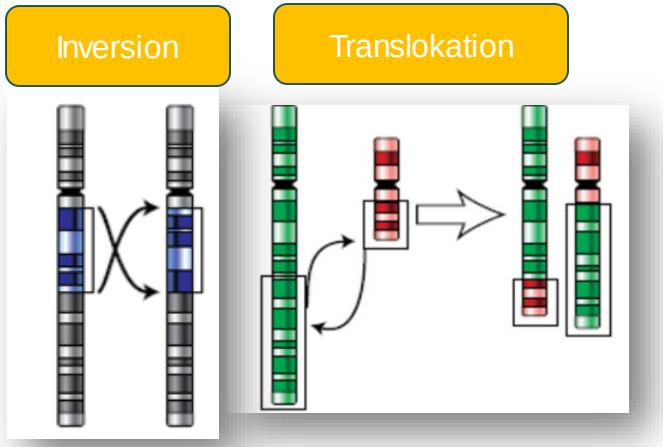


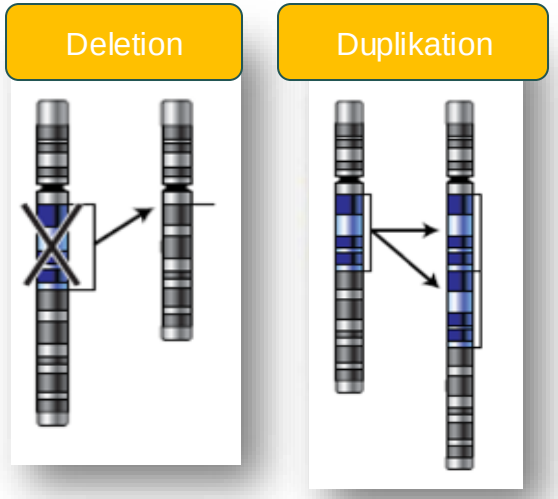
Kann:

Aneuploidie (Trisomien) erkennen

balancierte Veränderung in der Größe von **5-10 Mb** entdecken

unbalancierte Veränderungen genauso **in der Größe von 5-10 Mb** entdecken





Molekulare Zytogenetik - FISH

Sonden auf den Objektträger applizieren

↓

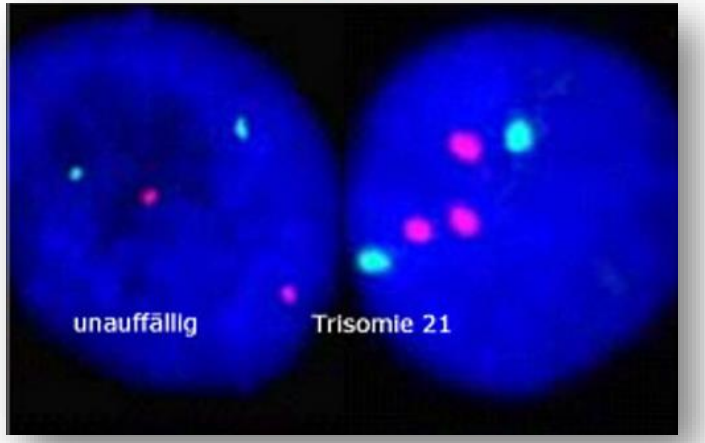
Denaturierung + Hybridisierung

↓

Waschen + Gegenfärbung (DAPI)

↓

Fluoreszenzmikroskopie



Kann:

Mikroskopisch sichtbare Aberrationen (konventionelle Zytogenetik)

Trisomien (T21, T18, T13) pränatal und postnatal

Cri-du-Chat-Syndrom	del(5)(p)	1:50.000

Mikroskopisch nicht sichtbare Aberrationen (Mikrodeletionen/duplikationen):

Nicht mittels konventioneller Zytogenetik nachweisbar > FISH, SNP Array verwenden

DiGeorge-Syndrom	del(22)(q11.22)	1:5.000	häufigste Mikrodeletion!
Williams-Beuren-Syndrom	del(7)(q11.23)	1:20.000	

Prader-Willi-Syndrom (väterliche Deletion 15q11.2q12)

Angelman-Syndrom (mütterliche Deletion 15q11.2q12) - 1:20.000

nun mehr methylierungspezif. MLPA

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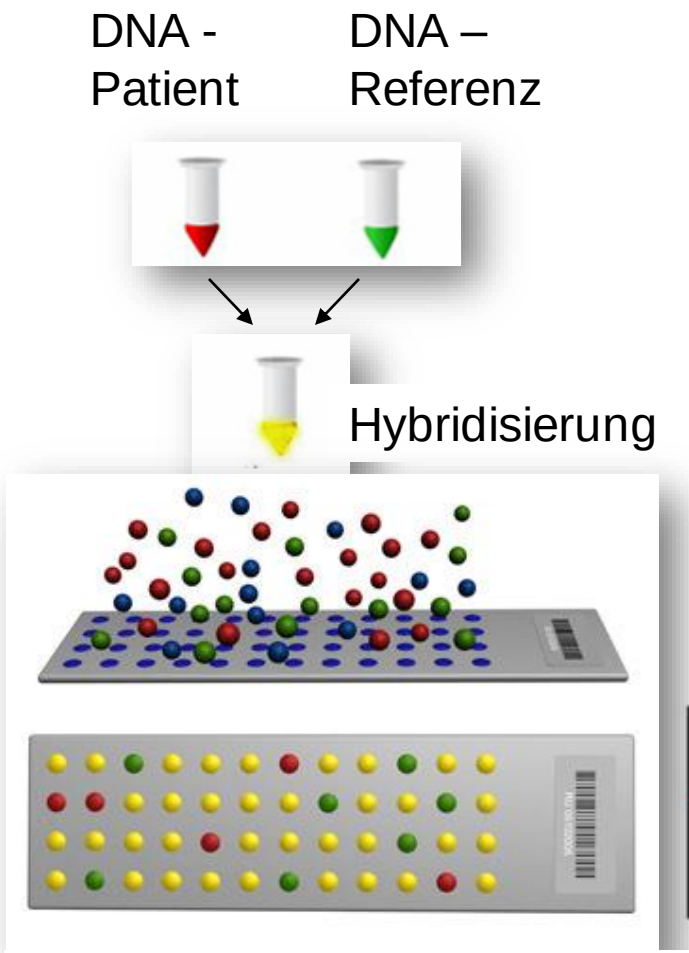
SNP Array

