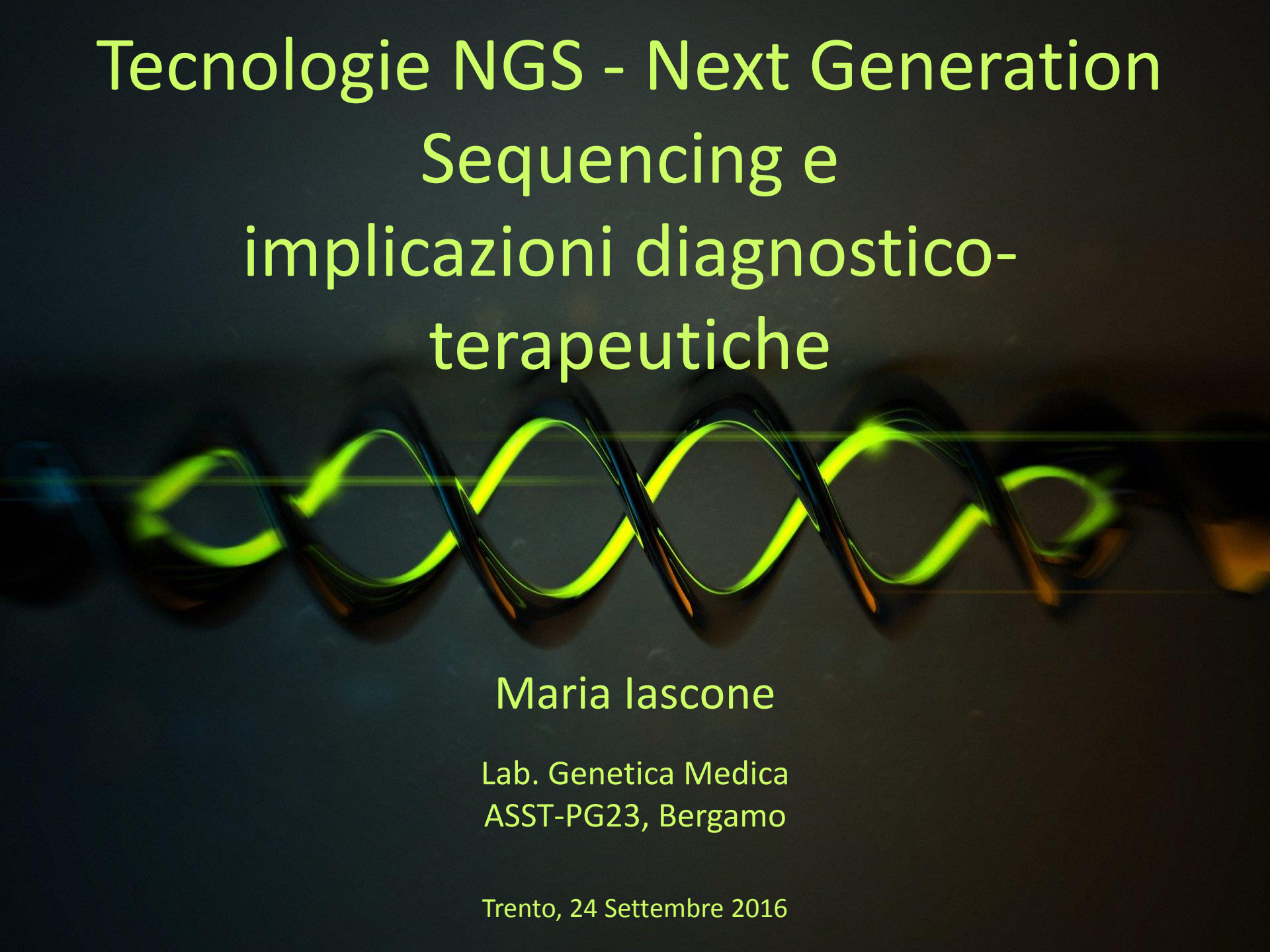
Tecnologie NGS - Next Generation Sequencing e implicazioni diagnostico-terapeutiche

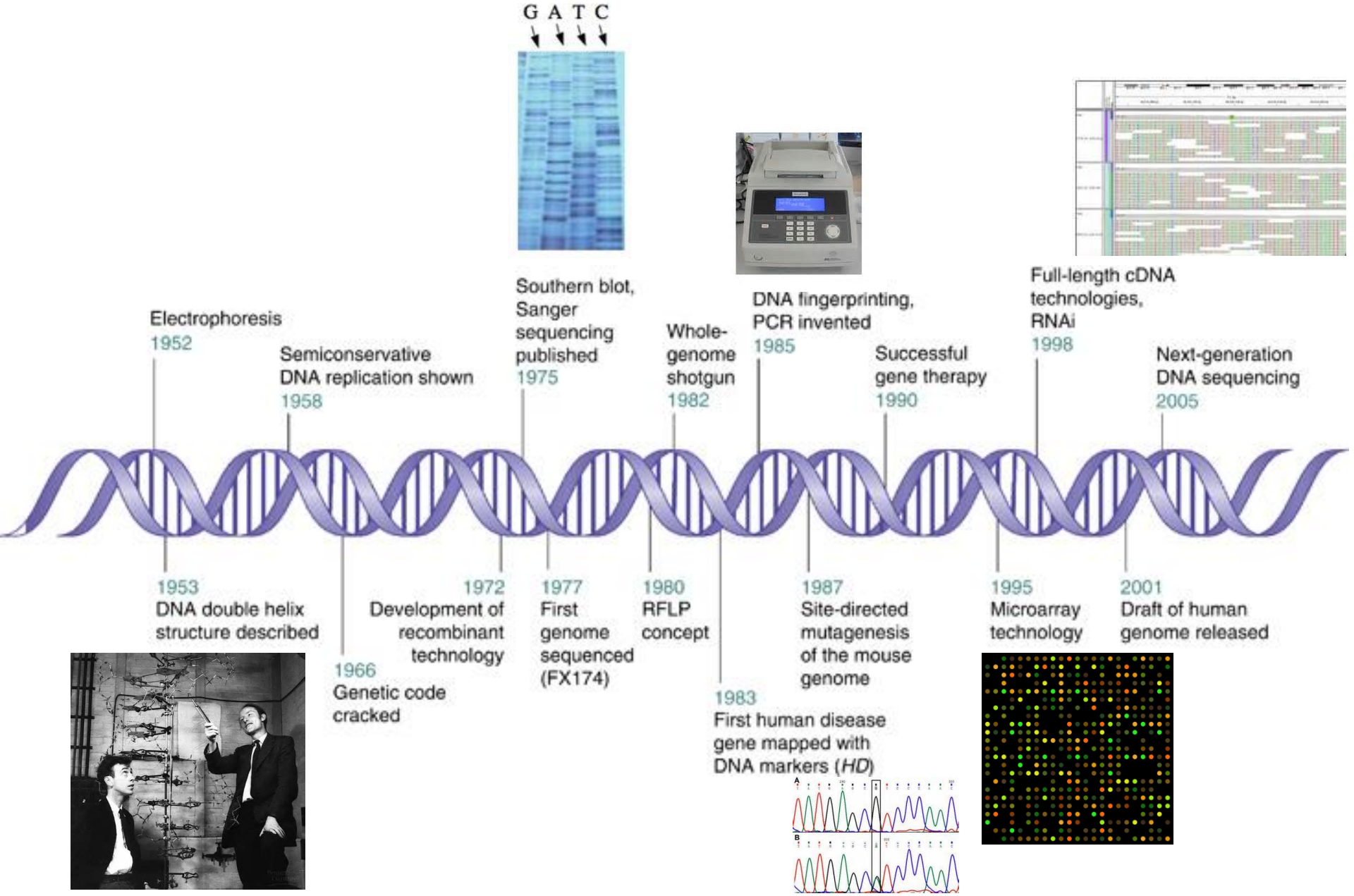


Maria Iascone

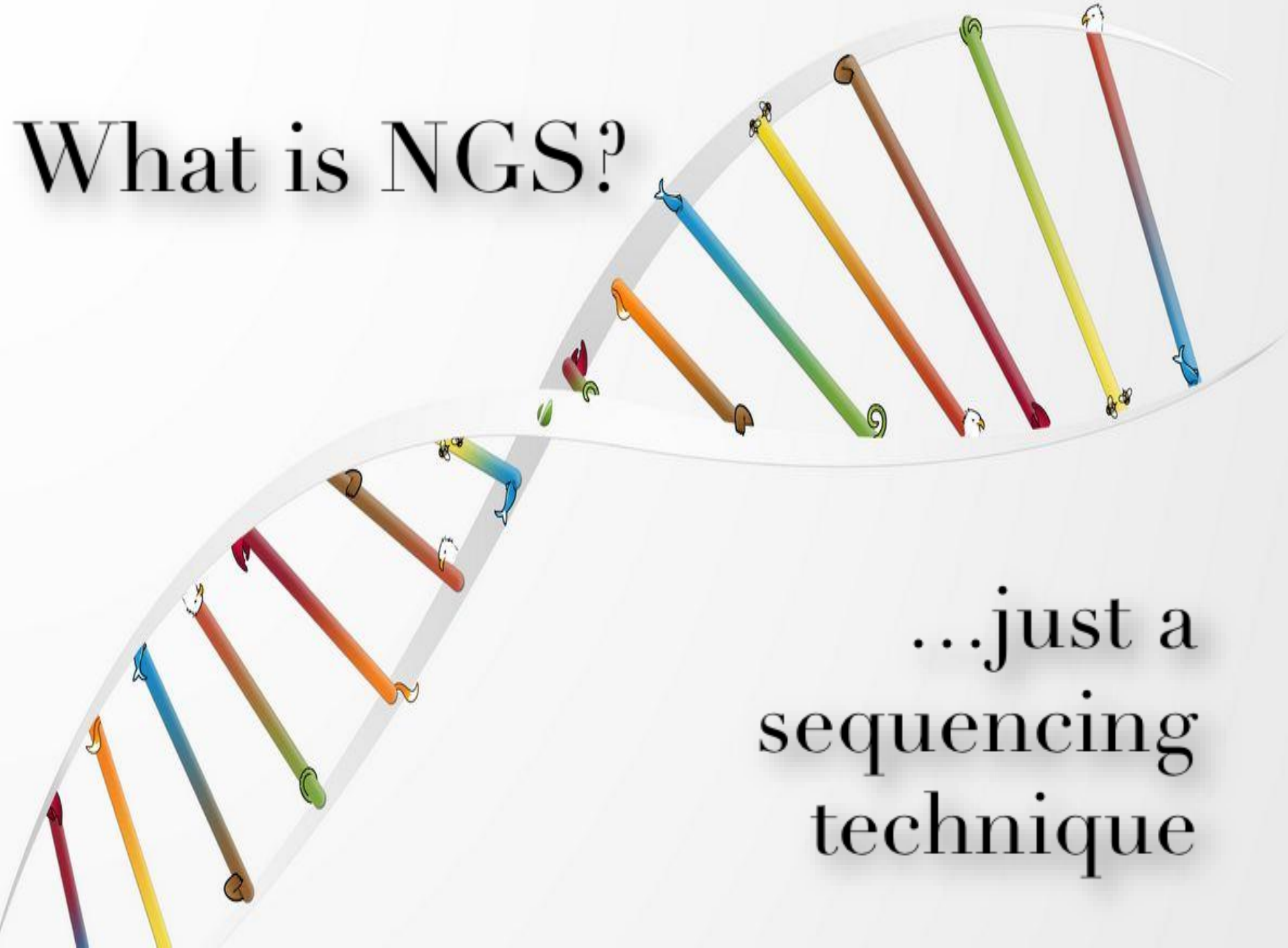
Lab. Genetica Medica
ASST-PG23, Bergamo

Trento, 24 Settembre 2016

Genetic Discoveries Timeline



What is NGS?