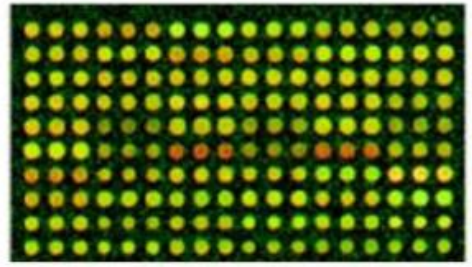
Kann:

- das **gesamte Genom** auf **Kopienzahlveränderungen** (d.h. Deletionen oder Zugewinne) untersuchen
- **Zygotie-Status** ermitteln (z.B. bei uniparentaler Disomie)
- Bis zu <50 kb kleine Deletionen /Dupl. entdecken

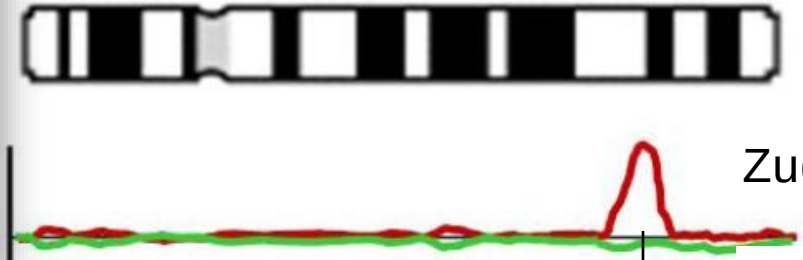
Kann nicht:

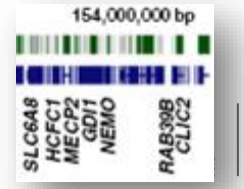
balancierte Translokationen detektieren





Analyse mit Computer





Arrays der verschiedener Densität

40-100 Kb (hohe Densität)
1 Mb (mittlere Densität)
3-4 Mb (niedrige Densität)

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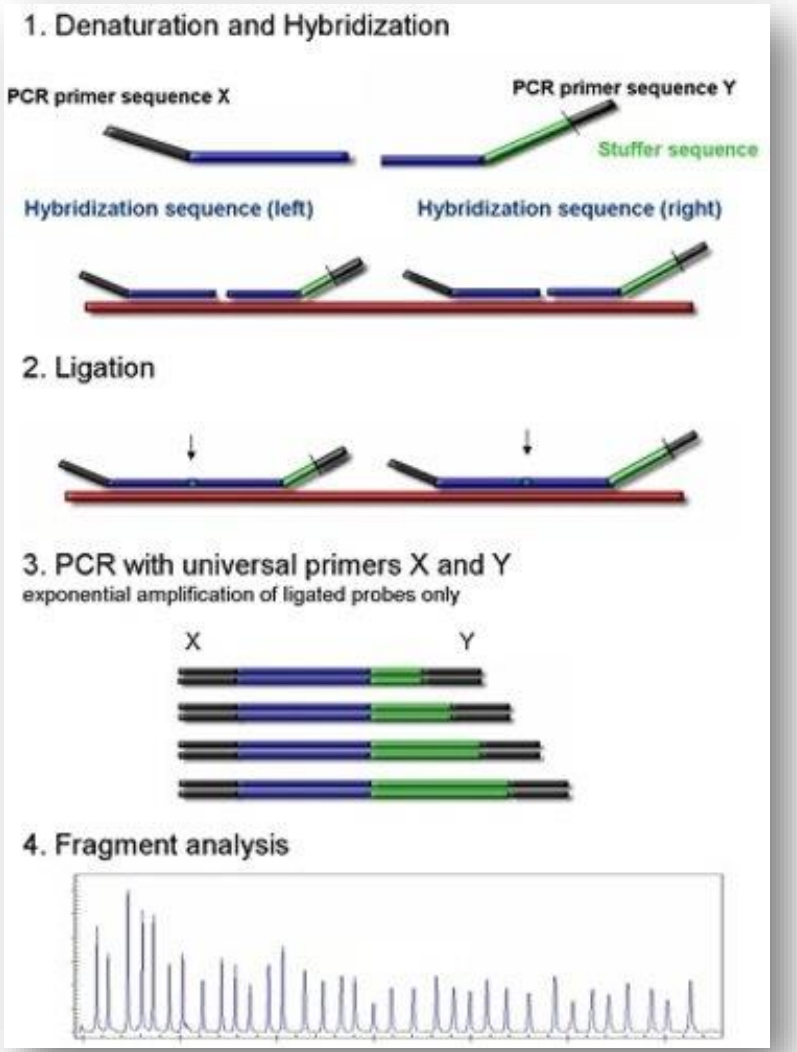
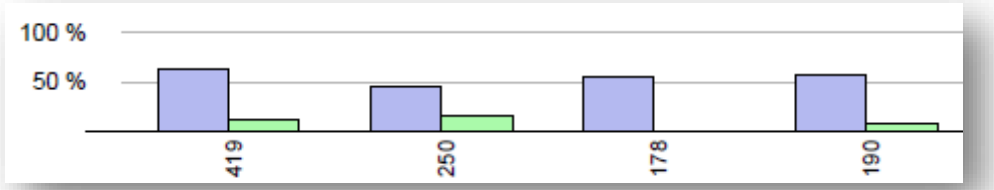
Molekulargenetik – MLPA **(Multiplex Ligation Dependent Probe Amplification)**



Kann:

- Kopienzahlveränderungen einzelner oder mehrerer Exone (CNV) nachweisen
- Mittels Methylierungsspezifische MLPA Deletionen/Duplikation sowie methylierte Bereiche untersuchen
- Wird für Duchenne/Becker, SMA, CMT/HNPP verwendet
- die **Methylierung** von Genen untersuchen; ein Beispiel ist das *SNRPN*-Gen, welches paternal exprimiert wird (imprinting)

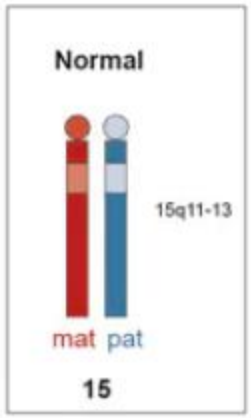


Violett = Kontrollperson

Grün = Patient mit Angelman

www.mlpa.com, Dr. G. Webersinke

Komplexe Diagnostik: SNP Array, MLPA, Sequenzierung, WES, TRIO-WES



Prader-Willi-Syndrom

Angelman-Syndrom

methylierung spezifische MLPA

UPD14 Syndrom

postpartale Hypotonie,
späte Adipositas,
kleine Hände und Füße,
teilweise Kleinwuchs,
EWR
Pubertas praecox
kein Hypogonadismus