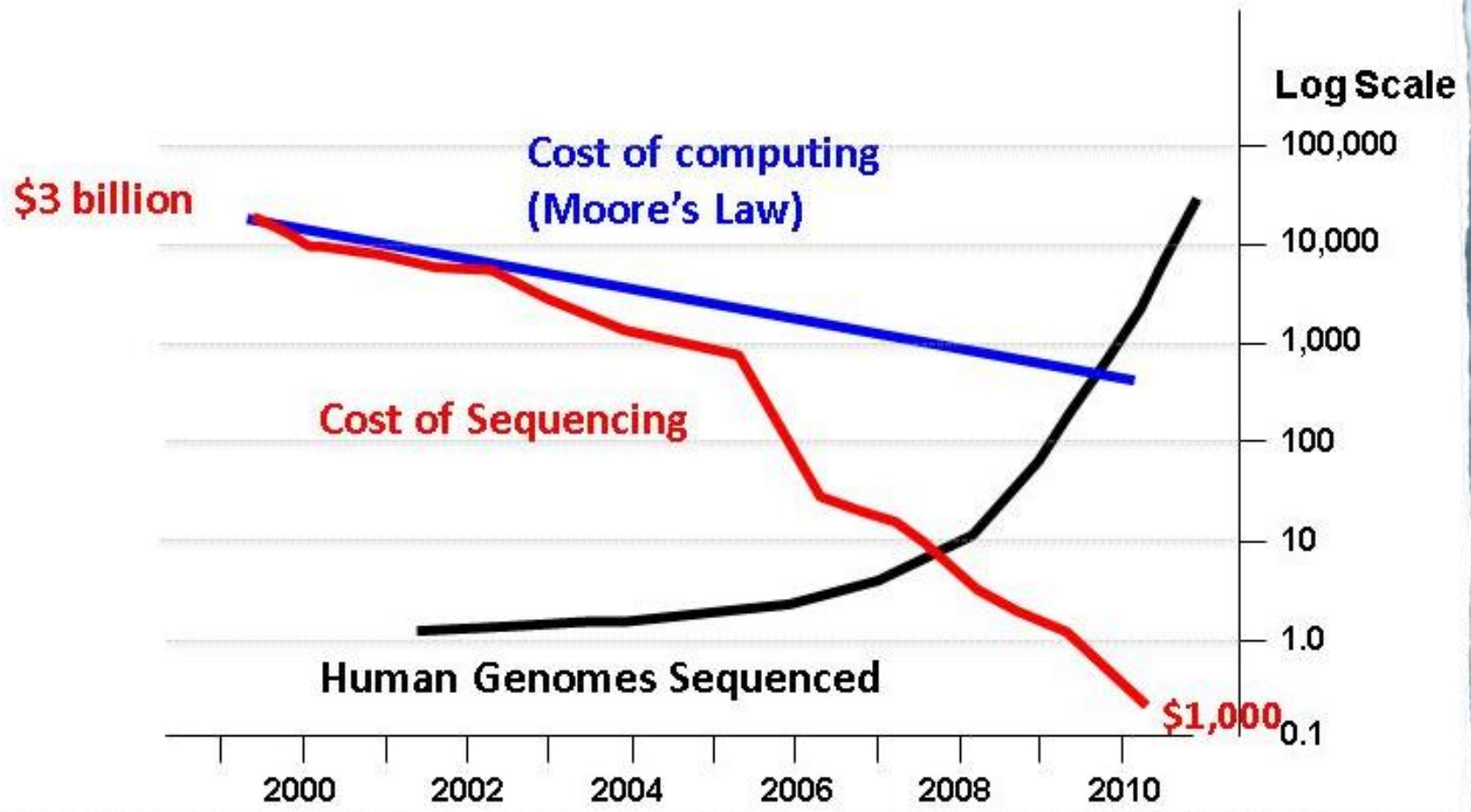


…just a sequencing technique

Adapted from

The Economist

The Sequencing Explosion



2001
HGP

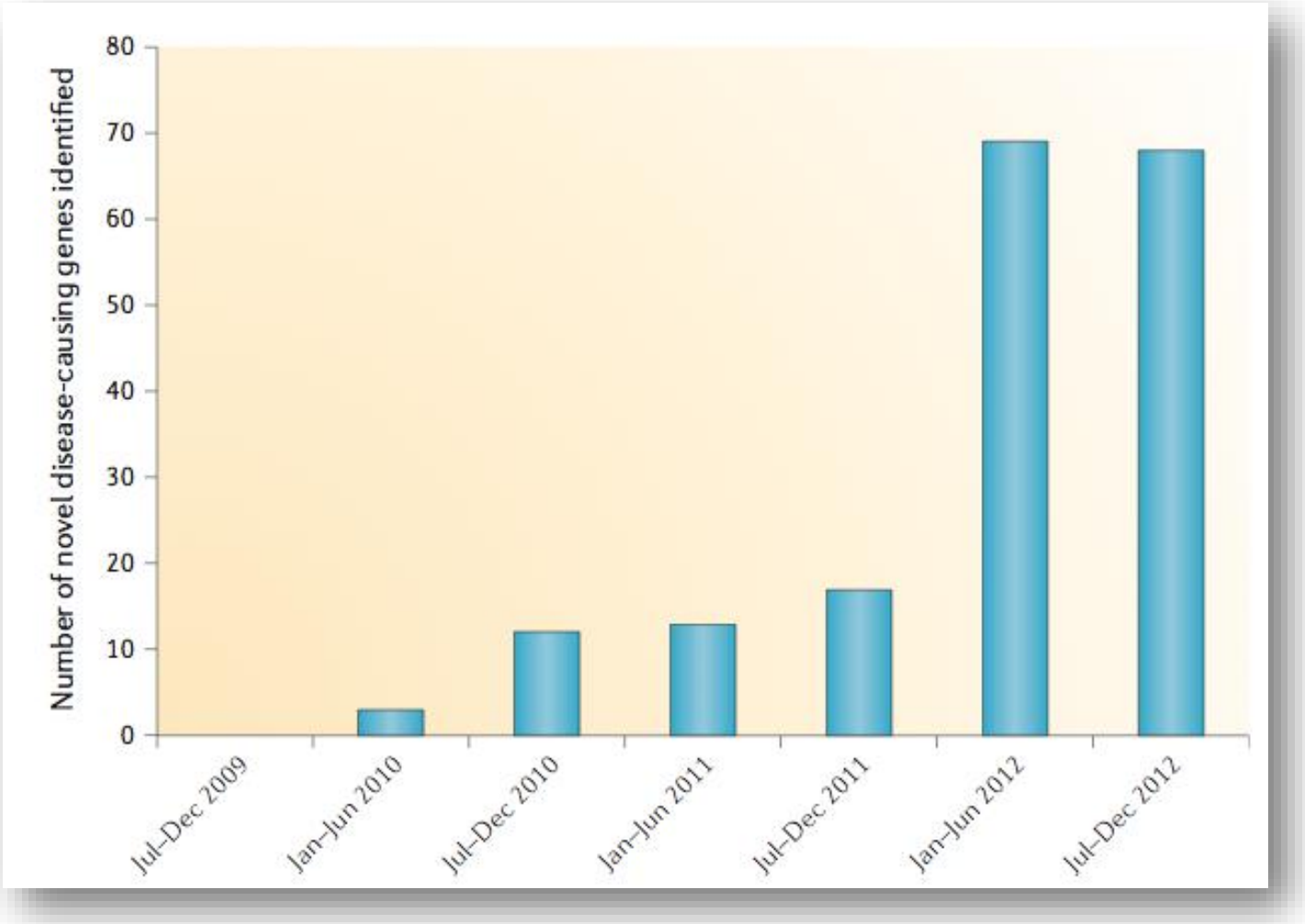
$ 3 billion
13 years

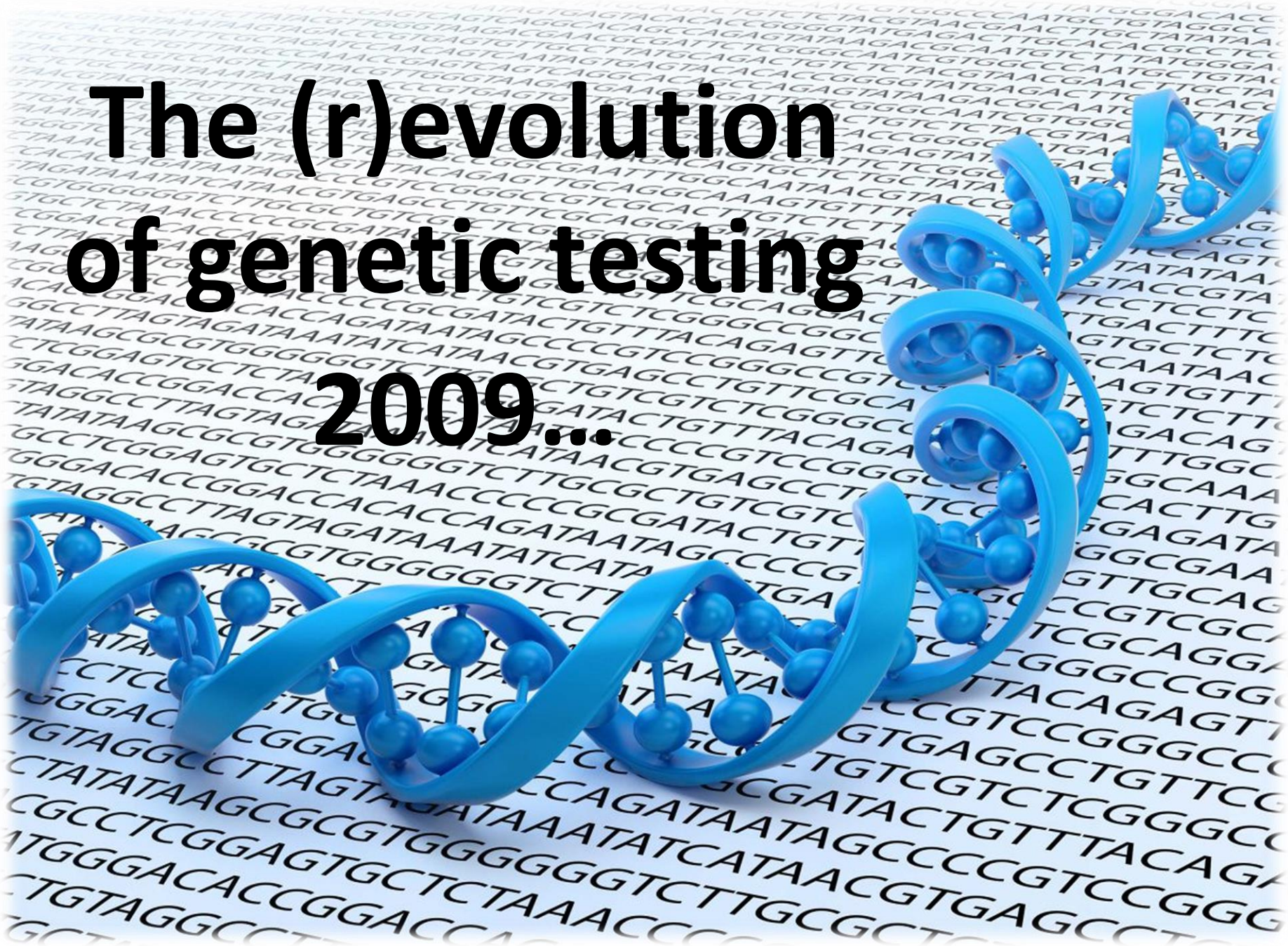
2010
1000's GP

$ 120 million
2 years

The (r)evolution of sequencing

Pace of discovery of novel rare-disease-causing genes using whole-exome sequencing

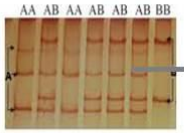


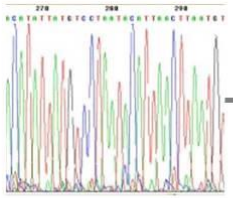

The (r)evolution
of genetic testing
2009…

Bergamo Experience 2001-2015

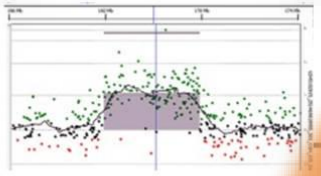
2001 → 2008 → 2011 → 2014 ⇢ 2016

SSCP



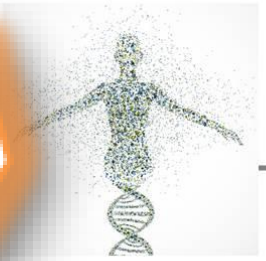


SEQUENCE ANALYSIS



SEQUENCE ANALYSIS
+ MLPA
+ ARRAY

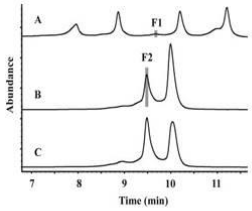
Analysis of entire coding sequences



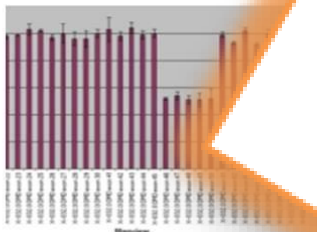
WHOLE EXOME SEQUENCING (clinical)

NGS WATERSHED

DHPLC



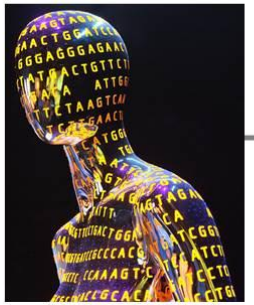
SEQUENCE ANALYSIS
+ MLPA



JAG1 and NOTCH1 analysis for point mutation and deletions and insertion

TARGETED SEQUENCING

Exome analysis of gene-panel



WHOLE GENOME SEQUENCING (clinical)

Genetic Testing & Diagnostic Yield

DECREASING DIAGNOSTIC YIELD

SINGLE GENE DISORDERS WITH HIGH DIAGNOSTIC YIELD → CYSTIC FIBROSIS

Targeted NGS

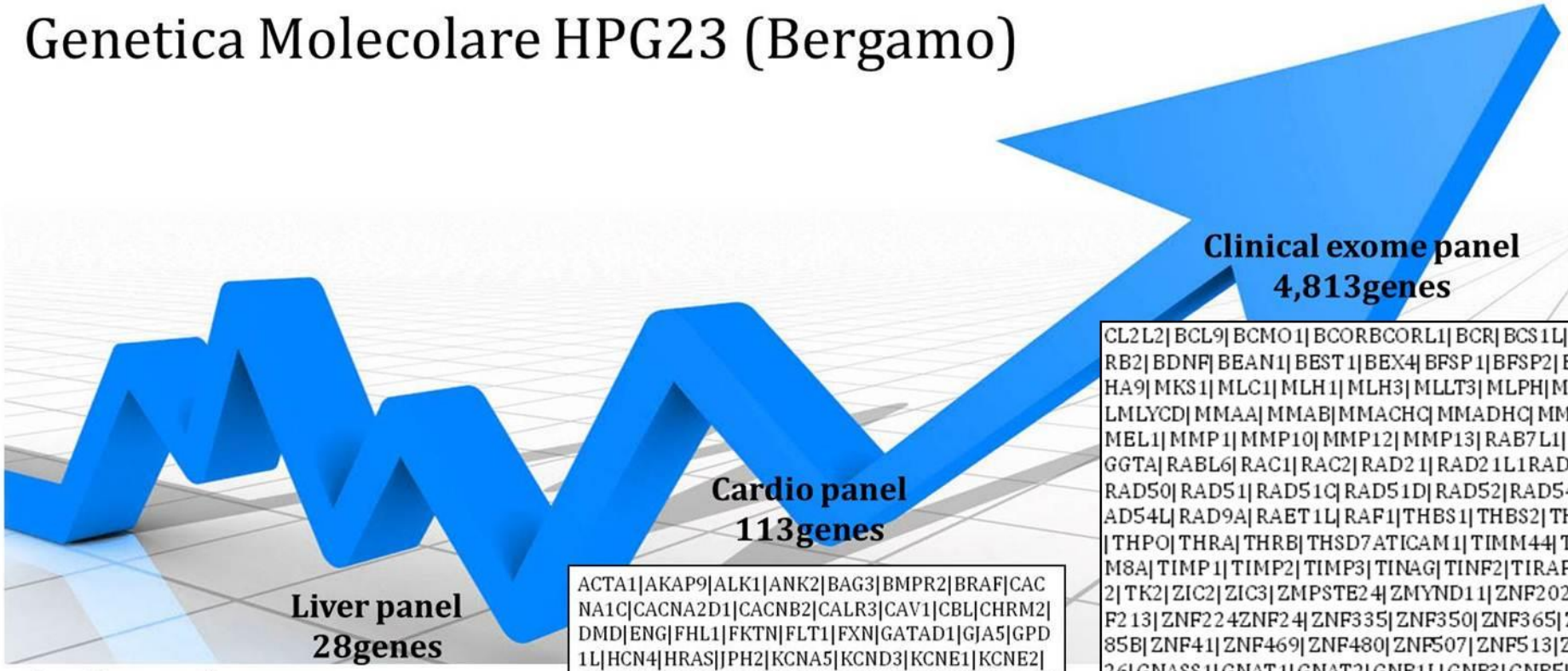
HOMOGENEOUS DISORDERS WHERE ≥1 OF A LARGE N° OF GENES MAY BE IMPLICATED → CARDIOMYOPATHIES, EPILEPSIES, XLMR

Targeted NGS

GENETIC DISORDERS WHERE AN APPROPRIATE SET OF GENES TO TEST CANNOT BE CLINICALLY DEFINED or VERY RARE DISORDERS → AUTISM, CHD, MALFORMATIONS, …

Whole Exome NGS

Genetica Molecolare HPG23 (Bergamo)



Aortic panel 19genes

ACTA2|CBS|COL1A2|COL3A1|COL5A1|COL5A2|EFEMP2|FBN1|FBN2|MYH11|MYLK|NOTCH1|PLOD1|SLC2A10|SMAD3|TGFB2|TGFBR1

ABCB11|ABCB4|ACADM|AKR1D1|ALDOB|AMACR|ATP7B|ATP8B1|BAAT|CYP7B1|DGUOK|EPHX1|FCYT/PKHD1|GAA|GALT|GBA|HSD3B7|JAG1|LIPA|NOTCH2|NPC1|NPC2|POLG|SERPINA1|SLC25A13|SMPD1|TJP2|UGT1A1

Liver panel 28genes

Cardio panel 113genes

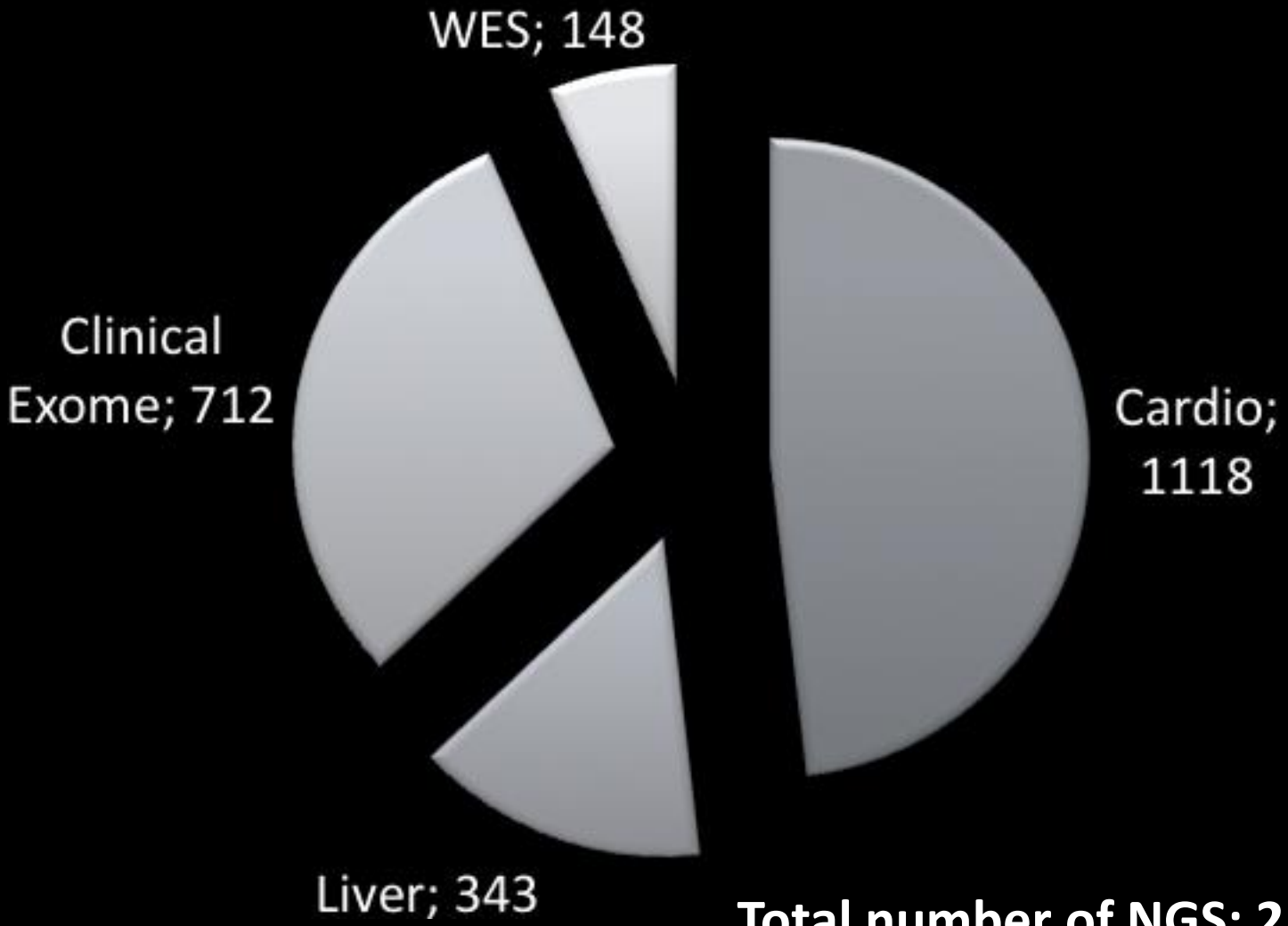
ACTA1|AKAP9|ALK1|ANK2|BAG3|BMPR2|BRAF|CACNA1C|CACNA2D1|CACNB2|CALR3|CAV1|CBL|CHRM2|DMD|ENG|FHL1|FKTN|FLT1|FXN|GATAD1|GJA5|GPD1L|HCN4|HRAS|JPH2|KCNA5|KCND3|KCNE1|KCNE2|KCNE3|KCNH2|KCNJ2|KCNJ5|KCNJ8|KCNK3|KCNQ1|KRAS|LAMA2|MAP2K1|MAP2K2|MYOT|MYPN|NPPA|NRAS|PDLIM3|PTPN11|RAF1|RIT1|RYR1|SCN1B|SCN3B|SCN4B|SCN5A|SGCA|SGCB|SGCG|SHOC2|SMAD9|SNTA1|SOS1|SYNE1|TGFB3|TMPO|TRDN|TRIM63|TRPM4|ABCC9|ACTC1|ACTN2|ANKRD1|CASQ2|CAV3|CRYAB|CSRP3|CTF1|DES|DSC2|DSG2|DSP|DTNA|EMD|FHL2|GLA|JUP|LAMA4|LAMP2|LDB3|LMNA|MYBPC3|MYH6|MYH7|MYL2|MYL3|MYLK2|MYOZ2|NEXN|PKP2|PLN|PRKAG2|RBM20|RYR2|SGCD|TAZ|TCAP|TMEM43|TNNC1|TNNI3|TNNT2|TPM1|TTN|TTR|VCL

Clinical exome panel 4,813genes

CL2L2|BCL9|BCMO1|BCORBCORL1|BCR|BCS1L|BDKRB2|BDNF|BEAN1|BEST1|BEX4|BFSP1|BFSP2|BHLHA9|MKS1|MLC1|MLH1|MLH3|MLLT3|MLPH|MLXIPLMLYCD|MMAA|MMAB|MMACHC|MMADHC|MME|MMEL1|MMP1|MMP10|MMP12|MMP13|RAB7L1|RABGGTA|RABL6|RAC1|RAC2|RAD21|RAD21L1RAD23B|RAD50|RAD51|RAD51C|RAD51D|RAD52|RAD54B|RAD54L|RAD9A|RAET1L|RAF1|THBS1|THBS2|THBS4|THPO|THRA|THRB|THSD7ATICAM1|TIMM44|TIMM8A|TIMP1|TIMP2|TIMP3|TINAG|TINF2|TIRAP|TJP2|TK2|ZIC2|ZIC3|ZMPSTE24|ZMYND11|ZNF202|ZNF213|ZNF224ZNF24|ZNF335|ZNF350|ZNF365|ZNF385B|ZNF41|ZNF469|ZNF480|ZNF507|ZNF513|ZNF526|GNASS1|GNAT1|GNAT2|GNB1L|GNB3|GNB5|GNEGNMT|GNPAT|GNPTAB|GNPTG|GNRH1|GNRHR|GNS|GOLGA3|GOLGA5|GON4L|GORAB|CLCN5|CLCN7|CLCNKA|CLCNKB|CLDN1|CLDN14|CLDN16CLDN19|CLEC11A|CLEC2D|CLEC4M|CLEC7A|CLIC2|CLK2|CLMP|CLN3|CLN5|CLN6|HSP90AA1|HSP90B1|HSPA1A|HSPA1B|HSPA1L|HSPA5|HSPA8HSPA9|HSPB1|HSPB3|HSPB6|HSPB7|HSPB8|HSPD1|HSPG2|HTN3|HTR1A|HTR1B|DAPK1|DARC|DARS2|DAZ1|DAZ2|DAZ3|DAZLDBH|DBI|DBT|DCAF13|DCAF17|DCC|DCDC2|DCK|DCLK1|DCLRE1C|DCN|...plus 3000 genes

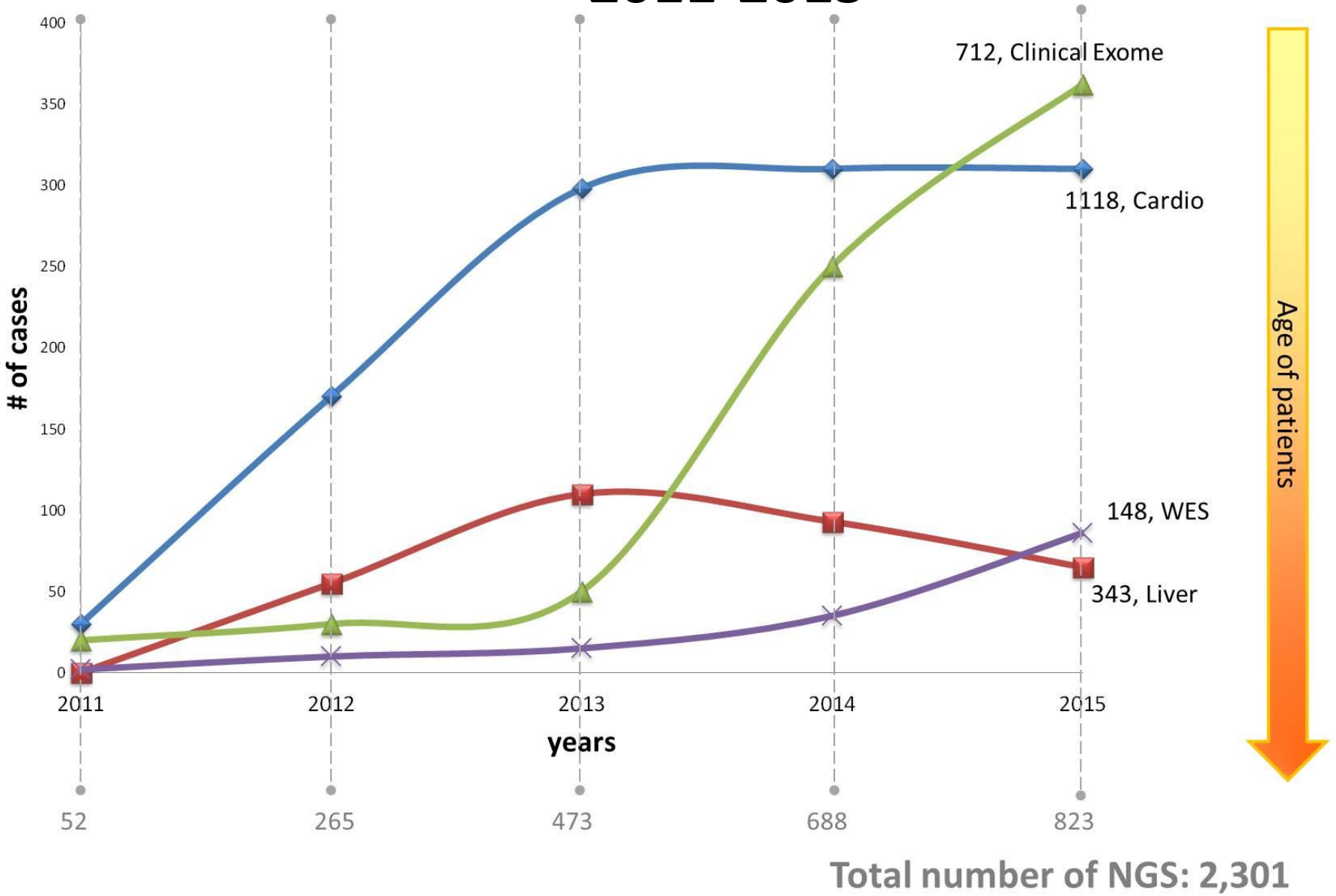
2009-2015…

NGS Bergamo Experience 2011-2015



Total number of NGS: 2,301

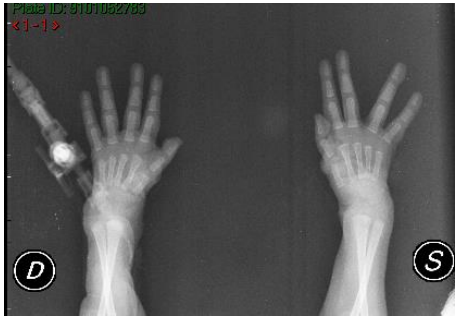
NGS Bergamo Experience 2011-2015

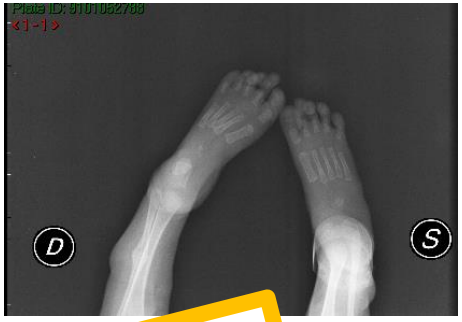


Total number of NGS: 2,301

The (r)evolution of genetic lab

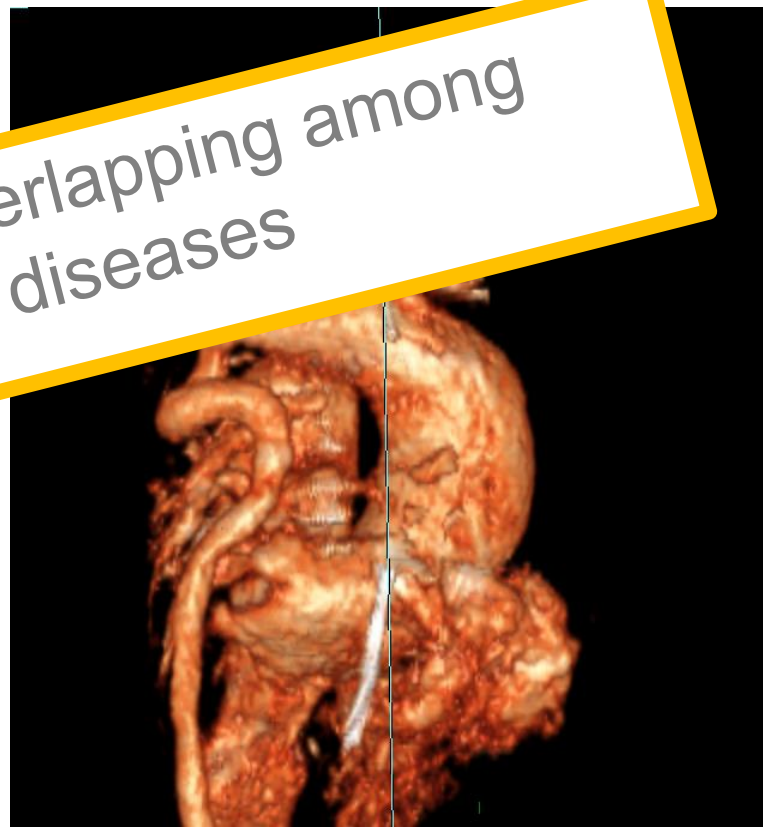
2011: 1st WES in clinic...





O.A., female, 6 months

- Arterial tortuosity
- Aortic aneurism
- Joint laxity
- Arachnodactyly
- Pectus excavatu
- Dis
-
- gulnity



Extensive phenotypic overlapping among several different diseases

Filtering Types... driven by

- Biology
- Genetics
- Phenotype

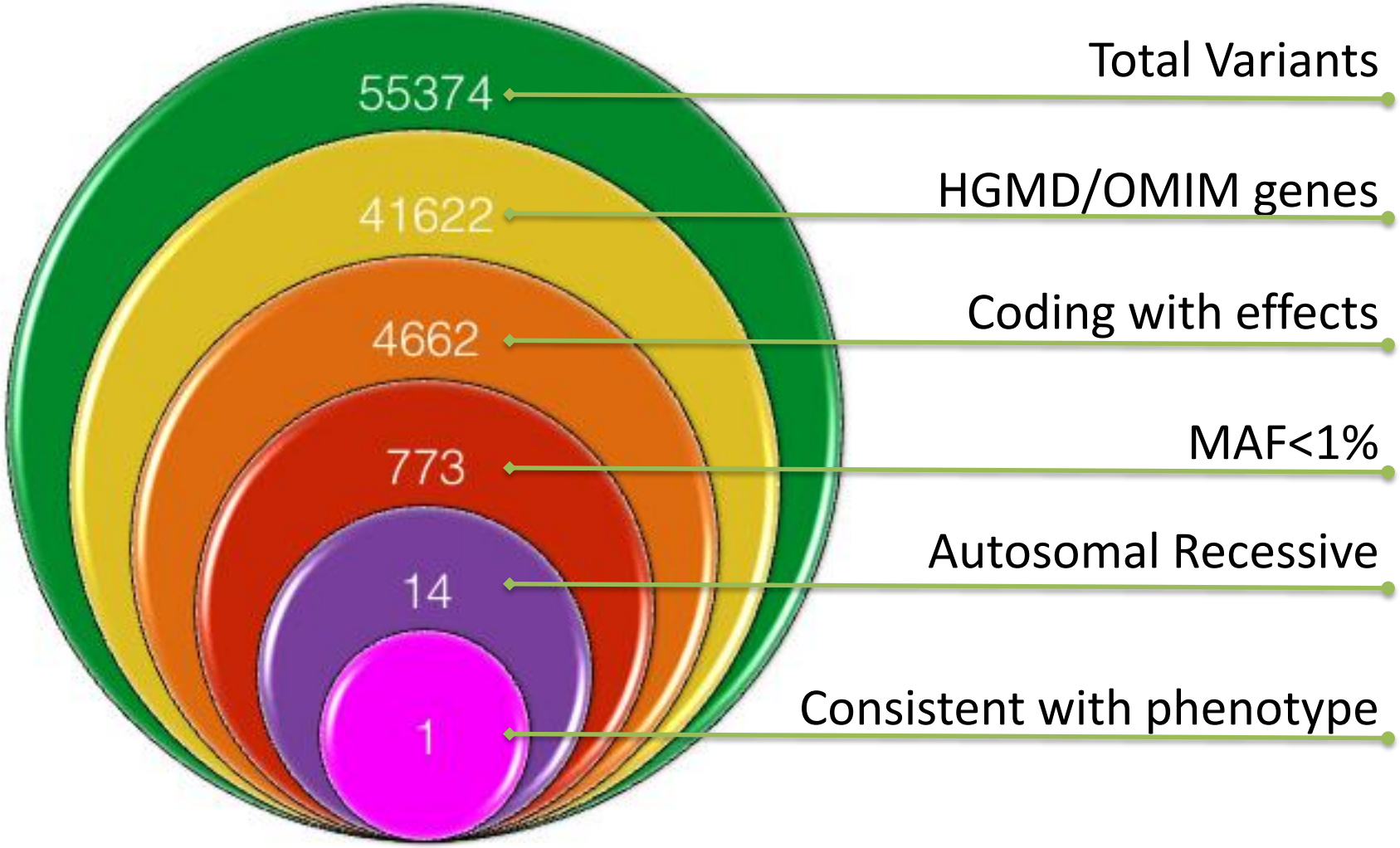
Biology & Genetics

- Effect on protein/transcript
- Minor Allele Frequency
- ...
- Recessive, X-linked, dominant , “de novo” model
- ...

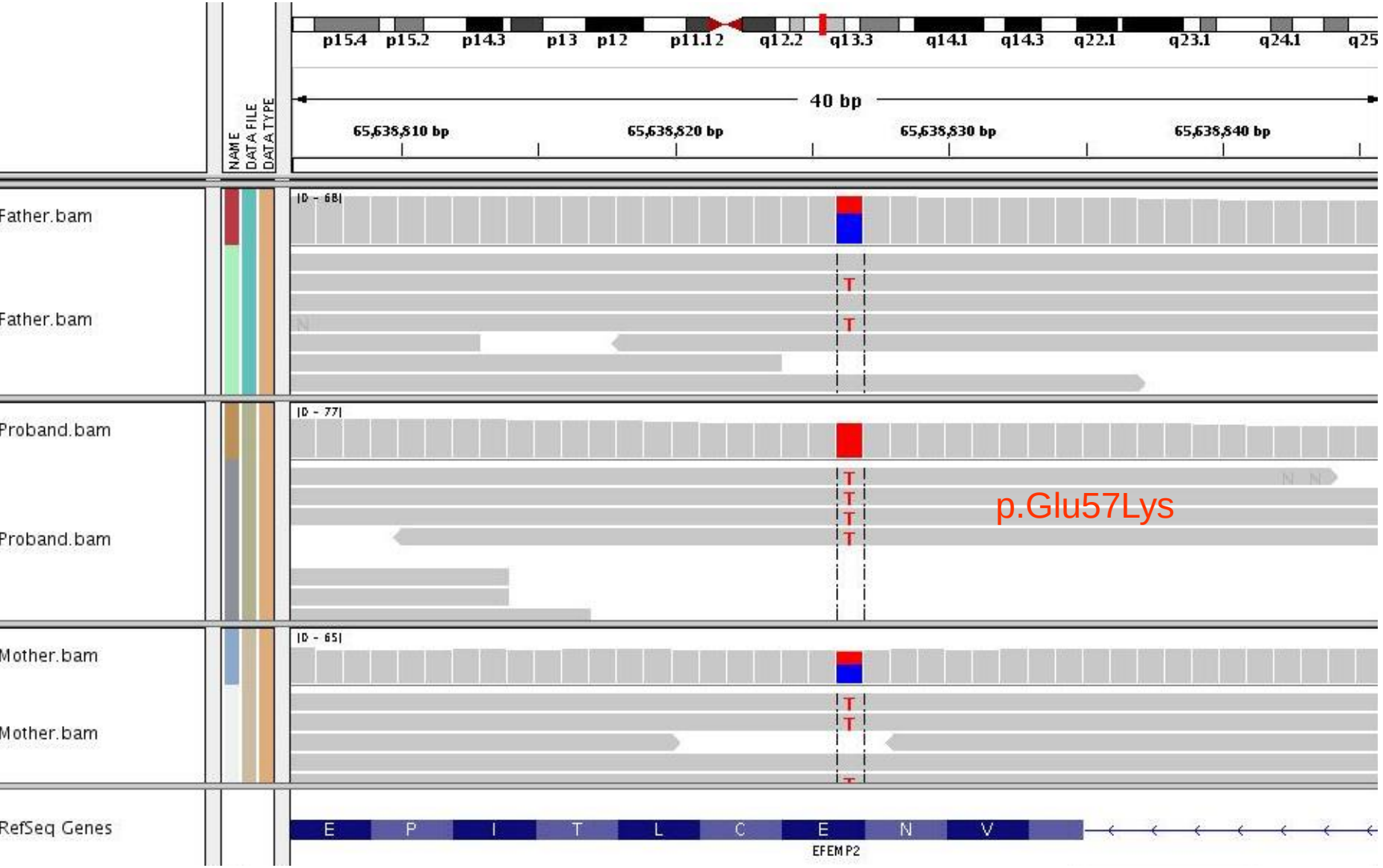
Clinical-driven Filtering strategies...

- Inheritance model (family history, etc)
- Clinical suspicion
- Phenotype-driven classification (HPO terms)
- Clinical Follow-up

Results


55374
41622
4662
773
14
1
Total Variants
HGMD/OMIM genes
Coding with effects
MAF<1%
Autosomal Recessive
Consistent with phenotype

Whole-exome sequencing



EGF containing fibulin-like extracellular matrix protein 2

Am J Hum Genet 2006

O.A, 6 months

- Autosomal recessive cutis laxa 1B (#614437)
- Only 6 cases reported in literature

Iascone et al Circulation 2012

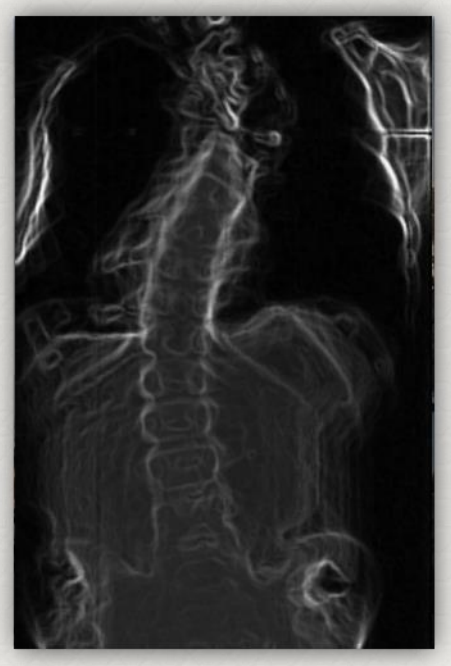
Pros	Cons
• Unlimited	• Costs • Turn Around Time


WHOSE
WHEN
WHO
WHOM
WHY
WHAT
HOW
WHAT
WHY
WHEN
WHO
HOW
WHERE
Clinical
Exome
Sequencing

F.B, male 6 yrs

2012

- Hypotonia at birth
- Mild dysmorphism
- Hyperelastic skin
- Joints hypermobility
- Kyphoscoliosis
- Myopathy and muscular atrophy
- Bladder diverticulum
- Myopia
- Congenital sensorineural deafness



Rupture of hypogastric artery aneurysm

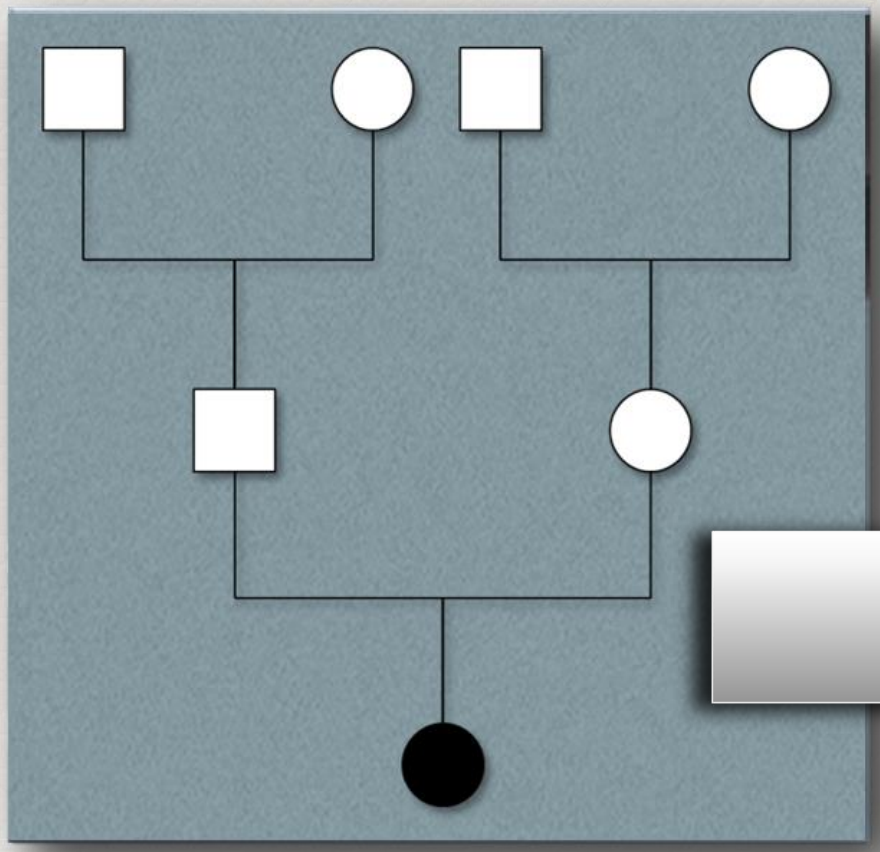
F.B, male 6 yrs

2012

2008. Karyotype: negative
2008. PWS / AS methylation test: negative
2009. Targeted mutation analysis of GJB2: heterozygous mutation
2009. Urinary assay of lysyl-and hydroxy-lysyl pyridinoline (Zurich Children's Hospital Suspected Ehlers-Danlos): negative
2009. Muscle biopsy: nemaline myopathy ?
2010. Sequencing analysis ACTA1, TPM2, TPM3: negative
2011. array CGH: negative