SNP Array

WES

ähnliche Syndrome

MAGEL2 Syndrom

Temple Syndrom

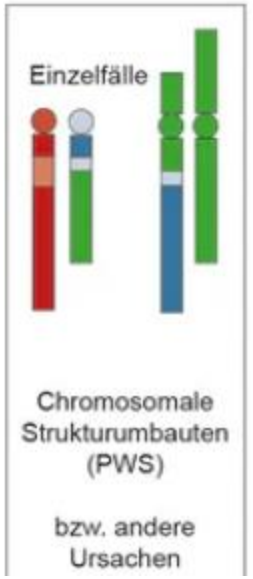
MYT1L Syndrom

SIM1 Syndrom

MORM Syndrom

Cohen Syndrom

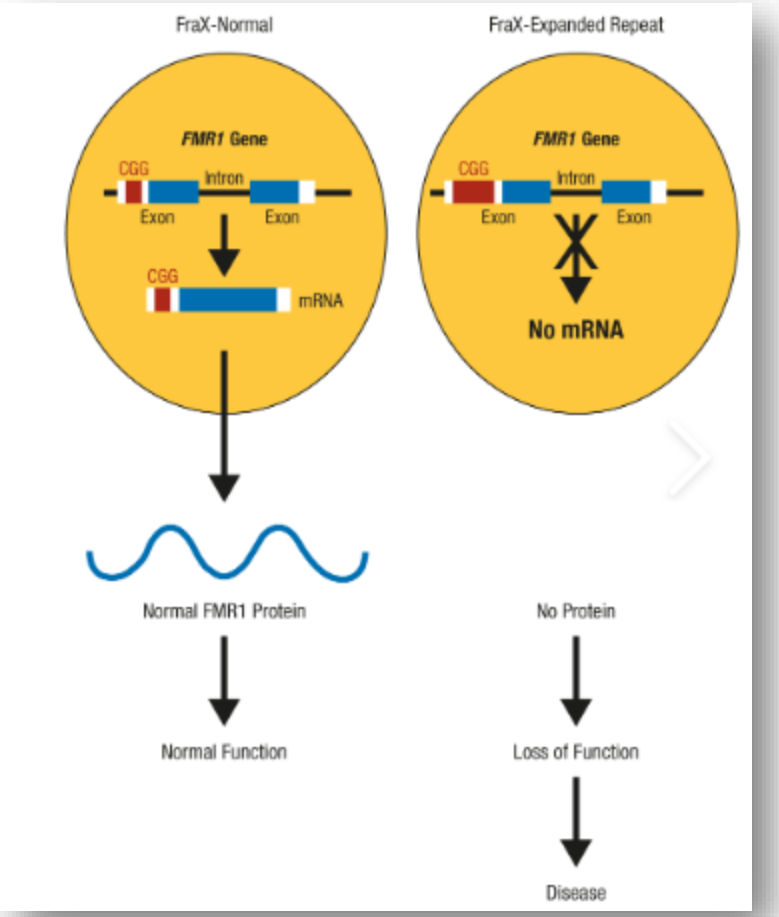
TRIO-WES



- Re-Evaluation TRIO-WES
- WGS (TRIO-WES)

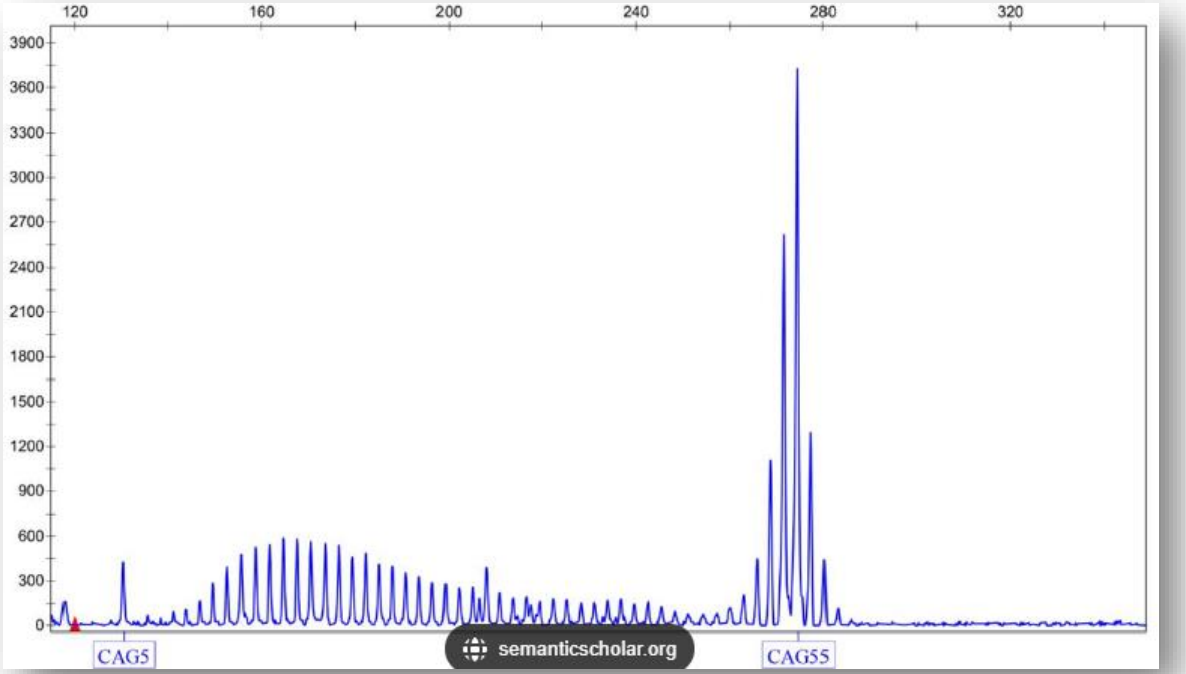
Carvalho L.M.L. et. al, Current Obesity Reports, 2024; 13:313-337

Repeat-Expansionserkrankungen



Kann:

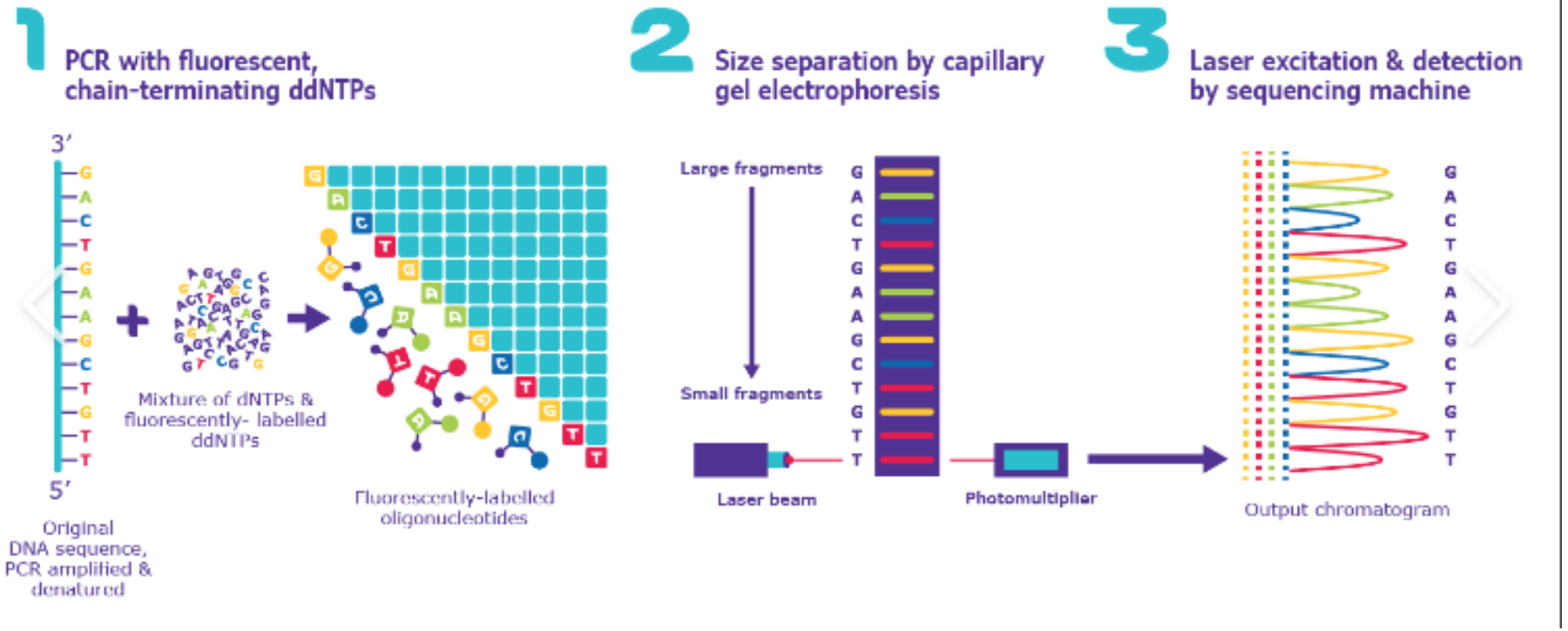
Fragiles X Syndrom (CGG Repeats im *FMR1*-Gen, Krankheit bricht ab 200 R. auf)
Friedreich Ataxie (homozygote GAA Triplettexpansion im *FXN*-Gen, ab 82 R. auf)
Myotone Dystrophie I (CTG Repeatsexpansion im *DMPK*-Gen, ab 51 R.)
Kennedy Erkrankung (CAG Repeatsexpansion im *AR*-Gen, ab 44 R.)
ALS (GGGGCC) hexanucleotide repeat expansion in *C9orf72*-Gen**)** diagnostizieren

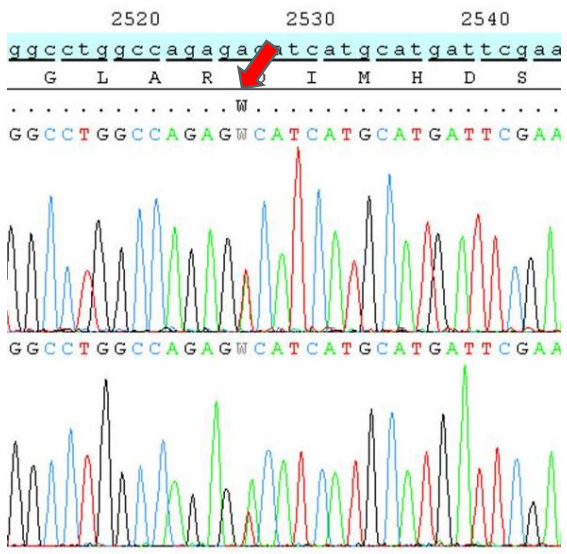


Barbé und Finkbeiner Genetic and Epigenetic Interplay Define Disease Onset and Severity in Repeat Diseases Front Aging Neurosci 2022