F.B, male 6 yrs

2012



Clinical Exome
2.761 disease-genes

F.B, male 6 yrs

2012

Clinical Exome
2.761 disease-genes

F.B, male 6 yrs

2008. Karyotype: negative
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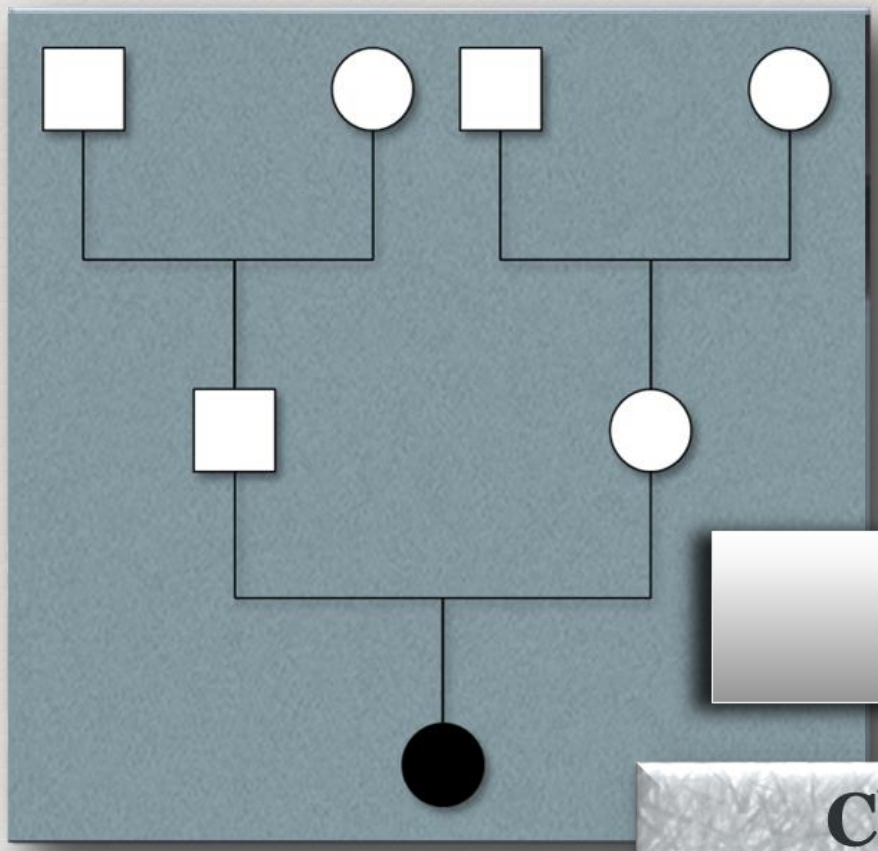
2009. Urinary assay of lysyl-and hydroxy-lysyl pyridinoline (Zurich Children's Hospital Suspected Ehlers-Danlos): negative
2009. Muscle biopsy: nemaline myopathy ?
2010. Sequencing analysis ACTA1, TPM2, TPM3: negative
2011. array CGH: negative
2012. NGS Analysis of 2.761 disease-genes negative


but technology
progresses *more*
& *more* becoming
always *more* &
more cheaper…

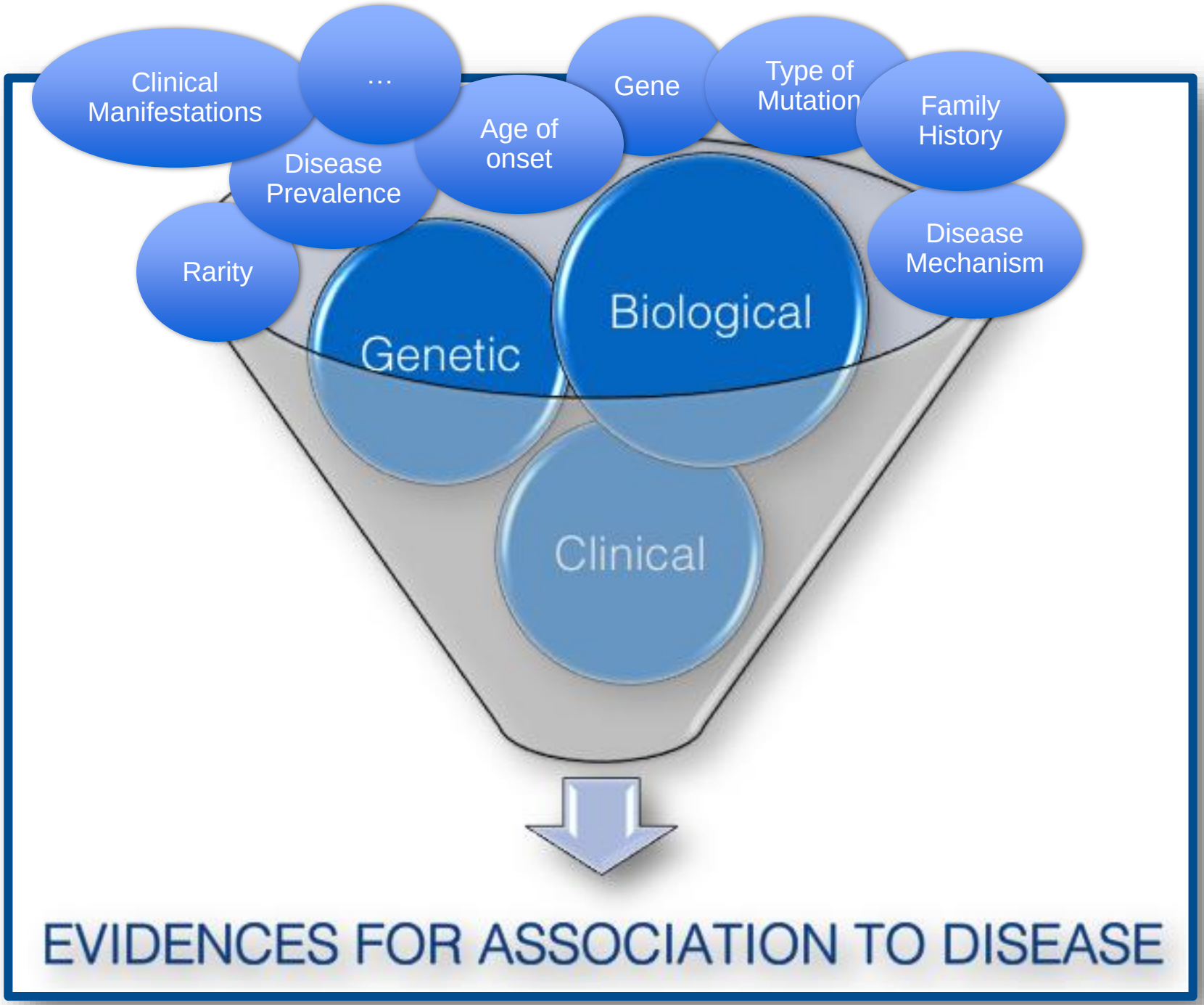
…expanding the number of analyzed genes

F.B, male 6 yrs

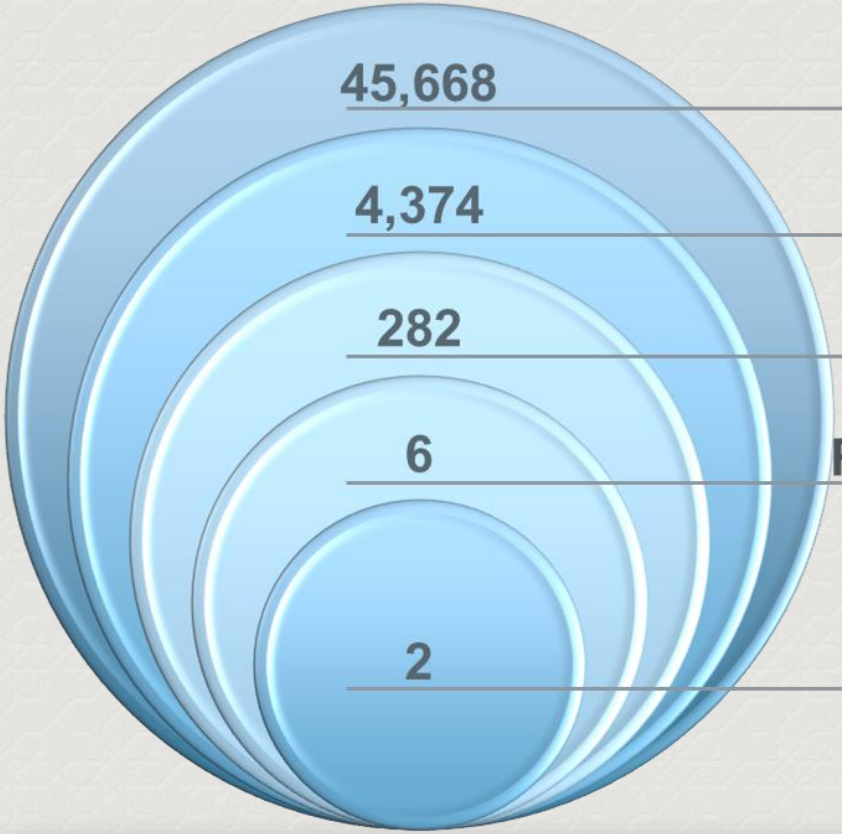


Clinical Exome
2.761 disease-genes

Clinical Exome
4,813 disease-genes

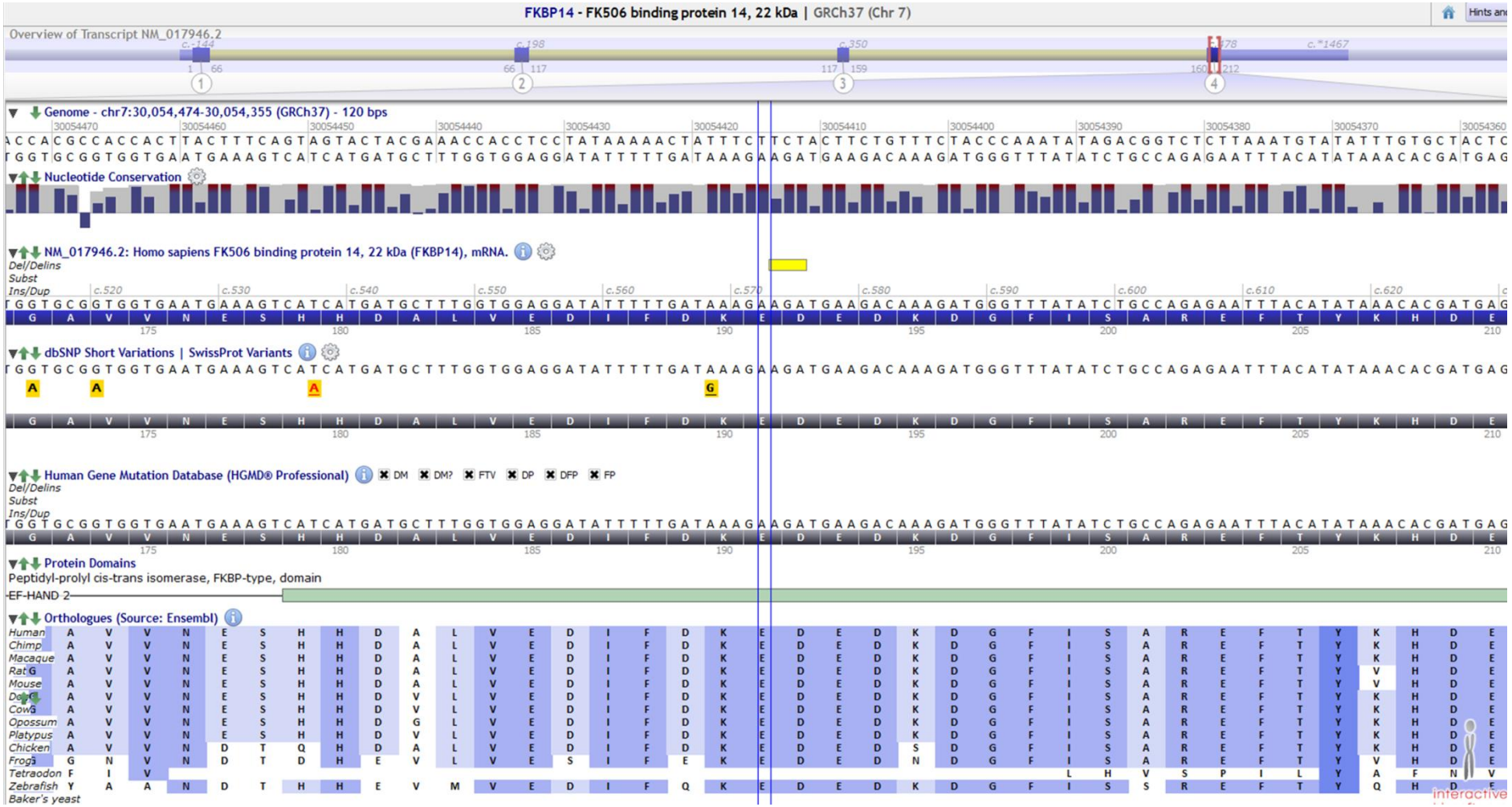

Clinical Manifestations
…
Gene
Type of Mutation
Family History
Age of onset
Disease Prevalence
Rarity
Disease Mechanism
Genetic
Biological
Clinical
EVIDENCES FOR ASSOCIATION TO DISEASE

F.B, male 6 yrs



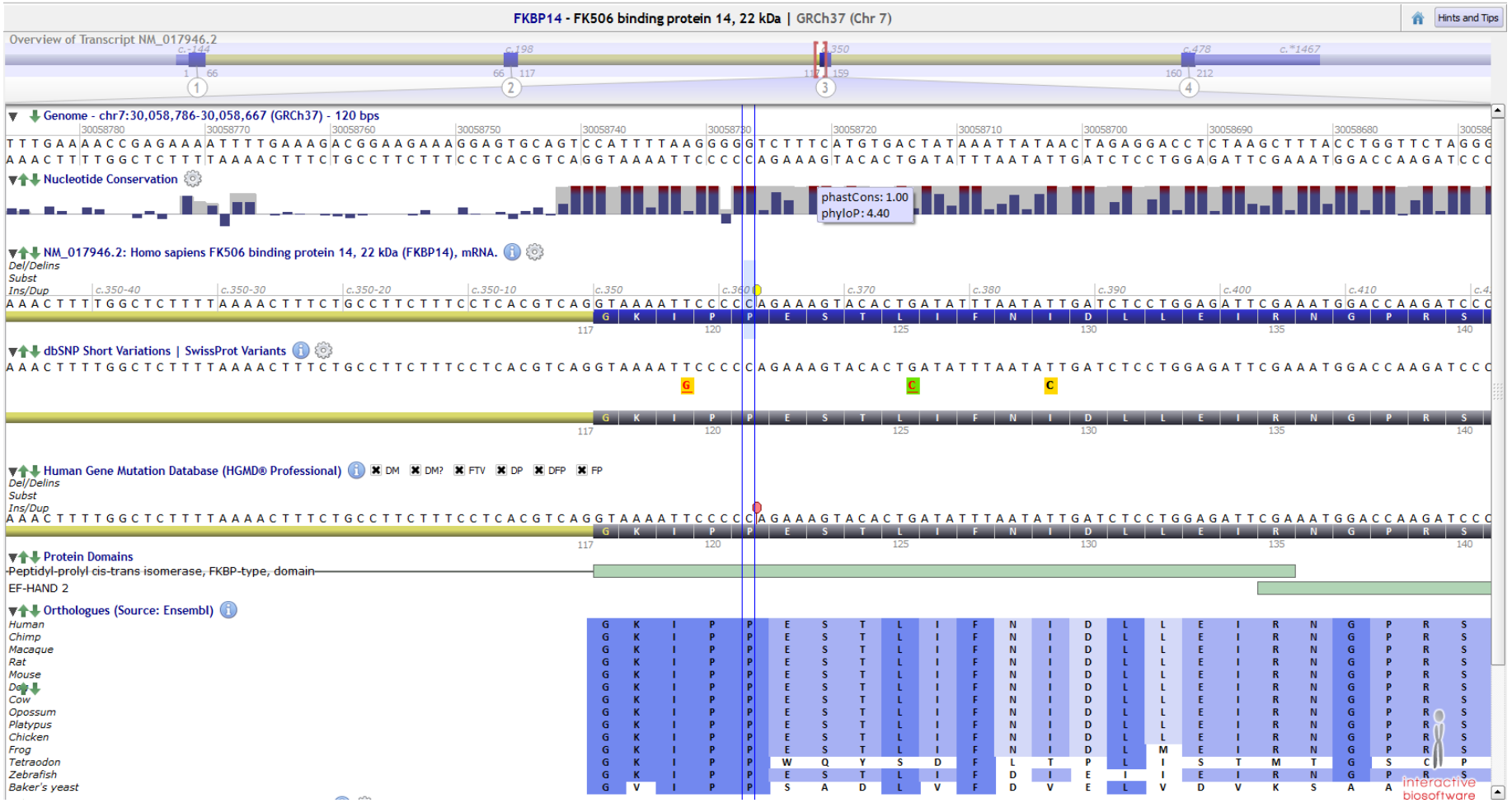
Clinical Exome
4,813 disease-genes

NM_017946.2(FKBP14):c.573_575del p.Glu191del (maternal)



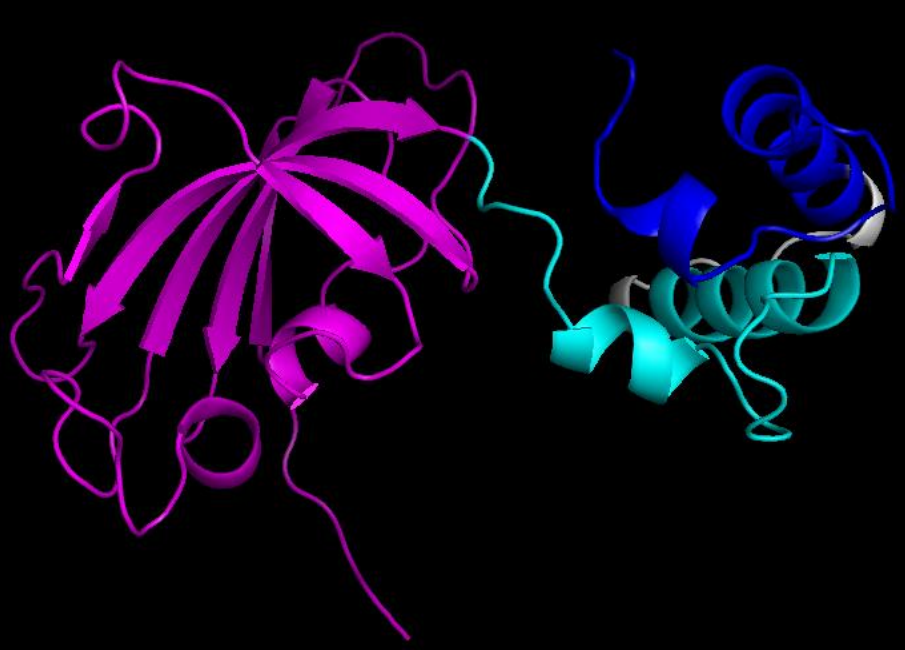
The protein encoded by this gene is a member of the FK506-binding protein family of peptidyl-prolyl cis-trans isomerases. The encoded protein is found in the lumen of the endoplasmic reticulum, where it is thought to accelerate protein folding

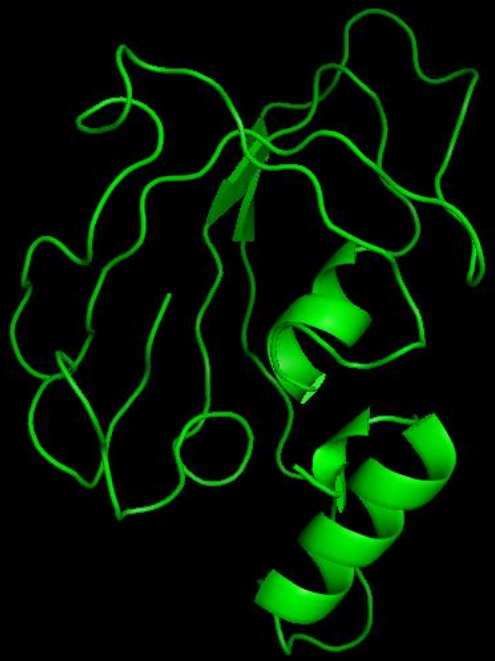
NM_017946.2(FKBP14):c.362dup p.Glu122Argfs*7 (paternal)