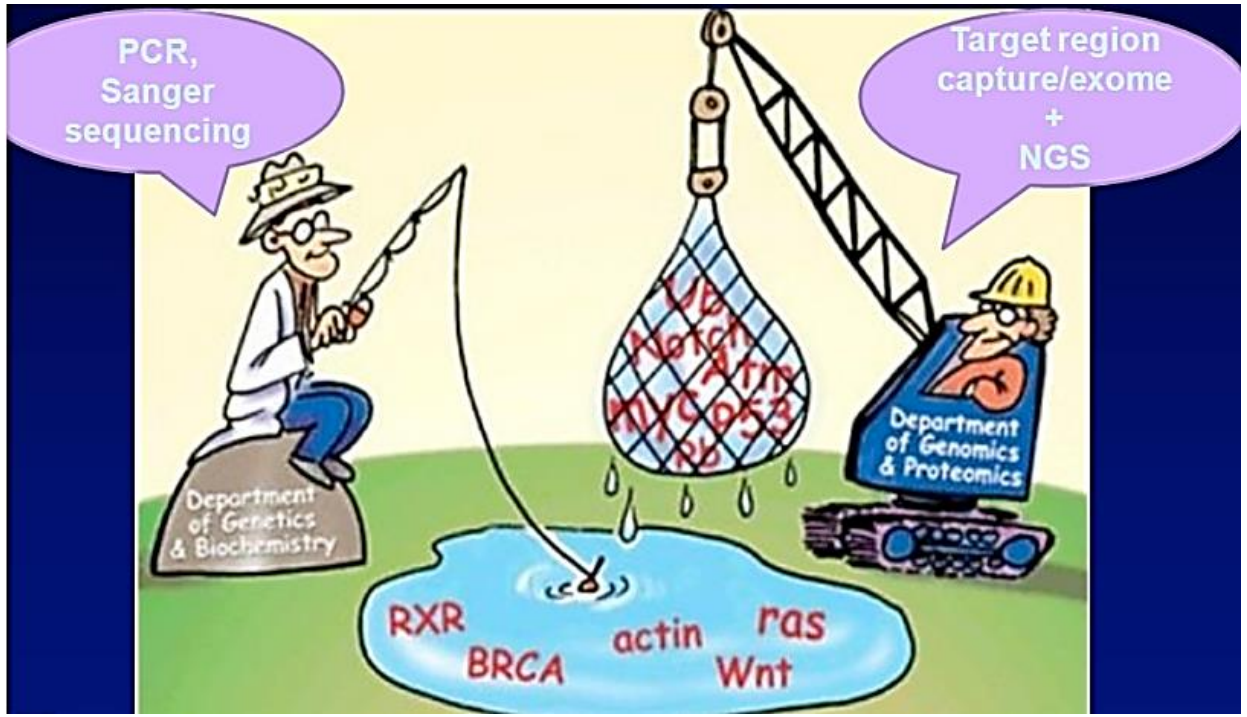
Sanger Sequenzierung

Fred Sanger
1918-2013
Nobelpreis 1980



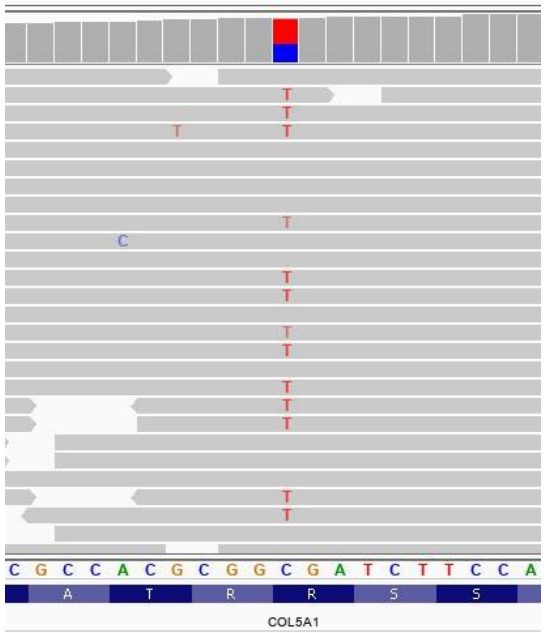


- Ein Gen
- Ein Exon
- Wenige Exone
- Gezielte Mutationen

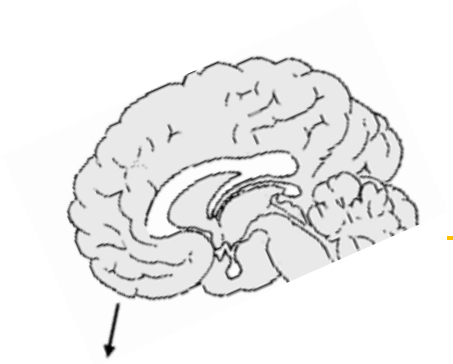


Genpanel	~2-200 Gene
Mendeliom / klinische Exom (CES)	~4 000-6 000 Gene
Whole Exom (WES)	~20 000 Gene

nur die kodierenden Regionen (Exons) + flankierende Sequenz



- Viele Gene parellel
- Whole Exome
- Whole Genome



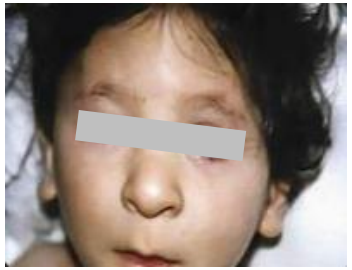
ZNS-Diagnostik mittels WES mit HPO-Terms

https://hpo.jax.org

Walker-Warburg-Syndrome

WES; neonatal hypotonia HPO: 0001319, abnormality of eye HPO: 0000478

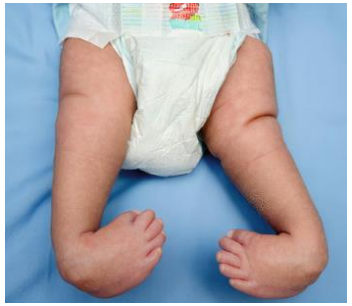
POMT1, POMGNT1, FKRP, FKTN, POMT2, LARGE1, ISPD, POMK, RXYLT1,B3GALNT2, B4GAT1, GMPPB, DAG1, POMGNT2



Arthrogryposis

WES; arthrogryposis HPO: 0002804, talipes equinovarus HPO: 0001763

MYH3, GLE1, TPM2, TNNT3, TNNI2, ACTA1, ECEL1, PIEZO2, MYBPC1

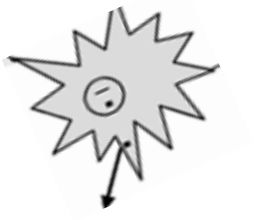


Fetal Akinesia

WES; fetal akinesia sequence HPO: 0001989

DOK7, MUSK, RAPSN, NUP88, MYOD1, GLE1, CNTNAP1, ADCY6, ADGRG6, GLDN, MYBPC1 (11)