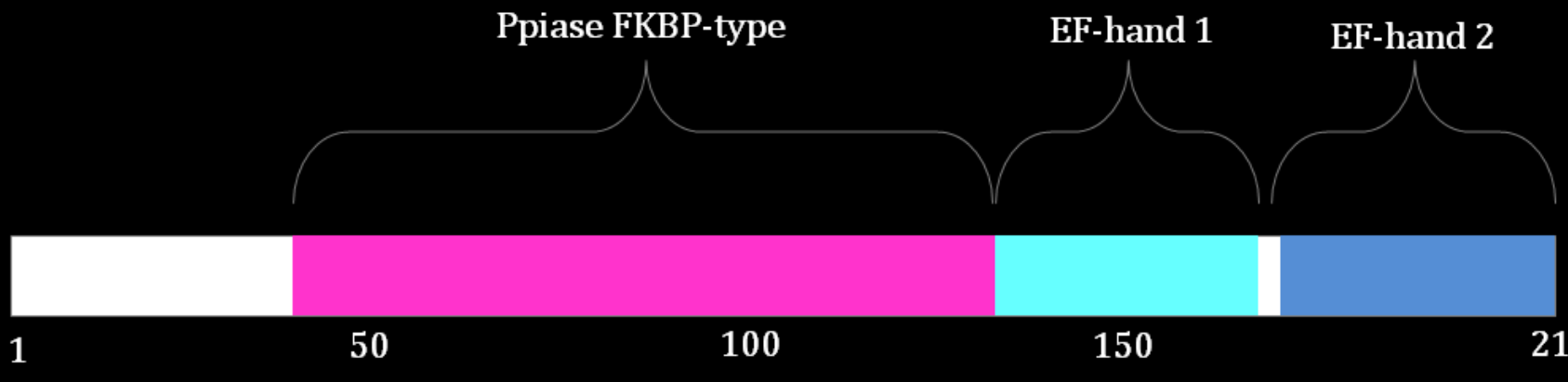


FKBP14 wild-type

FKBP14 Glu122Argfs*7

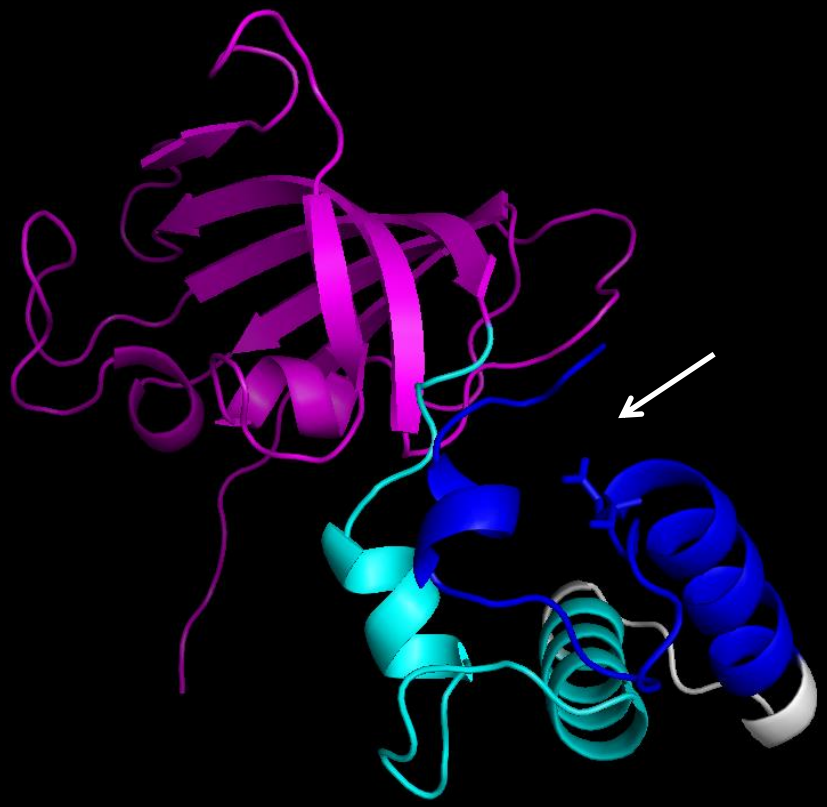


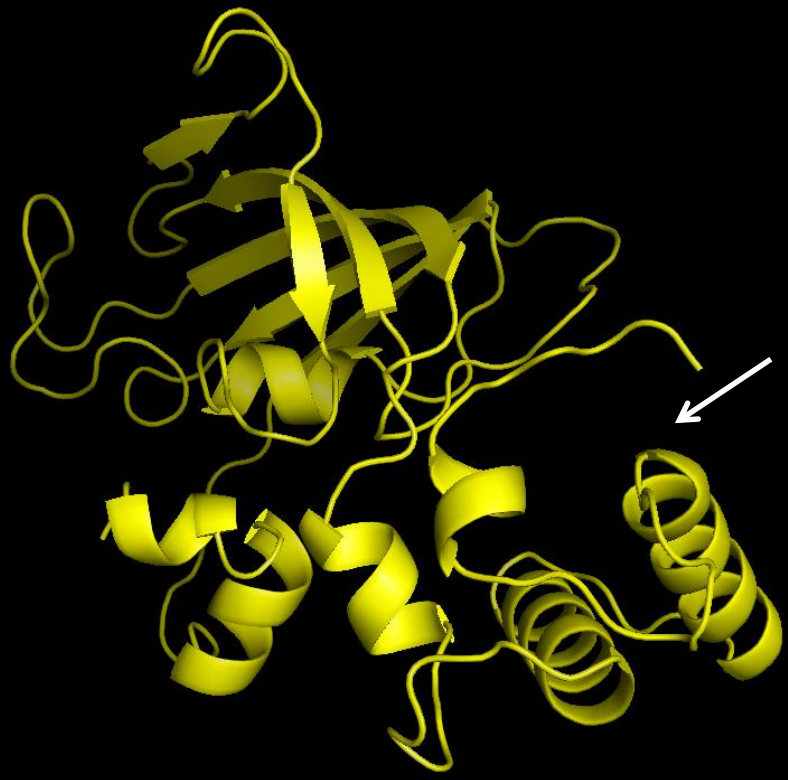


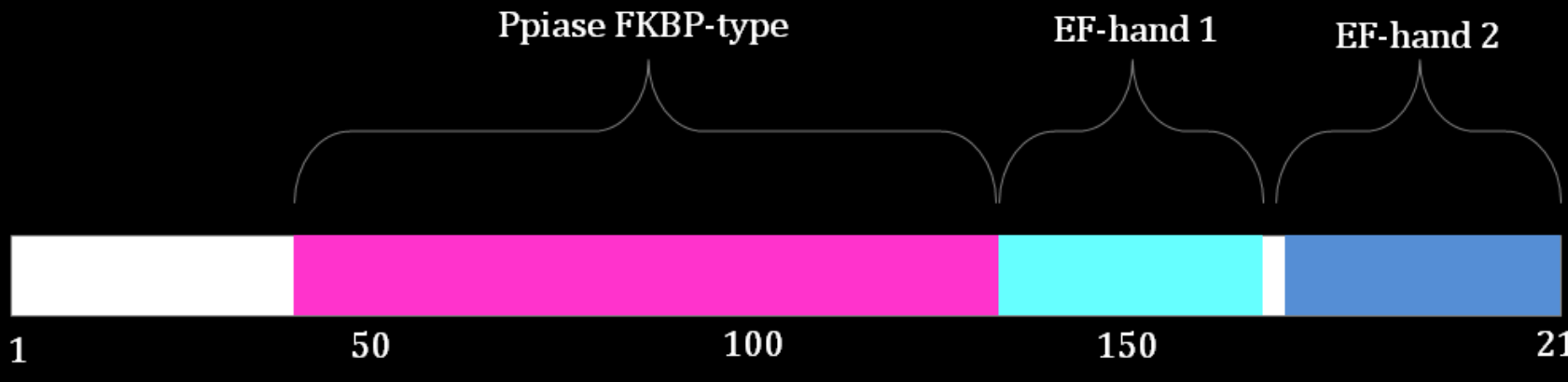


FKBP14 wild-type

FKBP14 Glu191del







Mutations in *FKBP14* Cause a Variant of Ehlers-Danlos Syndrome with Progressive Kyphoscoliosis, Myopathy, and Hearing Loss

Matthias Baumann,[1,14,*] Cecilia Giunta,[2,14] Birgit Krabichler,[3] Franz Rüschendorf,[4] Nicoletta Zoppi,[5] Marina Colombi,[5] Reginald E. Bittner,[6] Susana Quijano-Roy,[7] Francesco Muntoni,[8] Sebahattin Cirak,[8] Gudrun Schreiber,[9] Yaqun Zou,[10] Ying Hu,[10] Norma Beatriz Romero,[11] Robert Yves Carlier,[12] Albert Amberger,[3] Andrea Deutschmann,[3] Volker Straub,[13] Marianne Rohrbach,[2] Beat Steinmann,[2] Kevin Rostásy,[1] Daniela Karall,[1,3] Carsten G. Bönnemann,[10] Johannes Zschocke,[3] and Christine Fauth[3,*]

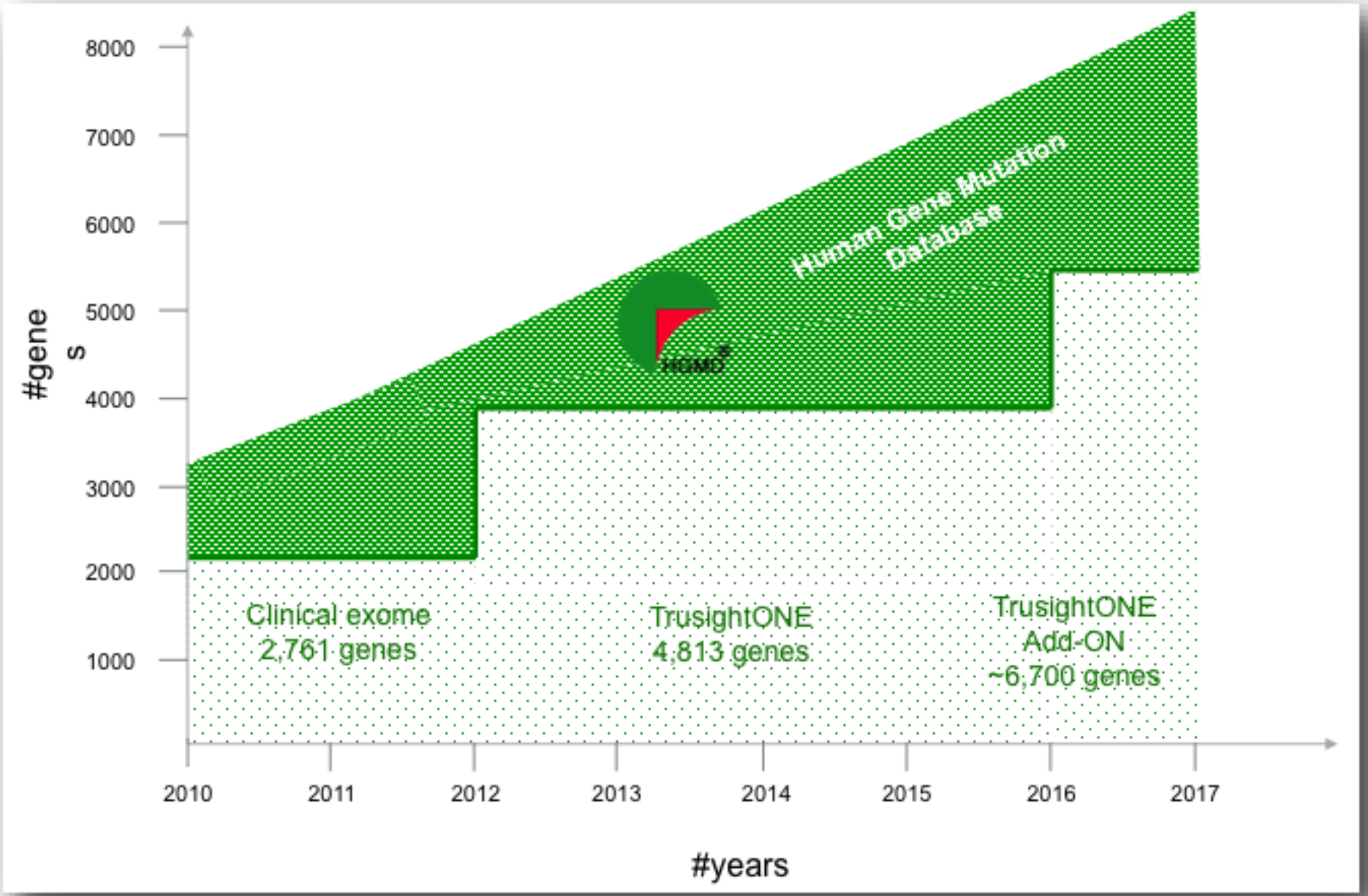
We report on an autosomal-recessive variant of Ehlers-Danlos syndrome (EDS) characterized by severe muscle hypotonia at birth, progressive scoliosis, joint hypermobility, hyperelastic skin, myopathy, sensorineural hearing impairment, and normal pyridinoline excretion in urine. Clinically, the disorder shares many features with the kyphoscoliotic type of EDS (EDS VIA) and Ullrich congenital muscular dystrophy. Linkage analysis in a large Tyrolean kindred identified a homozygous frameshift mutation in *FKBP14* in two affected individuals. Based on the cardinal clinical characteristics of the disorder, four additional individuals originating from different European countries were identified who carried either homozygous or compound heterozygous mutations in *FKBP14*. FKBP14 belongs to the family of FK506-binding peptidyl-prolyl *cis-trans* isomerases (PPIases). ER-resident FKBPs have been suggested to act as folding catalysts by accelerating *cis-trans* isomerization of peptidyl-prolyl bonds and to act occasionally also as chaperones. We demonstrate that FKBP14 is localized in the endoplasmic reticulum (ER) and that deficiency of FKBP14 leads to enlarged ER cisterns in dermal fibroblasts in vivo. Furthermore, indirect immunofluorescence of FKBP14-deficient fibroblasts indicated an altered assembly of the extracellular matrix in vitro. These findings suggest that a disturbance of protein folding in the ER affecting one or more components of the extracellular matrix might cause the generalized connective tissue involvement in this disorder. *FKBP14* mutation analysis should be considered in all individuals with apparent kyphoscoliotic type of EDS and normal urinary pyridinoline excretion, in particular in conjunction with sensorineural hearing impairment.

The American Journal of Human Genetics *90*, 201–216, February 10, 2012

Table 1. Salient Clinical Findings

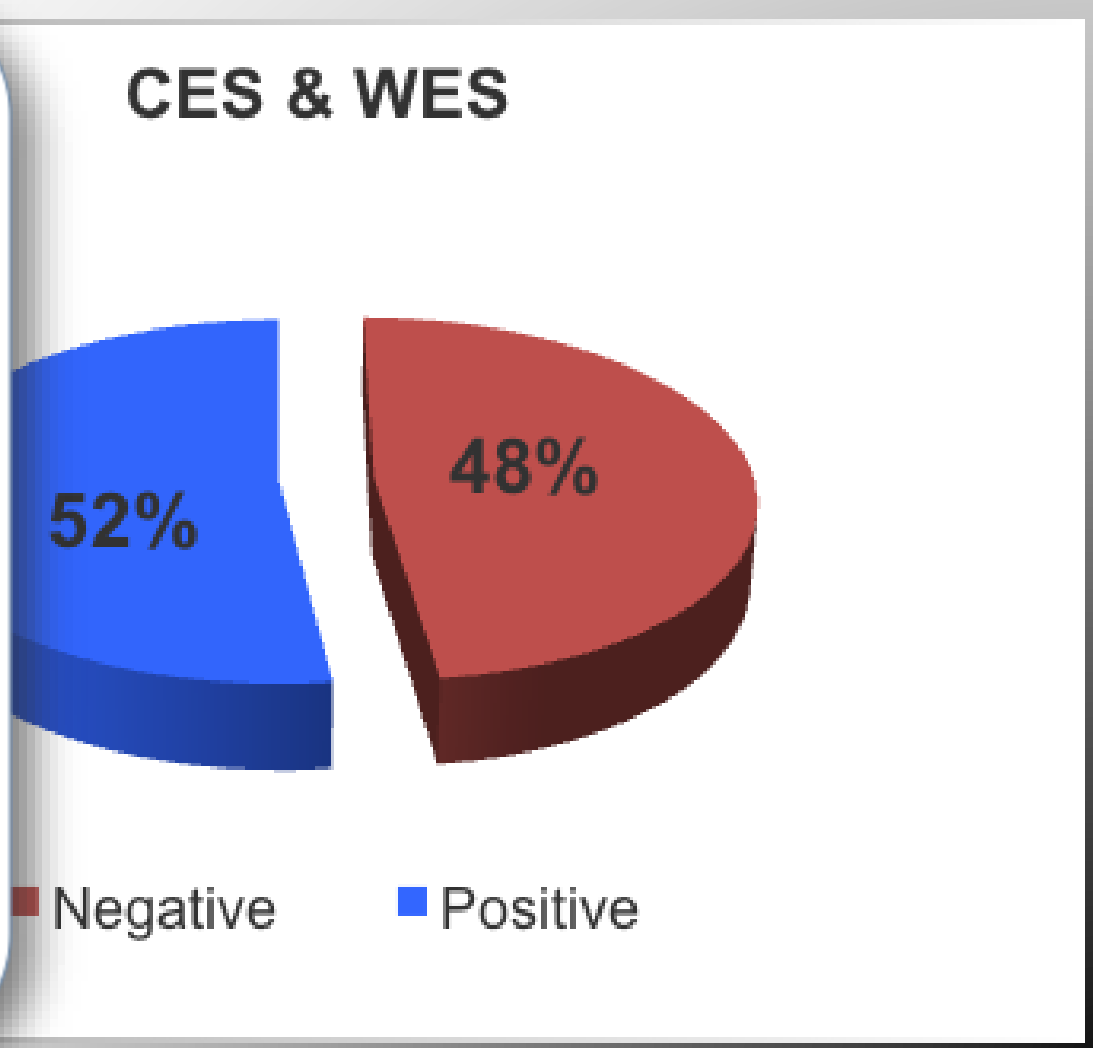
	P1	P2	P*	P3	P4	P5	P6
Current age/gender	**16 y/M**	**48 y/F**	**12 y/F[a]**	**11 y/F**	**16 y/F**	**11 y/M**	**3 y/F**
Origin	**Austria**	**Austria**	**Austria**	**Italy**	**France**	**Turkey**	**Germany**
Skin							
hyperelastic	+	(+)	nr	+	–	+	+
soft	+	+	nr	+	+	+	+
plantar softness	+	–	nr	+	+	+	+
follicular hyperkeratosis	+	–	+	+	+	–	+
easy bruising	–	+	nr	(+)	–	+	–
hypertrophic scars	(+)	–	nr	–	–	–	–
atrophic scars	–	–	nr	–	–	–	–
Joints							
hypermobility of large joints	+	+	+	+	++	++	+
hypermobility of small joints	++	+	+	++	++	++	++
Beighton score	6/9	6/9	nr	8/9	6/9	9/9	9/9
recurrent dislocations	–	–	–	–	–	++	–
joint contractures	–	–	–	–	–	–	–
Skeletal							
progressive kyphoscoliosis	++	++ (11 y op)	+	++ (4 y op)	++ (12 y op)	kyphosis	scoliosis
flat feet	+	+	+	+	+, club foot left	+	+, club foot left
fractures	–	–	–	–	–	(+)	–
Neuromuscular							
muscle hypotonia at birth	++	++	++	++	++	++	++
poor head control in infancy	+	+	+	+	+	+	+
weakness improving in infancy	+	+	+	+	+	+	+
delayed motor development	+	+	+	++	+	++	++
walking independently	2.5 y	2.5 y	2.5 y	4 y	2 y	4 y	3 y supported
muscular atrophy	+	+	(+)	(+)	+	(+)	(+)
current MRC muscle score	3–4	3–4	nr	4	4	3–4	3–4
Cardiovascular							
cardiomyopathy	–	nr	nr	–	–	–	–
valvular abnormalities	–	nr	nr	–	tricusp. insuf. I°	mitral and tricusp. insuf. I°	–
vascular abnormalities	–	–	aortic rupture	–	–	–	–
Eyes and Ears							
bluish sclerae	+ in infancy	–	nr	–	–	–	–
myopia	+	+	+	–	+	–	–
microcornea	–	–	nr	–	–	–	–
hearing impairment	sensorineural	sensorineural	nr	sensorineural	conductive	sensorineural	sensorineural
Miscellaneous							
herniae	inguinal	umbilical	–	umbilical	–	umbilical	–
bladder diverticulum	+	nr	nr	nr	nr	+	nr
cleft soft palate	–	–	–	–	–	+	+
retrogenia in infancy	–	–	–	–	–	+	+
	subdural hygroma					microcephaly, learning difficulties	

Biallelic mutations in FKBP14 cause a recessive form of Ehlers-Danlos syndrome characterized by progressive kyphoscoliosis, myopathy, and hearing loss.