Motoneuron Erkrankung



Infantile SMA + Diff. Dg 1. Schritt: *SMN1/SMN2* – MLPA

2. Schritt: ***WES; skeletal muscle atrophy HPO: 0003202***

IGHMBP2, PLEKHG5, ASAH1, BICD2, UBA1, TRPV4,
VRK1, SIGMAR1, ASCC1, DYNC1H1

Adulte SMA + Diff. Dg *SMN1/SMN2* – MLPA

WES; skeletal muscle atrophy HPO: 0003202

GARS1, BSCL2, CHCHD10, DCTN1, DNAJB2, FBXO38,
HSPB1, HSPB8, SLC5A7, REEP1, VAPB, HSPB3, AARS1, ATP7A

dHMN

WES; distal lower limb muscle weakness HPO: 0009053

IGHMBP2, HSPB1, BICD2, BSCL2, HSPB3, HSPB8, DCTN1,
GARS1, TRPV4, DNAJB2, FBXO38, REEP1, SIGMAR1, SLC5A7, PLEKHG5

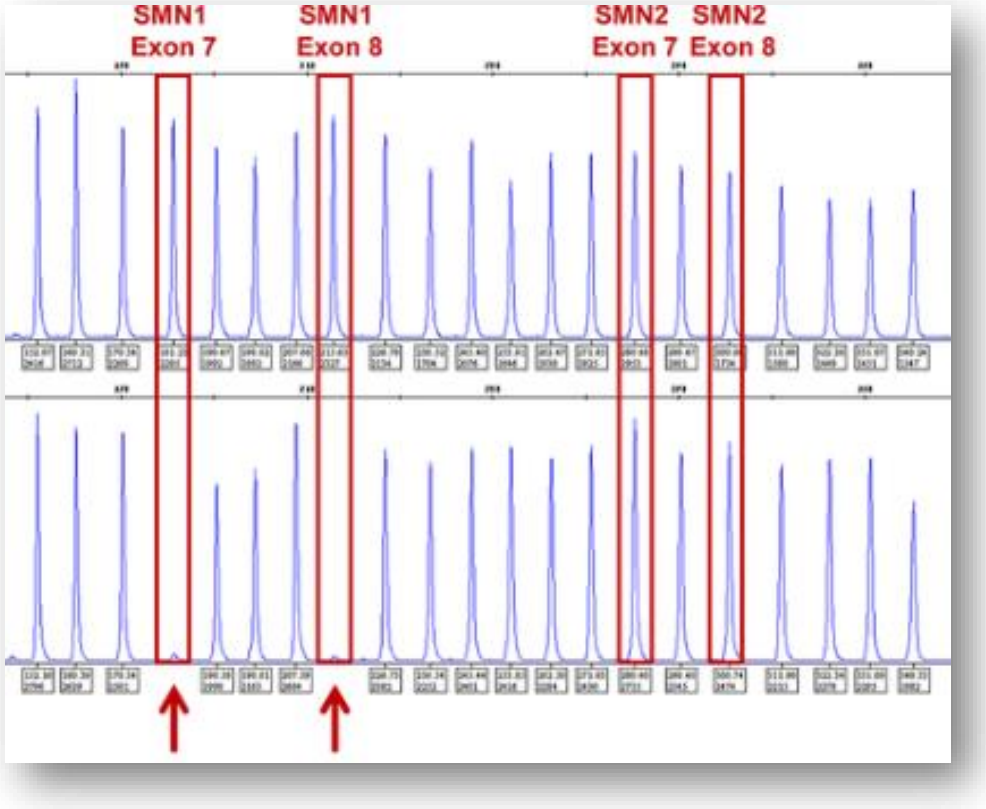
Kennedy Syndrome 1. Schritt: *AR*-Repeat-Analyse

ALS 1. Schritt *C9orf72* – Repeat Analyse

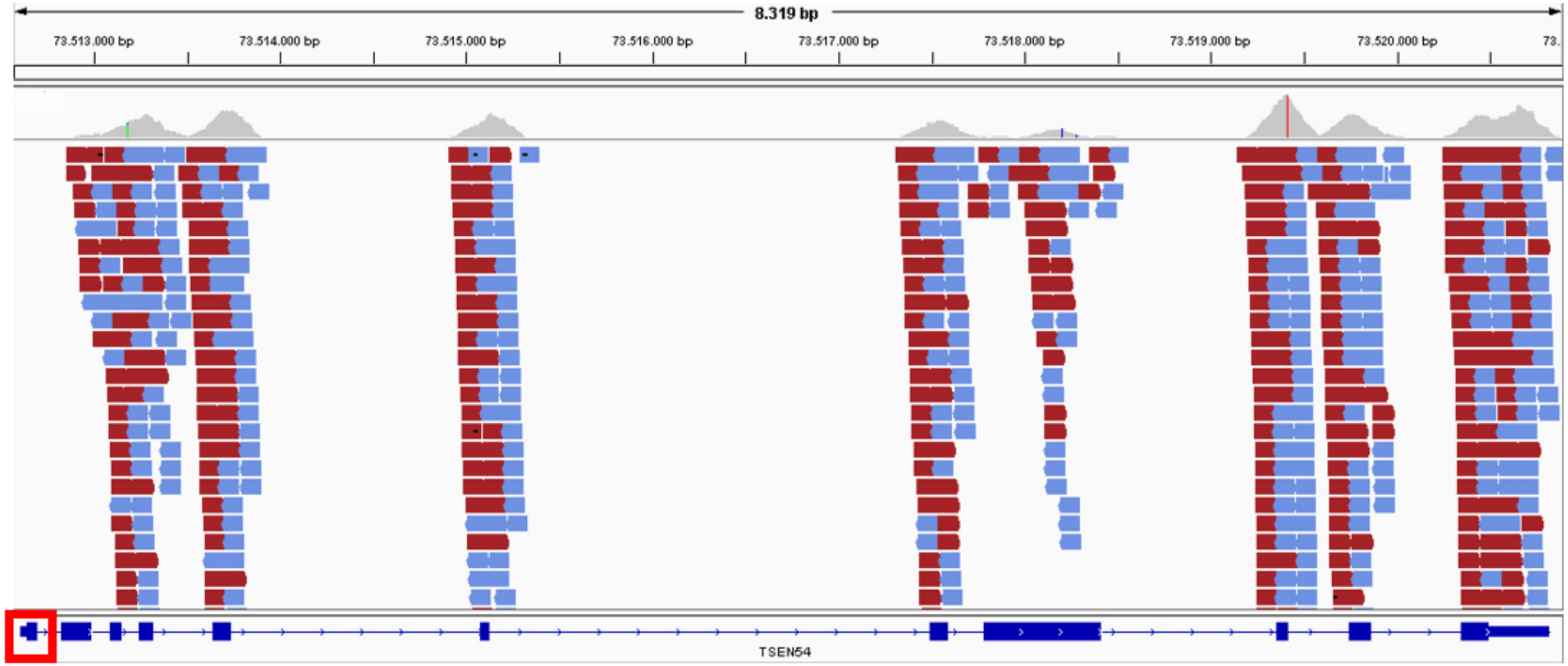
2. Schritt: *WES; SOD1, CHMP2B, CHCHD10, FUS, OPTN, TARDBP,*
PFN1, UBQLN2, VAPB, ANG, VCP, FIG4, ALS2, MATR3, TUBA4A

Spinale Paraparesis (AR / AD)

WES; paraparesis HPO:0002385



Abdeckung von Bereichen in der Sequenzierung = Coverage



Dr. Webersinke, Dr. Kranewitter, BHS Linz

WES – Analyse: Datenauswertung

40 000-60 000 VARIANTEN IN WES

SNPs, small Indels

VARIANTEN FILTERING

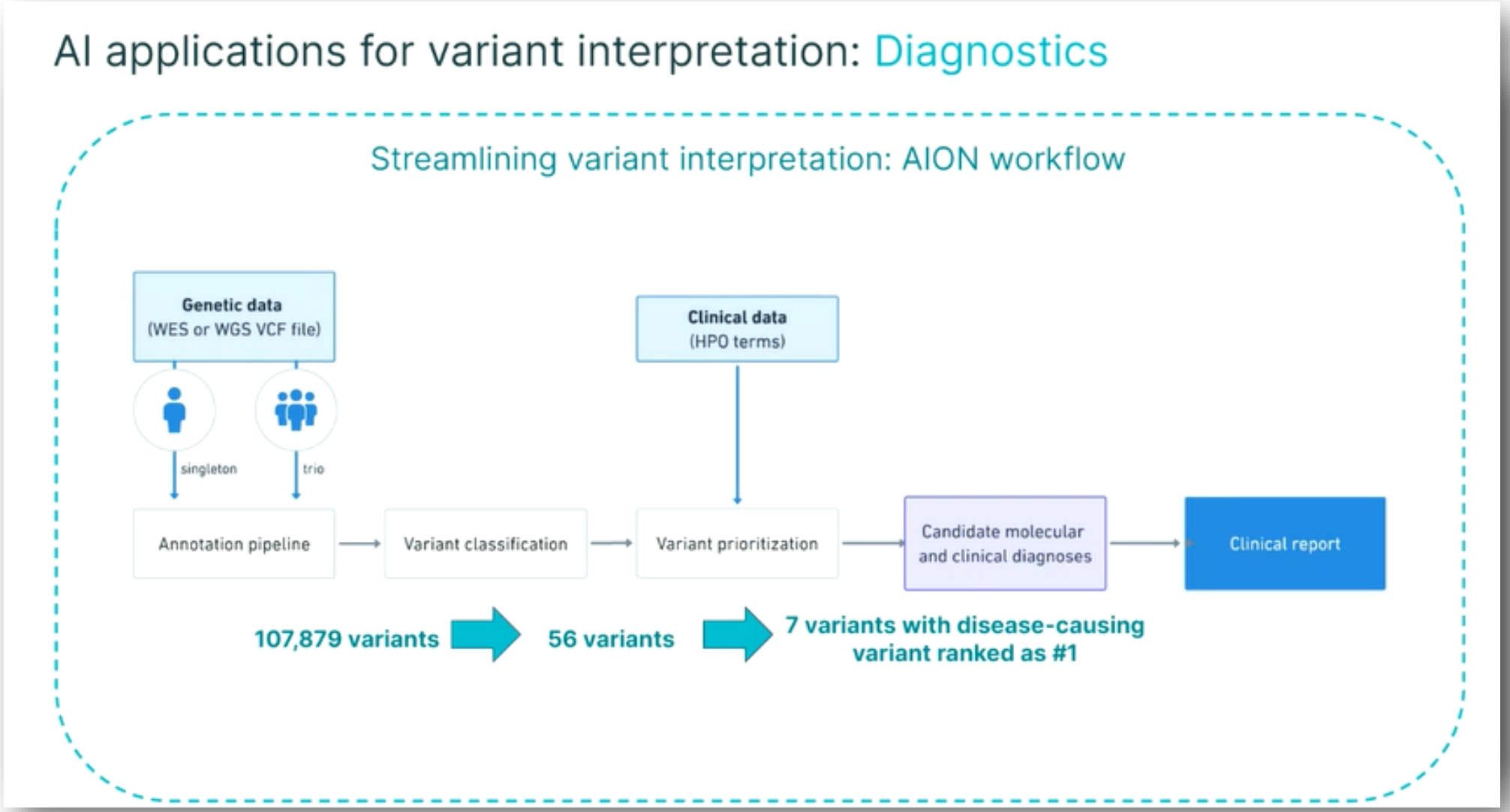
Lokalisation (Exon <–> Intron)
Synonyme <–> Non-synonyme Varianten
In Krankheits-assoziierte Gene<–> unbekannte Gene
In silico Prediktion des Varianteneffekts
Frequenz in Datenbanken für allgemeine Bevölkerung
- <1% für autosomal rezessiv
- <0,1-0,5% für autosomal dominant

50-150 VARIANTEN

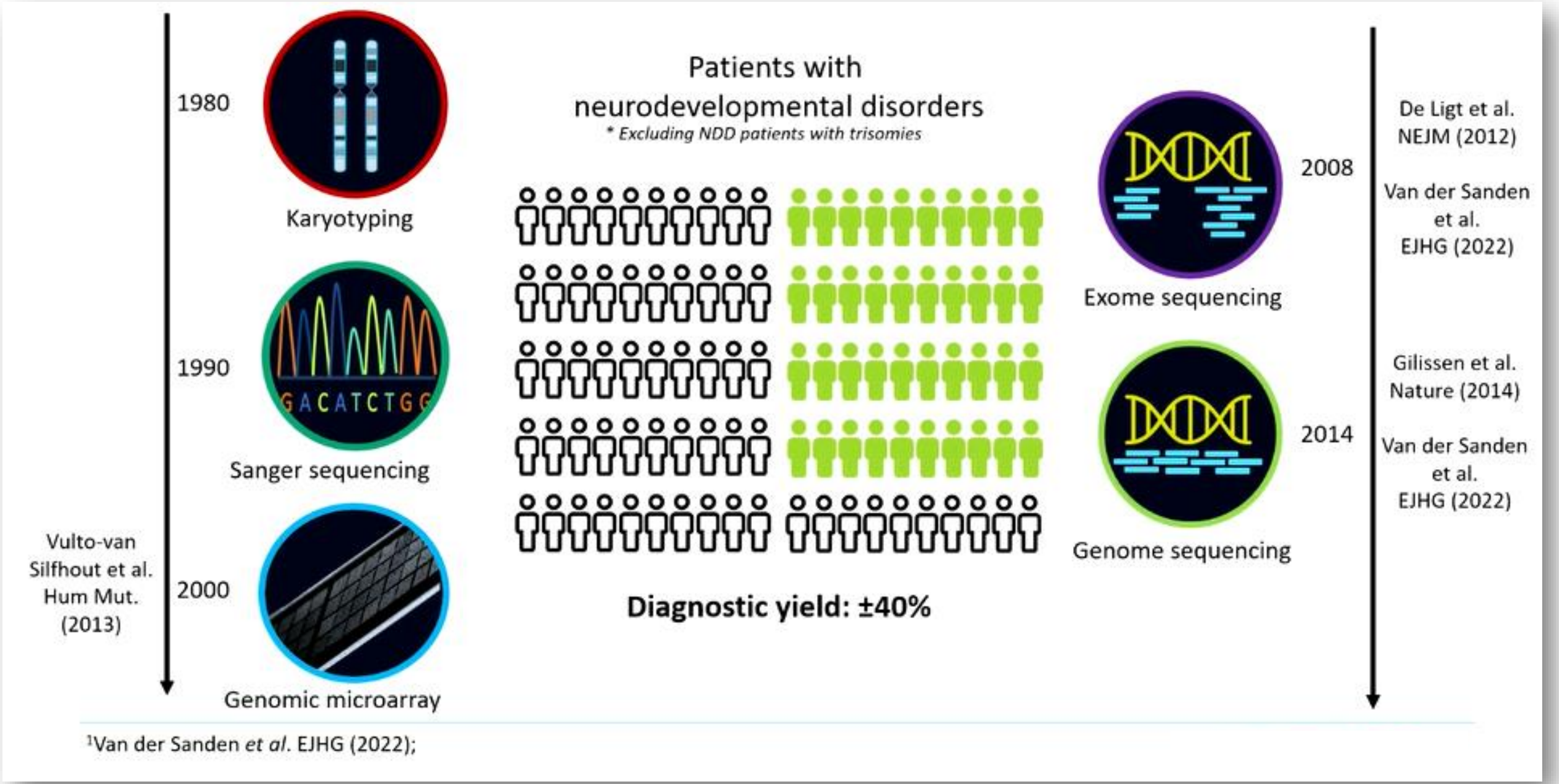
PATHOGENE V. VUS

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

Warum Data-Reevaluation?