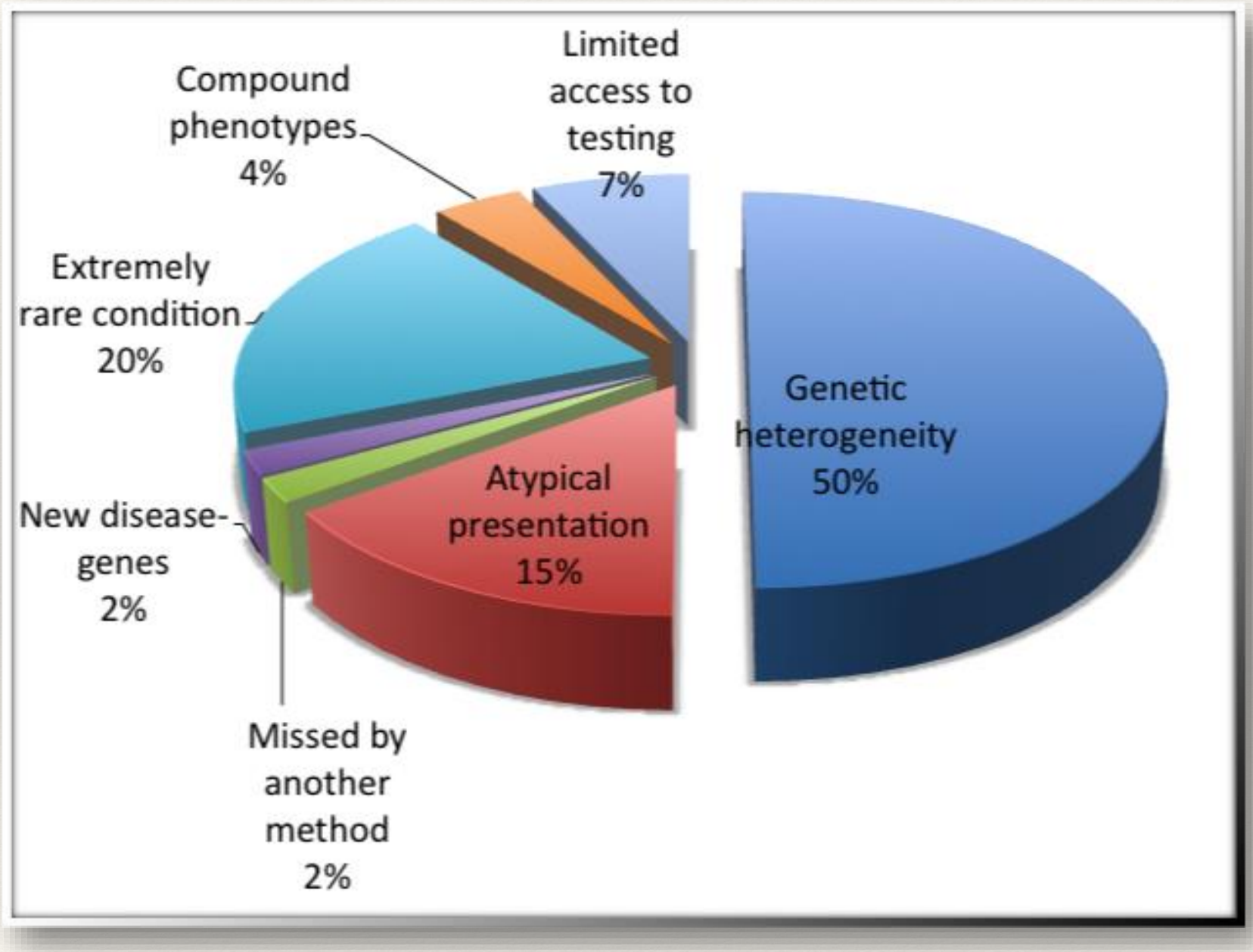

8000
7000
6000
5000
4000
3000
2000
1000
#genes
Human Gene Mutation Database
HGMD®
Clinical exome
2,761 genes
TrusightONE
4,813 genes
TrusightONE
Add-ON
~6,700 genes
2010
2011
2012
2013
2014
2015
2016
2017
#years

Bergamo Experience 2011-2015

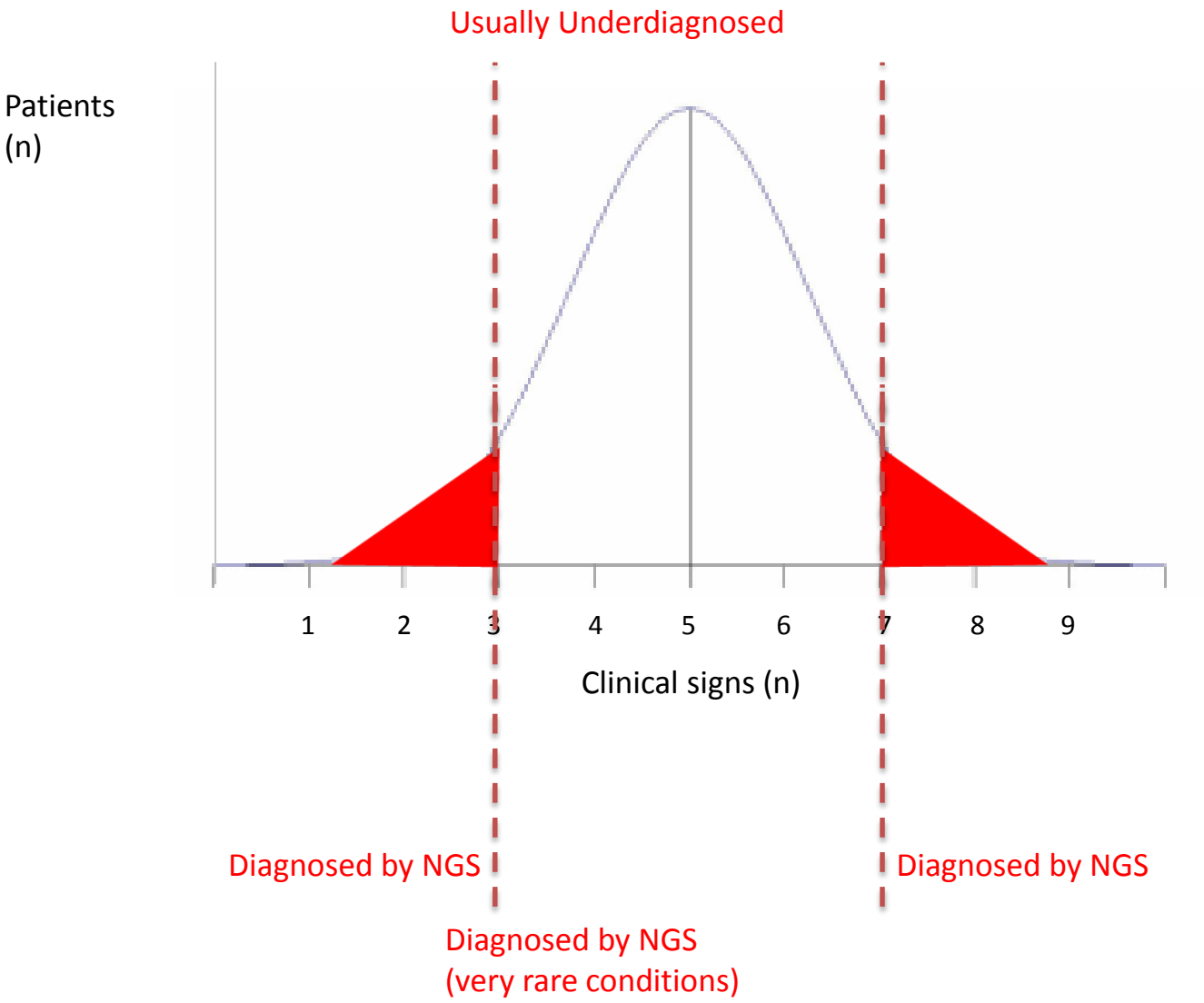
- Congenital lactase deficiency
- Adams-Oliver syndrome
- Crouzon syndrome
- Cutis laxa syndrome
- Tuberous sclerosis
- Craniofacial dysostosis
- Smith-Magenis syndrome
- Congenital disorder of glycosylation
- CHARGE syndrome
- Sotos syndrome
- Gaucher disease
- Niemann-Pick, NPC2
- Mitochondrial DNA depletion syndrome 6 (hepatocerebral type)
- Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia
- ....



Bergamo Experience 2011-2015



Spectrum of Clinical Manifestations




Analytic Validity
Clinical Validity
Clinical Utility
Turn Around Time
Cost

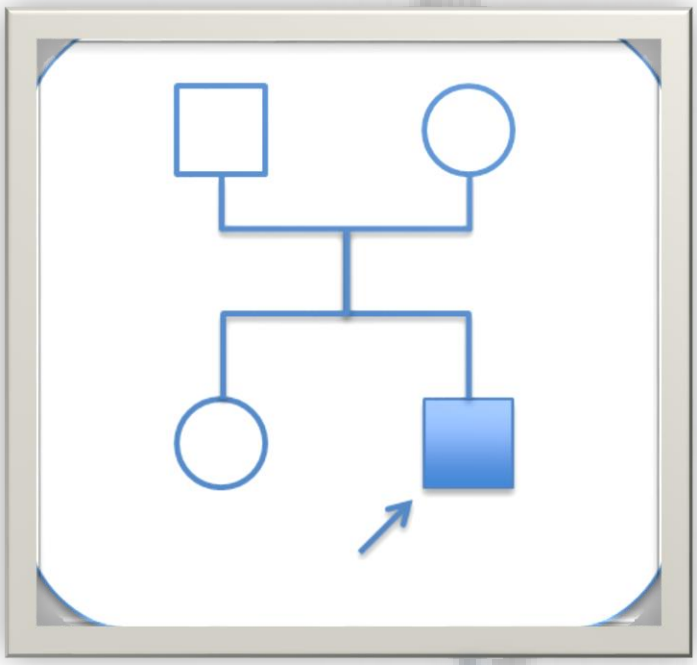
...time to diagnosis

… just an example

- Second child of healthy non consanguineous parents

- Age: 0 days
- Hypertonia
- Rigidity, axial and limb
- Lack of volitional movement
- Myoclonic seizures

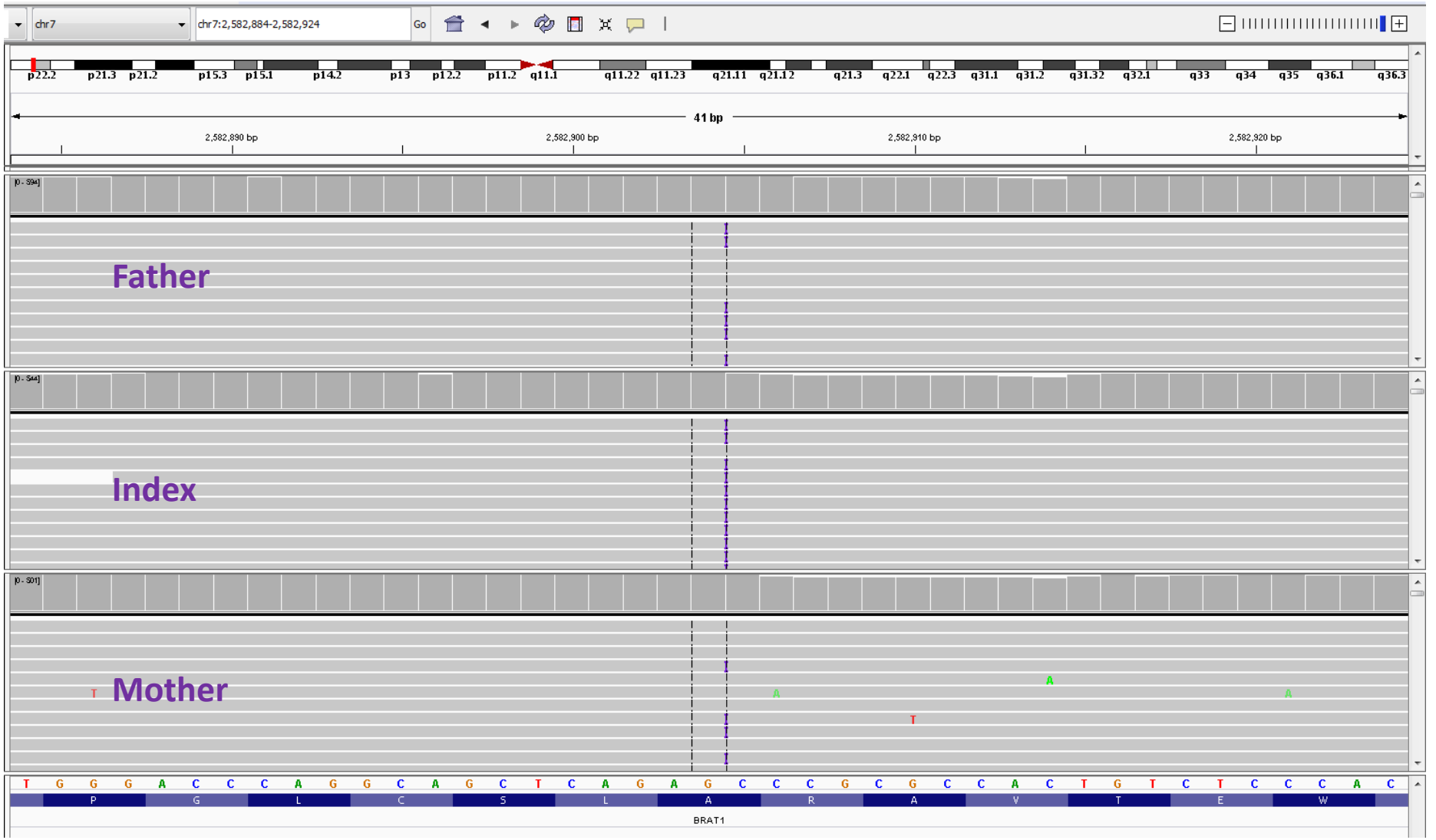
- Age: 15 days
- Worsening of neurological condition
- No response to any therapy



Supportive Care

Routine Metabolic Screening

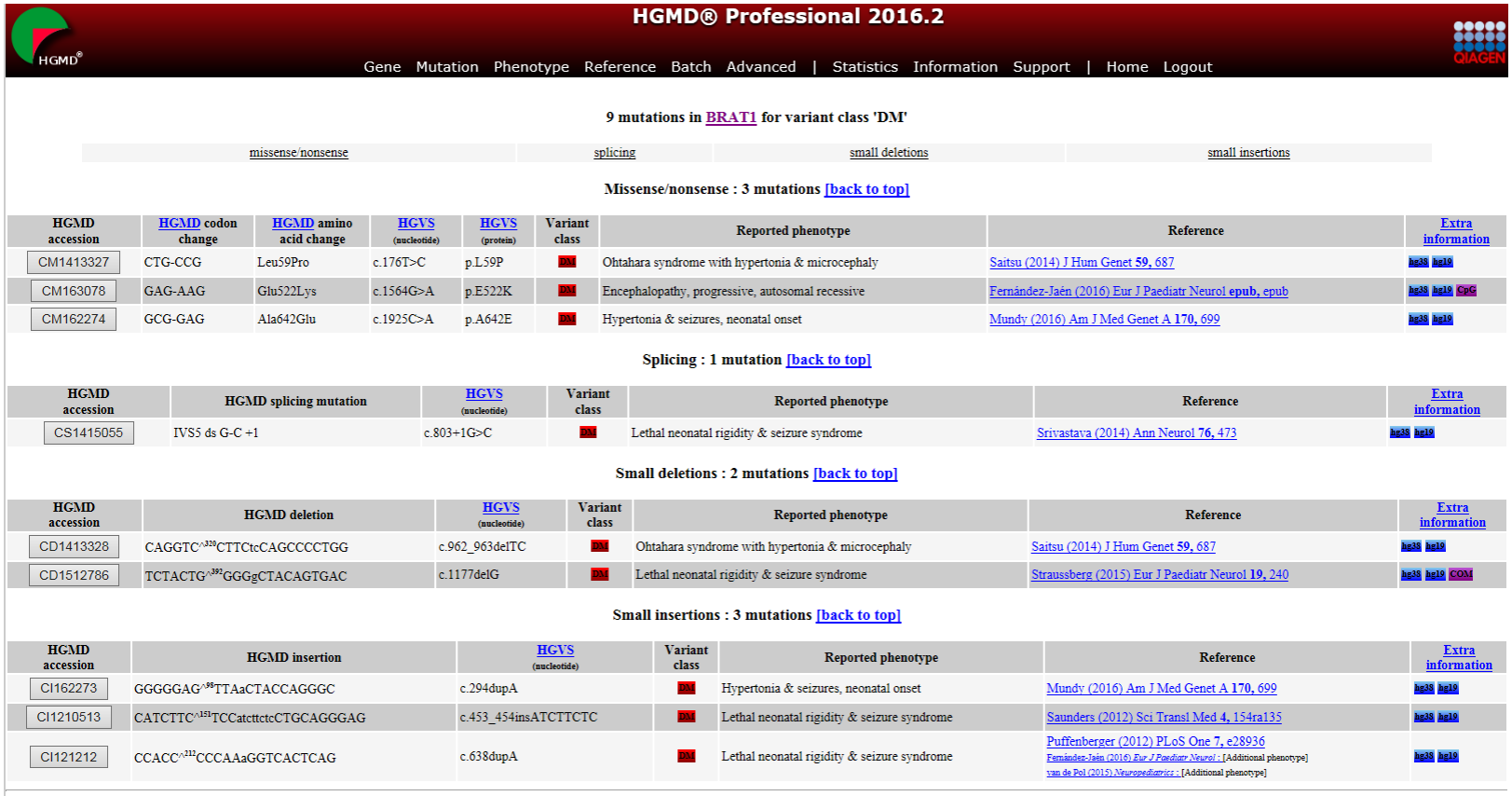
…Clinical Exome Sequencing Required



Homozygous

NM_152743.3:c.857dup

p.Leu287fs in *BRAT1* gene



HGMD® Professional 2016.2

Gene Mutation Phenotype Reference Batch Advanced | Statistics Information Support | Home Logout

9 mutations in BRAT1 for variant class 'DM'

missense/nonsense | splicing | small deletions | small insertions

Missense/nonsense : 3 mutations [back to top]

HGMD accession	HGMD codon change	HGMD amino acid change	HGVS (nucleotide)	HGVS (protein)	Variant class	Reported phenotype	Reference	Extra information
CM1413327	CTG-CCG	Leu59Pro	c.176T>C	p.L59P	DM	Ohtahara syndrome with hypertonia & microcephaly	Saitsu (2014) J Hum Genet 59, 687	hg38 hg19
CM163078	GAG-AAG	Glu522Lys	c.1564G>A	p.E522K	DM	Encephalopathy, progressive, autosomal recessive	Fernández-Jaén (2016) Eur J Paediatr Neurol epub, epub	hg38 hg19 CpG
CM162274	GCG-GAG	Ala642Glu	c.1925C>A	p.A642E	DM	Hypertonia & seizures, neonatal onset	Mundy (2016) Am J Med Genet A 170, 699	hg38 hg19

Splicing : 1 mutation [back to top]