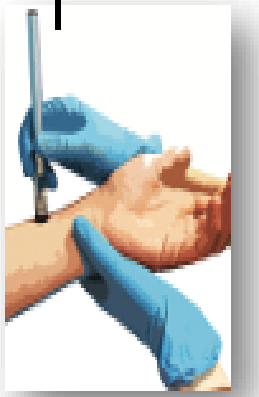
Warum nach WES noch WGS?



[1]Van der Sanden *et al*. EJHG (2022);

Zukunft: Integrative Analyse Genvariationen, DNA Methylierung, Genexpression

Patient derived fibroblasts

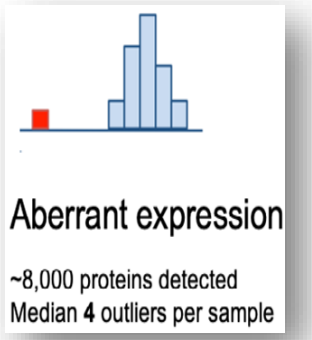


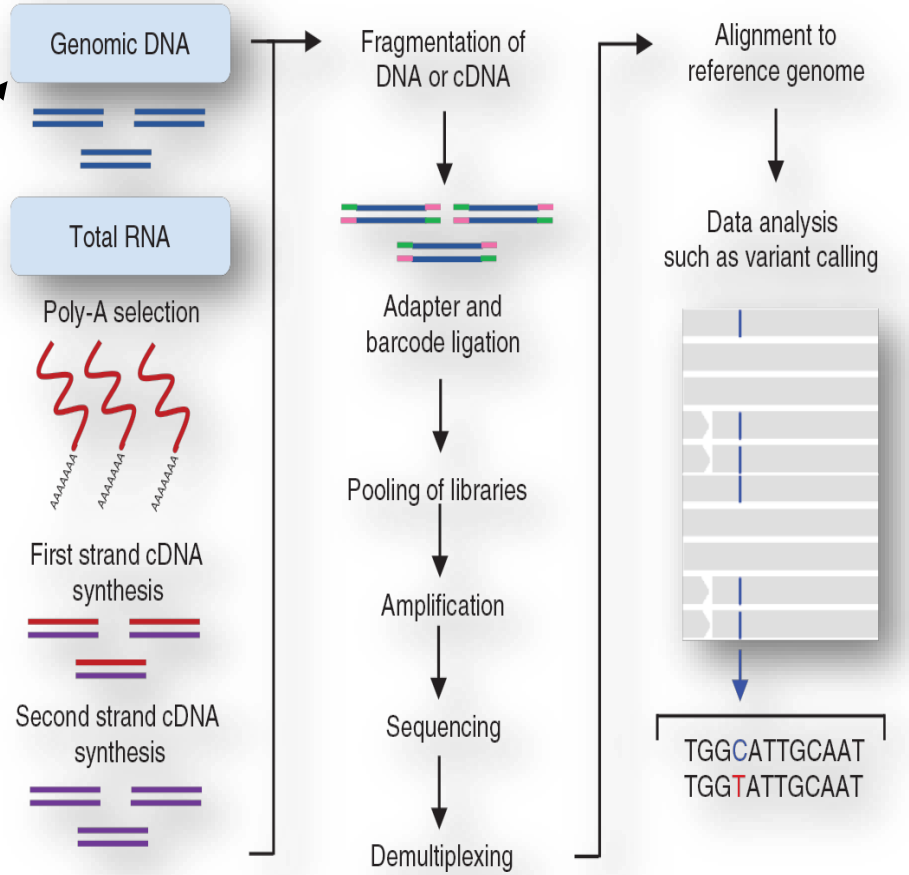
Patient blood

WGS and WES

RNA seq

Proteomics





Adapted from J Inherit Metab Dis. 2020;43: 25-35 and J Vis Exp. 2023; 125: 56146.

Welche genetische Methode soll man wählen?

Bestimmte Krankheiten haben bekannte genetische Veränderungen

Chronologische Analyse

Genetiker fragen…

Methoden in der Diagnostik

	Was untersuchen wir?	Wann indizieren wir?
Karyogramm	Translokationen, Deletionen, Duplikationen >5-10 Mb, Mosaik (10-20%)	Entwicklungstörung,ID, Hypotonie, Muskelschwäche, faziale Dysmorphie
SNParray (mol. Karyotyping)	Mikro-Deletionen und Duplikationen <50 kb (Exondeletion), UPD, Mosaik (> 20%)	- detto -
MLPA	1 und mehrere Gene, Methylierung, Mosaik (30-40%), UPD (methylspezif. MLPA)	SMA, PWS, Duchenne/Becker, Neuropathien, bestimmte Krankheiten
Sanger seq.	1 Gen, gezielte Mutation, Mosaik (20-30%)	bekannte Erkrankung
WES	20 000 Gene, SNVs, CNVs, Mosaik (5-10%)	Symptome mit HPO unbekannte Erkrankungen
WGS	Intronische Varianten, Regulationseinheiten, Mosaik (5-10%), Mikro-deletionen/Duplikationen	Forschung
Long read seq. Nanopore Seq.	Polymorphe Regionen, Methylationsanalysen, strukturelle Varianten, Gene mit Expansion/Repeats, RNA isoformen	Forschung, wenn WES negativ Gene mit Pseudogenen

Danke für Ihre Aufmerksamkeit

MEDIZINISCHE FAKULTÄT

Some cases need long to be solved

The future might bring a solution

Wait and look out

Be patient, don‘t panic

Always look at the bright side of life

(Prof. Serge Weis)

Denisa.weis@kepleruniklinikum.at
Ordination@praxis-weis.at

JOHANNES KEPLER UNIVERSITÄT LINZ
Altenberger Straße 69
4040 Linz, Österreich
jku.at