HGMD accession	HGMD splicing mutation	HGVS (nucleotide)	Variant class	Reported phenotype	Reference	Extra information
CS1415055	IVS5 ds G-C +1	c.803+1G>C	DM	Lethal neonatal rigidity & seizure syndrome	Srivastava (2014) Ann Neurol 76, 473	hg38 hg19

Small deletions : 2 mutations [back to top]

HGMD accession	HGMD deletion	HGVS (nucleotide)	Variant class	Reported phenotype	Reference	Extra information
CD1413328	CAGGTC^329CTTCtcCAGCCCCTGG	c.962_963delTC	DM	Ohtahara syndrome with hypertonia & microcephaly	Saitsu (2014) J Hum Genet 59, 687	hg38 hg19
CD1512786	TCTACTG^392GGGgCTACAGTGAC	c.1177delG	DM	Lethal neonatal rigidity & seizure syndrome	Straussberg (2015) Eur J Paediatr Neurol 19, 240	hg38 hg19 COM

Small insertions : 3 mutations [back to top]

HGMD accession	HGMD insertion	HGVS (nucleotide)	Variant class	Reported phenotype	Reference	Extra information
CI162273	GGGGGAG^98TTAaCTACCAGGGC	c.294dupA	DM	Hypertonia & seizures, neonatal onset	Mundy (2016) Am J Med Genet A 170, 699	hg38 hg19
CI1210513	CATCTTC^151TCCatcttctcCTGCAGGGAG	c.453_454insATCTTCTC	DM	Lethal neonatal rigidity & seizure syndrome	Saunders (2012) Sci Transl Med 4, 154ra135	hg38 hg19
CI121212	CCACC^212CCCAAaGGTCACTCAG	c.638dupA	DM	Lethal neonatal rigidity & seizure syndrome	Puffenberger (2012) PLoS One 7, e28936 Fernández-Jaén (2016) Eur J Paediatr Neurol : [Additional phenotype] van de Pol (2015) Neuropediatrics : [Additional phenotype]	hg38 hg19

- Recessive loss of function mutations in BRAT1 are recently described causes of a very severe neonatal encephalopathy

#614498

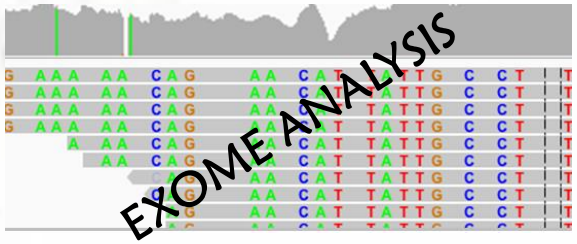
ORPHA: 435845

RIGIDITY AND MULTIFOCAL SEIZURE SYNDROME, LETHAL NEONATAL; RMFSL

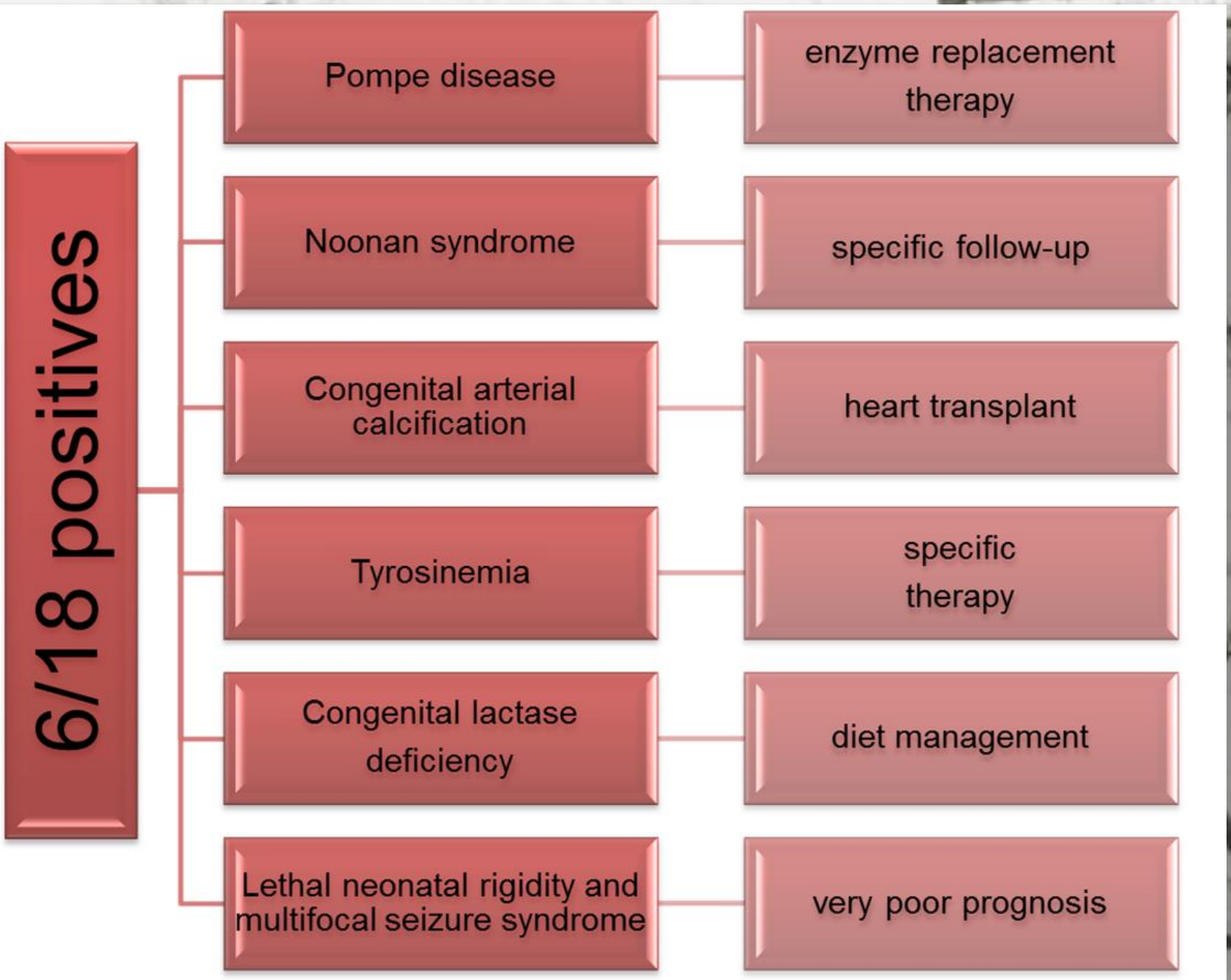
CATEGORY	SUBCATEGORY	FEATURES
Inheritance	-	Autosomal recessive
Head and Neck	Head	Small head (-1.5 to 2 SD)
		Microcephaly, progressive
	Face	Micrognathia (in some patients) [EoM image]
	Eyes	Optic atrophy (1 family)
Cardiovascular	Heart	Bradycardia
Respiratory	-	Apnea
Skeletal	-	
	Skull	
		...ge spikes over the temporal and central regions seen on EEG
		Suppression-burst pattern
		Multifocal seizures
		Background slowing
		Neuronal loss in the striatum, cerebral cortex, and cerebellum (in some patients)
		Astrogliosis (in some patients)
		Corticobasal degeneration (in some patients)
		Delayed myelination (in some patients)
Prenatal Manifestations	Movement	Episodic myoclonic spasms
Miscellaneous	-	Onset at or soon after birth
		Death in infancy
Molecular Basis	-	Caused by mutation in the BRCA1-associated ATM activator 1 gene (BRAT1, 614506.0001)

Worsening clinical conditions

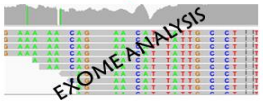
18 NICU cases



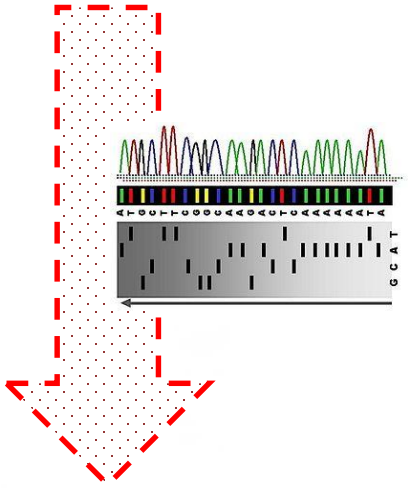
6/10 days
PRELIMINARY RESULTS


6/18 positives
Pompe disease
enzyme replacement therapy
Noonan syndrome
specific follow-up
Congenital arterial calcification
heart transplant
Tyrosinemia
specific therapy
Congenital lactase deficiency
diet management
Lethal neonatal rigidity and multifocal seizure syndrome
very poor prognosis

Worsening clinical conditions



6/10 DAYS
preliminary



20 days-3 months
FINAL REPORT

Limits

1. Technical
2. Bioinformatics
3. Knowledge

Limits

1. Technical
2. Bioinformatics
3. Knowledge

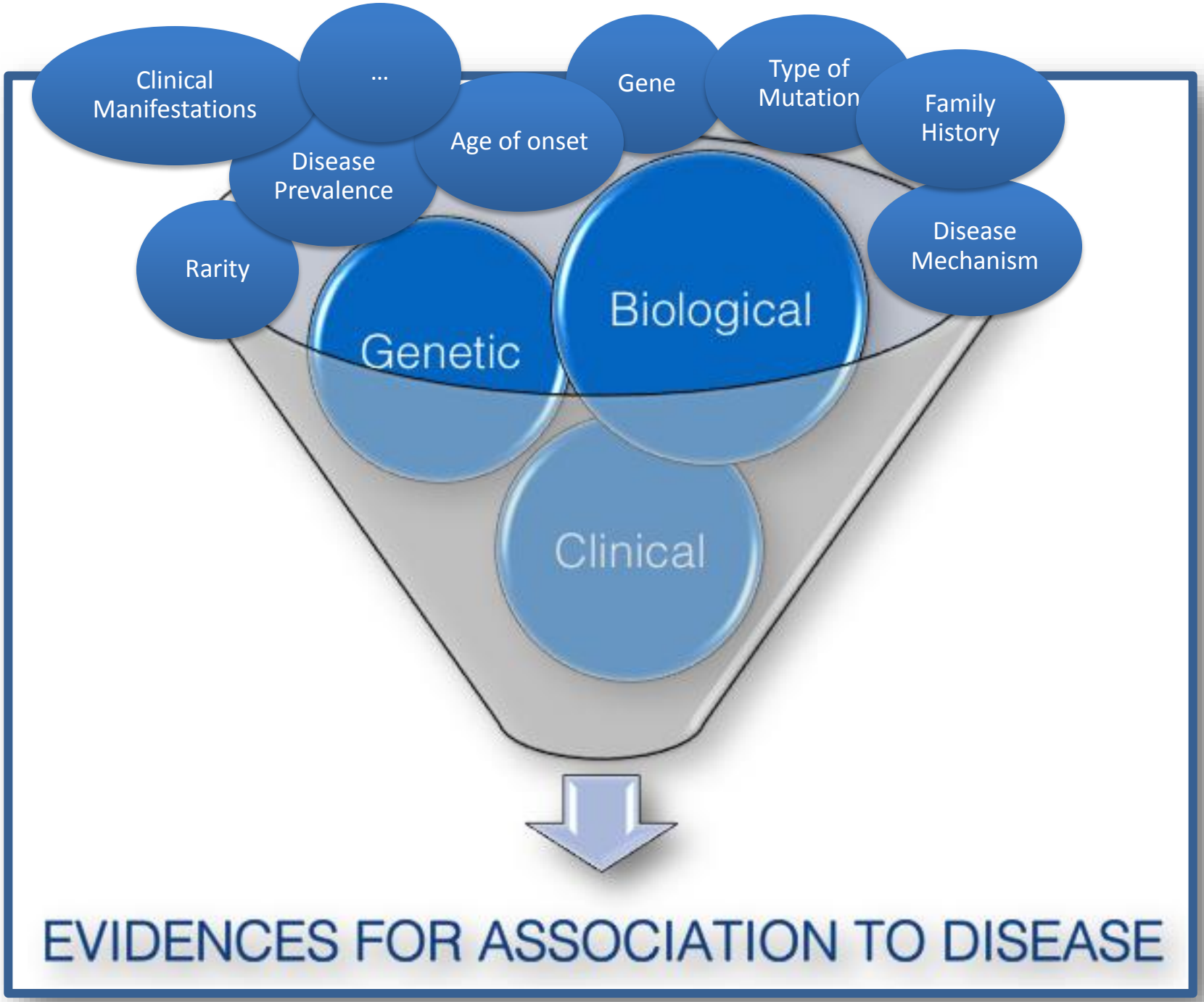
Limits

1. Technical
2. Bioinformatics
3. Knowledge
   - Gene not yet identified
   - Lack of recognition of the causal variant in genome sequence data by those reporting the results
   - Suggestive nature of the majority of findings

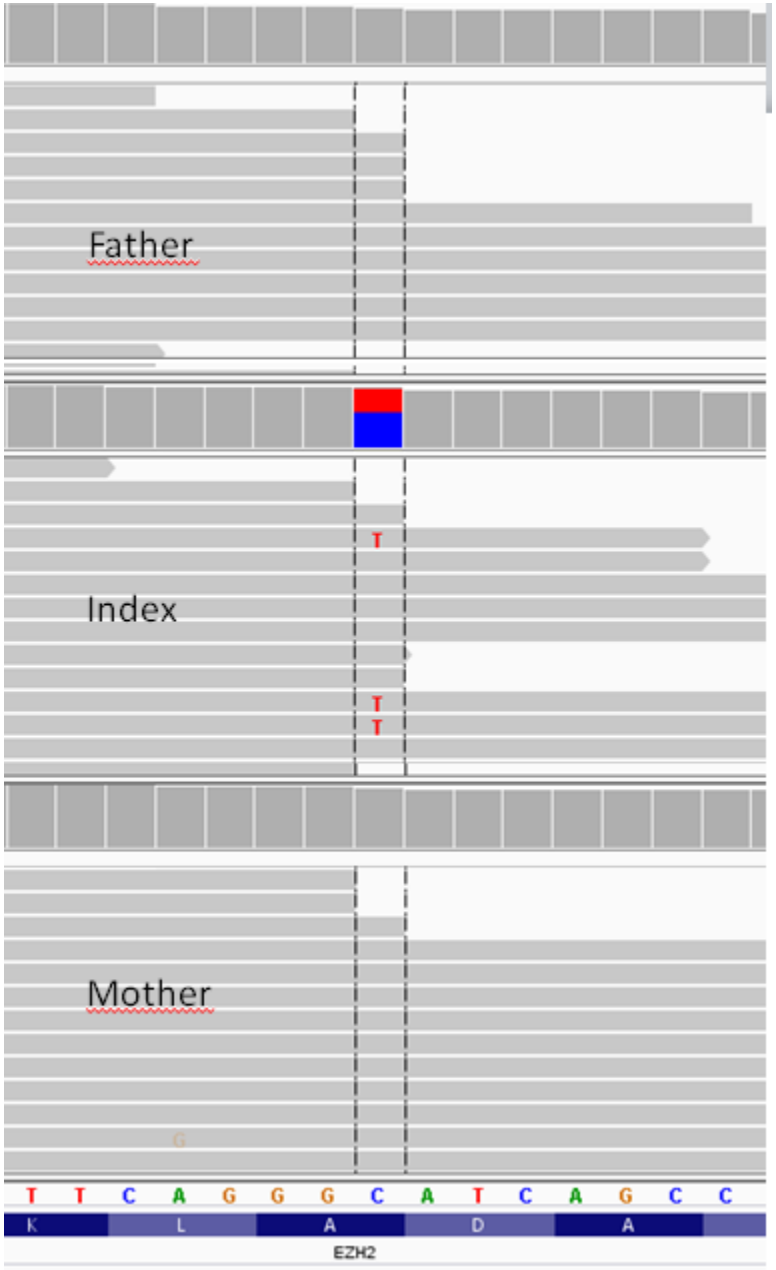
S.A, female 2 yrs

- Several congenital anomalies
- Negative family history
- 1st child, healthy non-consanguineous parents

WES analysis of trio


Clinical Manifestations
...
Gene
Type of Mutation
Family History
Age of onset
Disease Prevalence
Rarity
Disease Mechanism
Genetic
Biological
Clinical
EVIDENCES FOR ASSOCIATION TO DISEASE

A de novo “novel” EZH2 mutation



- *de novo*
- MAF=0
- computational 3D modeling
  - significative change in protein structure
- *In silico* prediction tools
  - disease causing

The American Journal of Human Genetics, 2012

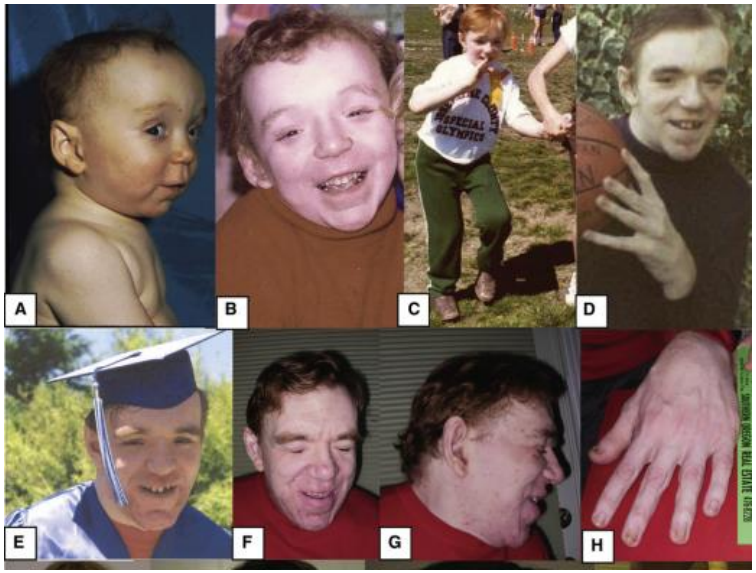
REPORT

Mutations in *EZH2* Cause Weaver Syndrome

William T. Gibson,[1,2,*] Rebecca L. Hood,[3,4] Shing Hei Zhan,[5] Dennis E. Bulman,[4] Anthony P. Fejes,[5] Richard Moore,[5] Andrew J. Mungall,[5] Patrice Eydoux,[1,6] Riyana Babul-Hirji,[7] Jianghong An,[5] Marco A. Marra,[1,5] FORGE Canada Consortium,[12] David Chitayat,[7,8] Kym M. Boycott,[9] David D. Weaver,[10] and Steven J.M. Jones[1,5,11]

We used trio-based whole-exome sequencing to analyze two families affected by Weaver syndrome, including one of the original families reported in 1974. Filtering of rare variants in the affected probands against the parental variants identified two different de novo mutations in the enhancer of zeste homolog 2 (*EZH2*). Sanger sequencing of *EZH2* in a third classically-affected proband identified a third de novo mutation in this gene. These data show that mutations in *EZH2* cause Weaver syndrome.

a rare congenital disorder associated with rapid growth beginning in the prenatal period and continuing through the toddler and youth years. It is characterized by advanced bone maturation, and distinctive craniofacial, skeletal, and neurological abnormalities



S.A, female 2 yrs

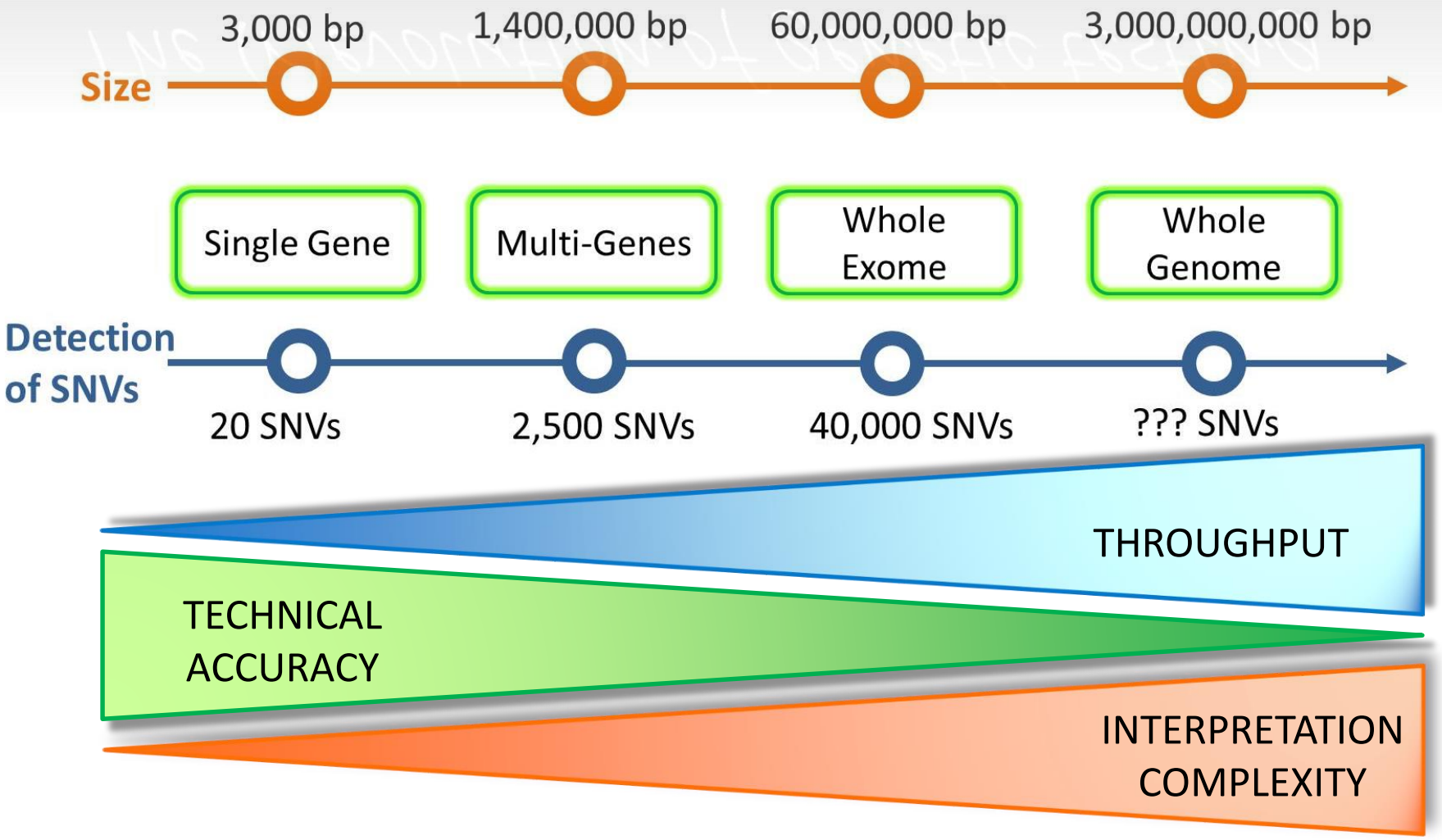
- COMING BACK TO CLINICIAN…

- Muscular hypotonia
- psychomotor impairment
- cranio facial features
- Weight 2,055gr/leng
- cc 33 cm (1
- C
-
-
- agia
- Brachydactyly
- Hypotrichosis

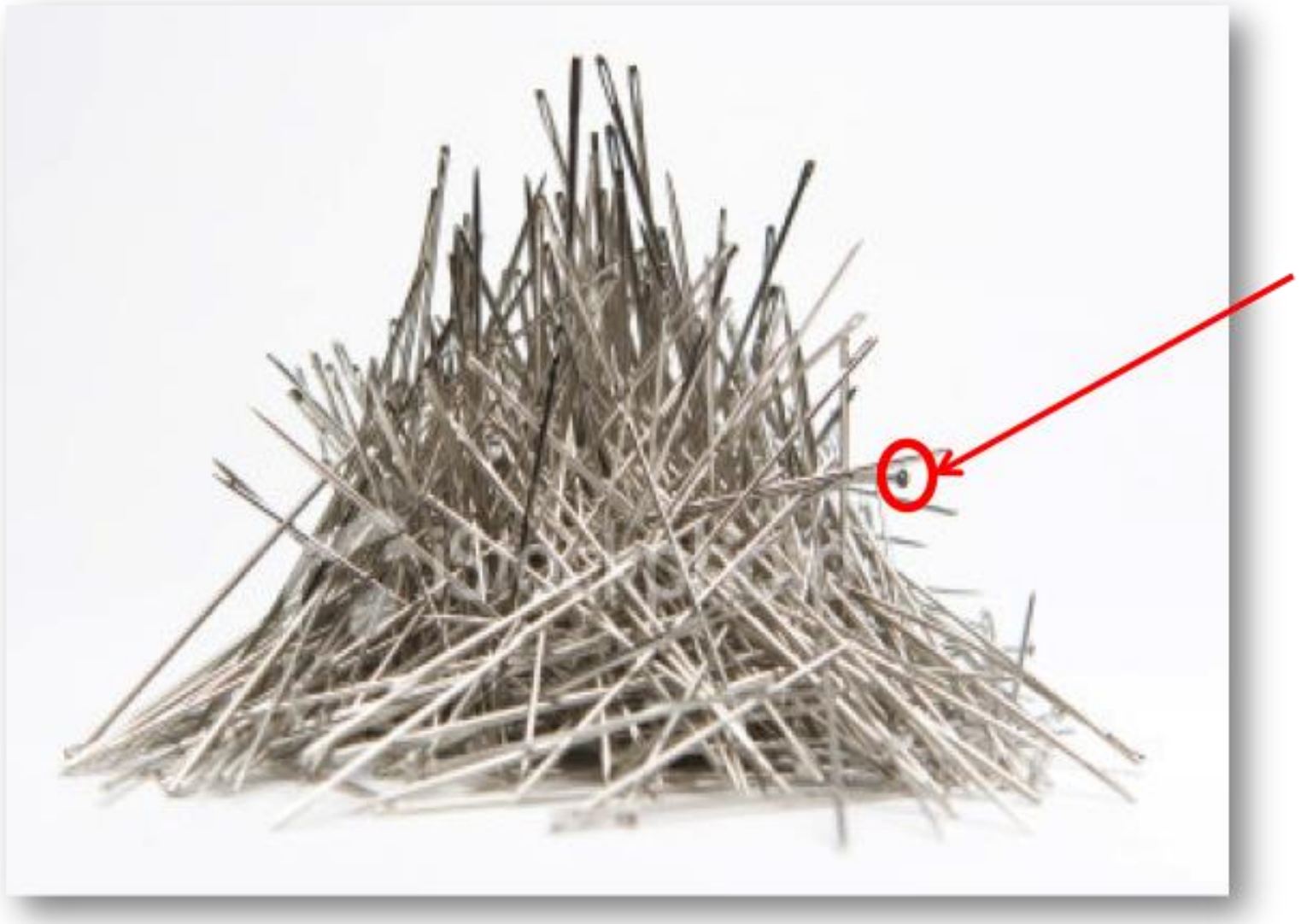
VERY SMALL FOR AGE
DELAYED BONE AGE

Back to Research…

The (r)evolution of genetic testing

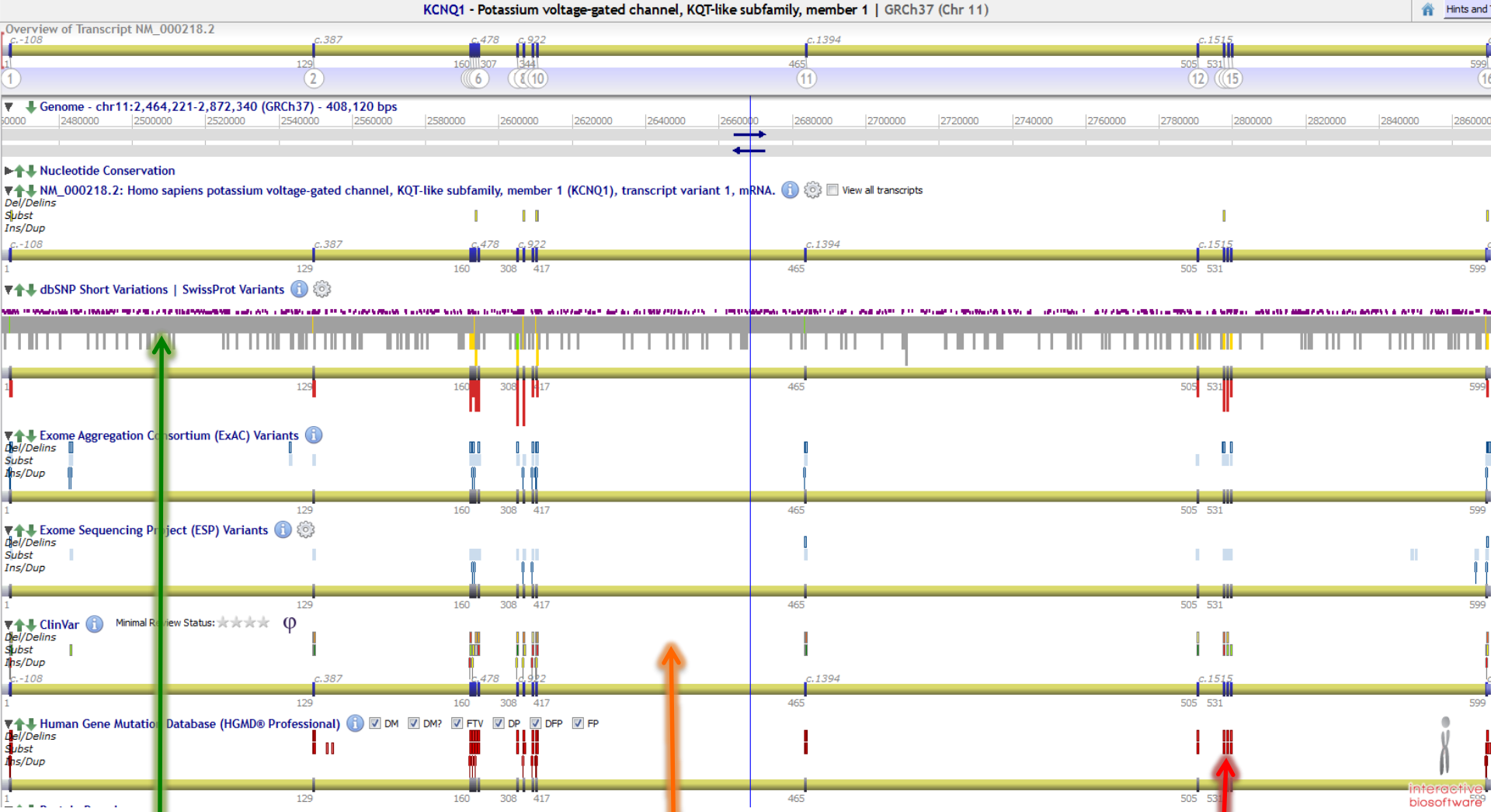


The challenge is finding a needle in a haystack of needles…



Human Variability

The problem is interpretation rather than identification



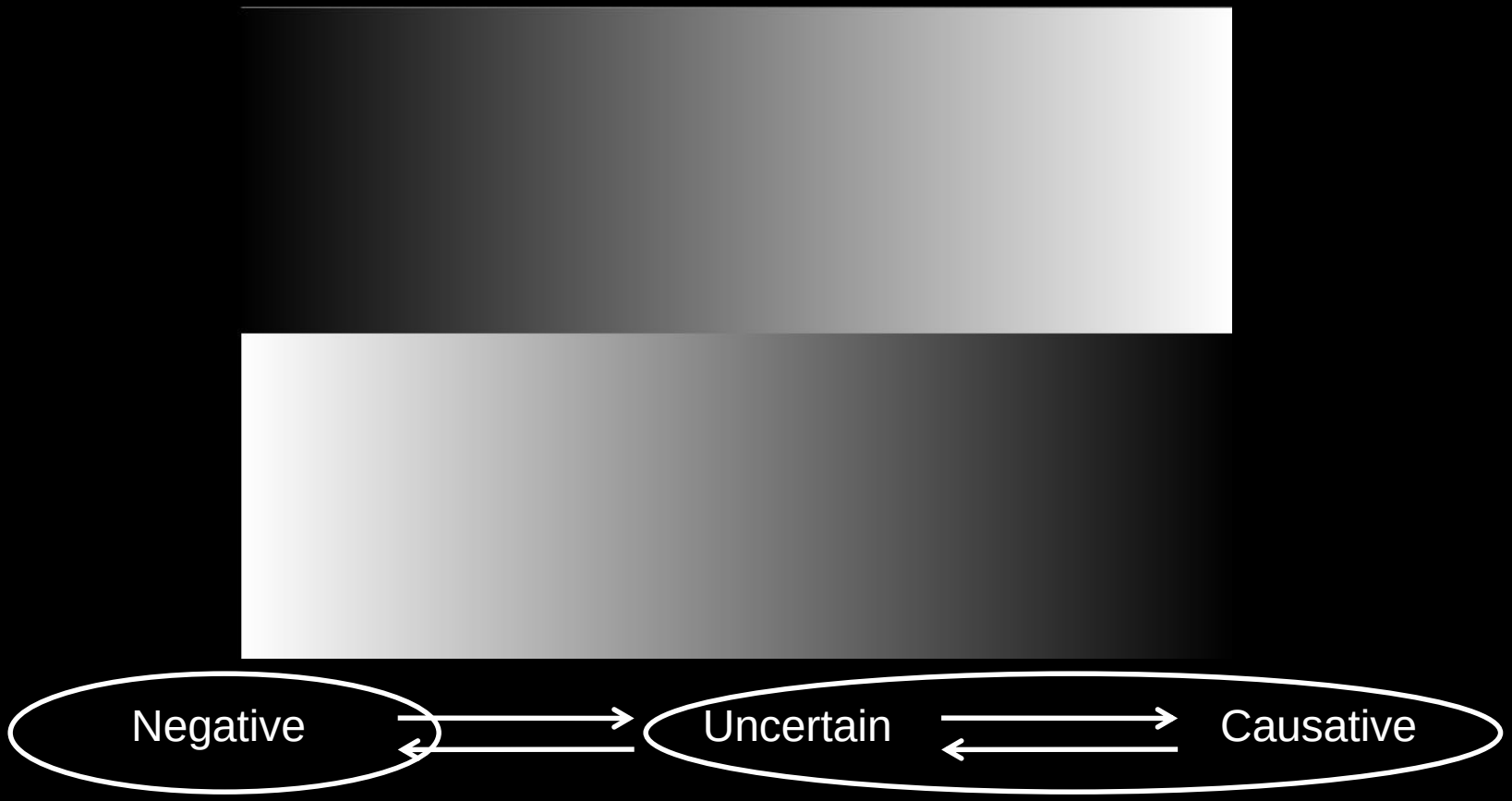
POTASSIUM CHANNEL, VOLTAGE-GATED, KQT-LIKE SUBFAMILY, MEMBER 1; KCNQ1

Likely benign

Re-classification

Reported as pathogenic

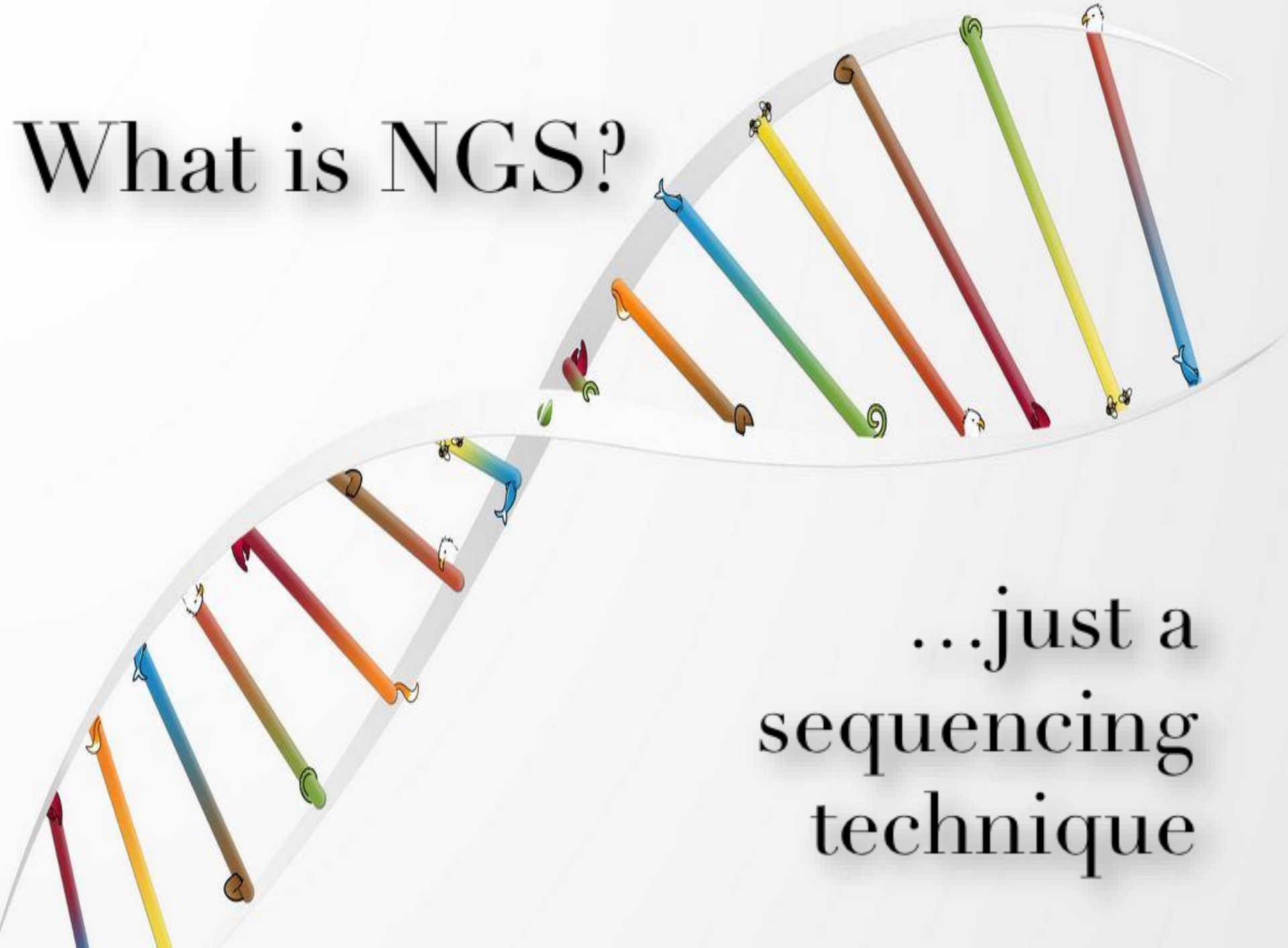
Result of DNA sequencing: a gradient of possible “dynamic” report



Not mean REALLY NEGATIVE

20-60%

What is NGS?



…just a sequencing technique


E E A E LA
M N C T
P R I V I T A’
S


PER
ME
SI
VA
NE
LA
CITTA’

Per me si va ne la città dolente,
per me si va ne l'etterno dolore,
per me si va tra la perduta gente

(Dante, Inferno III, 1-3)

The HPG23 Bergamo Lab

Daniela Marchetti	Lab Geneticist
Laura Pezzoli	Lab Geneticist
Chiara Lodrini	Lab Geneticist
Anna Rita Lincesso	Lab Technician
Loredana Perego	Lab Technician
Maria Elena Sana	Bioinformatician
Anna Cereda	Clinical Geneticist

miascone@asst-pg23.it